STUDIES OF CALCIUM AND OTHER STORAGE MINERALS IN EMBRYOS OF CUCURBITA MAXIMA, CUCURBITA ANDREANA AND THEIR RECIPROCAL HYBRIDS.

By

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ABSTRACT

Calcium is an essential element in plants and often it is the first nutrient to become limiting during seedling growth. Despite this requirement for Ca, embryos have been found to contain low amounts of Ca compared to the other macronutrients that they store. Cucurbita maxima (squash) and C. andreana (bitter gourd) were selected for the study of calcium storage within embryo tissues because it had already been shown that these two species have a 10-fold difference in seed size, a 3-fold difference in Ca level per gram embryo tissue and the reciprocal crosses between them produce viable hybrids.

Calcium was analyzed by atomic absorption spectroscopy and the four storage minerals (Mg, K, Ca and P) were analyzed by neutron activation analysis. Atomic absorption analysis required the destruction of the organic matrix of the samples. Dry ashing of cucurbit embryo tissues resulted in the discovery of some unique characteristics of these tissues. When ground embryo samples of less than 0.05 g weight were ashed in porcelain crucibles, the measured Ca levels were abnormally high due to sample-crucible interactions. The apparent enhancement of Ca level occurred because of changes in the K and perhaps Na levels of the samples. Although cucurbit embryo tissues were resistant to complete ashing at temperatures below 550°C, Ca was more easily extracted from the black ash obtained at these temperatures than from carbon-free ash obtained with higher temperature ashing. The Ca binding at the higher ashing temperatures, which occurred because of a low cation-to-P ratio within the cucurbit ash, was not present in other seed tissues with higher cation-to-P ratios. The problems were overcome by ashing samples of at

least 0.1 g and by acid digestion of the ash to convert Ca to more soluble forms.

Cucurbit embryos contained Mg, K, Ca and P but Ca made up less than 5% of the total mineral reserve while P made up about 50%. All four minerals were present in higher concentrations in the root-shoot axis than in the cotyledons, but the percent of the total Ca in the axis was higher than that of Mg, K and P. The embryos of C. maxima , the species with the large seeds, stored higher amounts of Ca in the axis than the smaller embryos of C. andreana . The Ca levels were more variable in the large-seeded species and were negatively correlated with embryo size. No correlation between embryo size and Ca level was found for C. andreana . The parent and hybrid embryos differed less in their Mg, K and P levels than in their Ca levels. The Ca levels in the hybrid embryos (C. maxima x C. andreana and C. andreana x C. maxima) were significantly different from the parental Ca levels indicating that the hybrid embryos exerted some influence on Ca uptake. However, the fact that hybrid embryos differed in Ca level from each other and that each hybrid approached the Ca level of its female parent showed that maternal influences on Ca level were also prominent. In both species mobilization of minerals from the cotyledons to the growing axis occurred only in seedlings grown in distilled water. In normal growth conditions the expanding cotyledons, which became the first photosynthetic leaves, assimilated nutrients from the medium. The assimilation was particularly marked for Ca.

This study has contributed toward the understanding of mineral storage in seeds of a group that has not been widely studied to date. The study has also emphasized the differences between the storage of Ca and the storage Mg, K and P.

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ABBREVIATIONS

AAS - atomic absorption spectroscopy

c.v. - coefficient of variation calculated by dividing the standard deviation by the mean and multiplying by 100

La/HCl - solution used for Ca measurement by AAS consisting of 0.5% lanthanum ions in 5% hydrochloric acid

MTB - Minitab statistical program (Ryan *et al*, 1985) used the Cyber 170/730 computer

NAA - neutron activation analysis

r - correlation coefficient (Pearson product-moment correlation coefficient)

SD - standard deviation

SE - standard error

Chapter 1.

ORIGIN OF THE STUDY AND GENERAL INFORMATION ON CUCURBITS.

INTRODUCTION

The main aim of this study was to determine whether genetic factors or seed size were more significant in controlling the accumulation of calcium in Cucurbita embryos. The species Cucurbita maxima and Cucurbita andreana provided a unique and useful system for this purpose. These species had embryos which were not only markedly different in size but also had about a three-fold difference in Ca level. Cross-pollination between these species produced viable hybrids. Previous studies had shown interesting differences in Ca distribution from that of the other storage minerals, Mg, K and P and had also shown that Ca distribution was related to embryo size (Lott, 1975; Lott and Vollmer, 1979). The earlier studies had localized Mg, K, Ca and P within the embryo storage cells using energy-dispersive x-ray analysis. In this study the emphasis was on whole embryos and embryo parts using quantitative procedures either for Ca alone or for all four of the main elements stored in cucurbit embryos. Since information on mineral storage in cucurbit embryos was minimal, the secondary aim of this study was to define the mineral storage pattern and overall variation in mineral levels in the embryos of the two parent species and the embryos of their reciprocal hybrids. Again the emphasis was on the differences in Ca storage from that of Mg, K and P. The first part of the thesis deals with the analytical aspects of the work. The second part discusses the results of the mineral analysis of the embryos.

REASONS FOR SELECTING THE ATOMIC ABSORPTION PROCEDURE.

The main choice in procedures for calcium analysis is between destructive and non-destructive techniques (Gorsuch, 1970). In the latter category, minimal sample preparation is needed and the minerals are analyzed within the original organic matrix. With destructive techniques some procedure for destroying the organic matrix is needed and as a result these procedures are usually more time-consuming. For this mineral study it was anticipated that a large number of embryo samples would have to be analyzed for calcium. Thus the chosen technique, in addition to being accurate and precise, had to be rapid and relatively inexpensive. Atomic absorption procedure was suitable but was not rapid since tissues had to be ashed. Two non-destructive procedures, which can be used to analyze a spectrum of minerals virtually simultaneously, were tried on the cucurbit embryos. Initial trials with neutron activation analysis (Hoffman, 1980; Wang et al , 1975) did not provide consistent results. X-ray fluorescence analysis (Evans, 1970; Knudsen et al , 1981) had a persistent matrix problem with cucurbit embryo tissues and could not be applied without much standardization. X-ray absorption technique (Cameron et al , 1976) could not be used to measure calcium in tissues with potassium levels as high relative to Ca as found in seed tissues (Lott, 1984). Atomic absorption spectroscopy has been widely used for calcium measurement on a wide variety of biological and non-biological samples and seemed to be the procedure of choice (Allen, 1974; Gorsuch, 1970; Robinson, 1975). Chapter 2 discusses the standardization of the atomic absorption procedure.

After selecting the analytical procedure, a choice had to be made among the various methods for destroying the organic matrix of the embryo

samples (Gorsuch, 1970). Dry ashing in a muffle furnace was chosen as the most suitable procedure. Problems, which were encountered in ashing of small samples, are explained in Chapter 4. The binding of Ca within the light-coloured ash from higher temperature ashing (Chapter 5) was found to be prevalent in the ash of various cucurbit embryos because of their low cation to phosphorus ratio (Chapter 6). The neutron activation procedure described in Chapter 3 was different from the procedure tried initially and was used to solve the problems arising from the ashing and analysis of cucurbit embryos using AAS. In addition NAA enabled the study of Ca to be extended to include the other minerals stored in the embryos.

IMPORTANCE OF CALCIUM AND OTHER STORAGE MINERALS IN SEEDS.

The regular presence of stores of macromutrients in seed tissues indicates the importance of these elements in germination and seedling establishment. Much of the Ca is found as calcium pectate in the middle lamella of cell walls (Christiansen and Foy, 1979; Marschner, 1983; Somers, 1973). Calcium is also adsorbed to the outside of the plasma membrane and functions in maintaining membrane integrity (Hanson, 1984). Calcium is involved in various tropic responses and is required in the assembly and disassembly of microtubules during cell division (Marme, 1983). In growing plant tissues phosphorus is necessary as a component of nucleic acids and phospholipids and as a source of chemical energy in the form of ATP and related esters (Bieleski, 1973; Clarkson and Hanson, 1980; Mengel and Kirkby, 1982). Magnesium is a vital component of the chlorophyll molecule, is needed to maintain ribosome integrity and is required as a co-factor by many enzymes (Clarkson and Hanson, 1980; Mengel

and Kirkby, 1982). Potassium is also required by a number of enzymes but in addition it plays a key role in maintaining the osmotic potential of cells (Clarkson and Hanson, 1980). Potassium is involved in cell turgor, cell expansion, anion neutralization and assimilate conduction (Mengel and Kirkby, 1982).

Calcium is different from the other minerals because it has distinct intra- and extra-cellular roles and is required inside the cells in micromolar quantities while extracellularly its optimum concentration is millimolar (Marme, 1983; Hanson, 1984). The characteristics of Ca ions have been extensively studied to explain its very broad and unique functions (Levine and Williams, 1980; Hanson, 1984; Hepler and Wayne, 1985; Williams, 1980). Although similar to magnesium, Ca has a larger ionic radius and smaller ionic potential. Calcium also has a more adaptable coordination sphere which results in a less stringent stereochemical demand permitting Ca to bind and cross-link with various anions which do not form regular lattices. Within cells, magnesium concentration is high relative to Ca but the special properties of Ca permit it to bind selectively to proteins such as calmodulin and activate specific enzyme systems (Marme, 1983; Poovaiah, 1985).
Calmodulin-dependent protein kinases have been detected in Cucurbita pepo hypocotyls (Salimath and Marme, 1983) and in some seeds in the early phases of germination (Cocucci, 1984). While much more has been written about Ca than about the other mineral nutrients, much remains unclear about its functions. How the low and variable presence of Ca in so many seed tissues meets the seedling's need for Ca also remains an enigma. This study makes an attempt to determine some of the reasons for the low Ca

levels in cucurbit embryos and to relate the levels to seed size or to the genetic makeup of the embryos.

CUCURBITA : GENERAL INFORMATION

The members of the family Cucurbitaceae are widely distributed over both temperate and tropical regions of the earth although the tropical species predominate (Barber, 1909). There are 90-100 genera and 700-1000 species (Bailey 1949; Heiser, 1979). The cucurbits produce a number of edible fruits such as pumpkins, melons and cucumbers as well as useful but inedible gourds (Heiser, 1979). Some fruits of the genus Cucurbita may be the largest fruits in the world (Barber, 1909; Coombe, 1976; Heiser, 1979; Whitaker and Davis, 1962). In addition to their use as food, some cucurbits also have ornamental and pharmaceutical value (Barber, 1909). The oil-rich seeds remain as yet underexploited for their oils (Vaughan, 1970).

The genus Cucurbita contains 27 species of which 5 are cultivated and 22 are wild (Singh, 1979) and includes squashes, pumpkins and gourds (Whitaker and Bemis, 1975). This genus is of particular importance in North America because five of its species are cultigens indigenous to the Americas (Whitaker and Bemis, 1975). The corn-bean-squash complex formed the agricultural basis of the pre-Columbian Indian cultures. Cucurbita moschata , C. mixta , C. ficifolia , C. pepo and C. maxima are the cultivated species, some of which appear in the archaeological record before 5000 BC (Cutler and Whitaker, 1961). C. andreana is not only closely related to C. maxima but has even been suggested as a potential ancestor of the cultivated species (Whitaker and Davis, 1962). C.

andreana grows wild in disturbed areas in South America and is characterized by particularly high concentrations of the bitter and toxic cucurbitacins (Metcalf et al , 1982). These allelochemicals act as insect repellents for many insect species but as a result of coevolution have become powerful insect feeding attractants for the Diabroticite beetles.

CUCURBIT FRUITS AND SEEDS.

The growth characteristics and anatomy of Cucurbita species have been described by Bushnell (1920), Esau (1977), Mann and Robinson (1950) and Whitaker and Davis (1962) and are described in Chapter 7. Successful fruit set depends on adequate pollination to ensure the fertilization of sufficient number of ovules to sustain fruit growth (Stephenson, 1981). Increasing the number of pollen grains increases the number of seeds produced up to the threshold for a full seed set and subsequent over-pollination may have continued beneficial or deleterious effects depending on the species and growth conditions (Snow, 1982,1986). Cucurbit pollen grains germinate within a few hours and bring about fertilization by the third day (Mann and Robinson, 1950). The cell division in the ovary is generally finished by about 5 days after anthesis (time of full flower opening) and subsequent growth is mainly by cell expansion (Crane, 1964; Nitsch, 1953; Pratt, 1971). Growth regulators play a major role in fruit expansion but how different growth regulators act in different species remains poorly defined (Crane, 1964; Gifford and Evans, 1981). Pratt (1971) stated that auxins, kinin-like factors and gibberellin-like factors are present in melons (Cucumis melo) and Bewley and Black (1985) report that all four of the main plant growth regulators have been found in

immature seeds. Fruits in which there is a lower than normal seed set may abort (Stephenson, 1981) while fruits with many seeds may develop faster than those with fewer seeds (Gorchov, 1985).

The relationships between fleshy fruit size, seed size and seed number are not always clear-cut (Pet and Garretsen, 1983). It has been stated that plants have to compromise between seed size and seed number as a response to environmental or internal stress (Harper et al , 1970). Fruit growth would thus be more dependent on the total seed mass made up of either a large number of small seeds or a smaller number of large seeds. Regulation of seed size and seed number has been studied mainly in grains and legumes while studies on fleshy fruits with several hundred seeds are lacking. Seed size varies over a wide range between species but within each species seed size is the least plastic characteristic (Harper et al , 1970). From the economic point of view, seed size is an important component of yield (Mengel and Kirkby, 1982). Larger seeds are also believed to be more vigorous and have been found to produce larger seedlings (Wulff, 1986) although the advantage is often only evident under stress (Fontes and Ohlrogge, 1972). Extent of predation, type of dispersal and adaptations to various environments are also dependent on seed size (Harper et al , 1970; Salisbury, 1974). Factors controlling seed size are not well understood but involve growth rate, duration of growth and cell number (Egli et al , 1981). Factors such as the supply of photosynthates (Egli et al , 1981; Wulff, 1981), photoperiod (Cook,1975) and temperature (Egli and Wardlaw, 1980) can affect seed size in some species under some conditions but genetic factors are generally more significant (Harper et al , 1970). The control of seed size is a highly complex and variable

process (Spaeth and Sinclair, 1983).

Fruit growth and seed filling appear to be supported by assimilates carried via the phloem (Thorne, 1985). Although fruits have both phloem and xylem connections with the parent plant, there appears to be a deliberate switch from xylem to predominantly phloem flow during fruit growth (Mengel and Kirkby, 1982). Phloem transport has been found to account for 97% of the carbon accumulated in cowpea embryos (Peoples et al , 1985). The transport of nitrogen can be via both xylem and phloem and in both organic and inorganic forms (Richardson and Baker, 1982). The four inorganic macronutrients P, K, Mg and Ca can be found in both xylem and phloem exudate, but P, Mg and Ca are higher in the xylem while K is mainly in the phloem (Richardson and Baker, 1982). The study of the accumulation of minerals in seeds has shown that phloem transport alone cannot account for the amount of Mg and Ca laid down in the seeds (Pate and Hocking, 1978). Interactions between phloem and xylem systems and the reverse flow of water and nutrients out of the fruits via the xylem further complicate the picture (Pate and Sharkey, 1975). It has been suggested that the switch to phloem transport and the resultant delivery of lower amounts of Ca is a necessary step in successful fruit growth (Marme, 1983; Mengel and Kirkby, 1982). A low Ca level would foster higher solute intake and permit rapid expansion by the fruit cells. If the Ca level becomes too low fruits succumb to various Ca deficiency disorders (DeKock et al , 1982; Guttridge, 1982; Simon, 1978). The very high growth rates of cucurbit fruits and the high rates of phloem flow necessary to sustain such growth have puzzled both early and contemporary researchers (Crafts and Lorenz, 1944 a,b; Pharr et al , 1985; Richardson et al , 1984).

Within the developing seeds the nutrients are converted to their storage forms within specific organelles (Lott, 1980; Weber and Neumann, 1980). In cucurbits the seed reserves are stored as oil, protein and phytin (Lott and Vollmer, 1973a,b). Lipid synthesis and lipid body (spherosome) formation appear to be associated with both plastids and endoplasmic reticulum (Wanner et al , 1981). As is typical of storage oils, Cucurbita embryo oil is rich in unsaturated fatty acids (Jacks et al , 1972). The main components of the oil are oleic, linoleic, palmitic and stearic acids (Tsuyuki et al ,1985). The proteins, synthesized on the rough endoplasmic reticulum, are deposited within vacuoles which subdivide to form the protein bodies (Bewley and Black, 1985; Lott, 1980). As much as 90% of the protein consists of cucurbitin, a salt soluble storage globulin (Hara et al , 1979 a,b; O'Kennedy et al , 1979; Pichl, 1976). It is currently believed that cucurbitin is a hexameric protein with a molecular weight of 340,000 and a sedimentation coefficient of 11-12S (Blagrove and Lilley, 1980; Hara-Nishimura et al , 1985). Water soluble albumin proteins are also present in low concentration (Pichl, 1978).

Carbon, in the form of myo -inositol also forms an integral component of the mineral reserves which are called phytin (Lott, 1984; Lott and Ockenden, 1986; Scott and Loewus, 1986). Phytin is a Ca, Mg and K salt of myo -inositol hexaphosphoric acid (Cosgrove, 1966; Maga, 1982). The salt has also been called myo -inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (IUPAC-IUB, 1968). Present evidence of Greenwood and Bewley (1984), derived from castor bean studies, suggests that phytic acid is synthesized in the cytoplasm in association with the cisternal endoplasmic reticulum. Cations are apparently bound to phytic acid at this

time also. The synthesis of phytin is a multi-step process that remains not fully elucidated (Loewus 1971; Loewus and Loewus, 1983; Scott and Loewus, 1986). The synthesized phytin is transported to the vacuoles, where protein is also accumulating, and may eventually condense into globoid crystals (Greenwood and Bewley, 1984). Globoid crystals are electron dense spheres embedded in the proteinaceous matrix of the protein bodies (Lott et al , 1971; Lott and Vollmer, 1973a).

FATE OF CUCURBIT RESERVES ON GERMINATION.

Dehydration of a seed, after the accumulation of its reserves is complete, signals the end of development and rehydration in appropriate conditions leads to germination (Simon, 1984). It has been established, mainly from work on cereals, that the breakdown of stored seed reserves is under the control of growth substances (Bewley and Black, 1985), but such control mechanisms remain less well defined for the dicot seeds. Davies and Slack (1981) reviewed the published work and concluded that the presence of the axis was needed for the maximum mobilization of nutrient reserves from dicot cotyledons. They proposed that the axis acted as a metabolic sink promoting continual breakdown and transport of the reserves. Chapman and Davies (1983) still favoured this "source-sink" hypotheses over the involvement of growth regulators in the germination of dicot seeds. Murray (1984) refers to the above as the feed-back inhibition mechanism and concludes that the information remains contradictory. He suggests that perhaps both mechanisms may be involved depending on the species and its stage of development.

Mobilization of protein and lipid reserves has been quite

extensively studied in cucurbits, especially in cucumber seeds (Cucumis sativus). Davies and Chapman (1979b) found that there was some degradation of cucumber lipids and proteins in the cotyledons with the axes removed but that higher amounts were degraded when the axes were present. The enzyme activities increased independent of the axes. Davies and Chapman (1979b, 1980) took these results as support for the function of the axis as a sink to remove the end-products of degradation. These findings for Cucumis sativus are in contrast to the work on Cucurbita maxima (Penner and Ashton, 1966,1967) in which the axis was found to be necessary for the development of proteolytic activity but the axis could be replaced by cytokinin. The above is in disagreement with the results on Cucurbita moschata (Splittstoesser, 1983) where the axis was found to have no effect on protein degradation. Legocka et al (1985) found that cytokinin (kinetin) was needed for the mobilization of lipids and proteins in isolated, cultured Cucumis sativus cotyledons. Davies and Chapman (1979b) point out that externally applied kinetin stimulates cotyledon expansion and the expansion may result in an apparent enhancement of lipid and protein degradation. Chapman and Galleschi (1985) cite evidence of starch accumulation in the cotyledons germinating without axes, as support for the cotyledons acting as a source and the axes as a sink. Thus the problem remains unresolved.

On germination the water insoluble cucurbit globulin (Cucurbita sp.) is partially degraded to more soluble components (Hara et al ,1976b) which are then further hydrolyzed to peptides and amino acids (Hara and Matsubara, 1980). Some enzymes for protein degradation have been detected in dry, ungerminated seeds but the activity increases rapidly after water

uptake. The first type of limited hydrolysis apparently occurs within the protein bodies (Hara-Nishimura *et al*, 1982). The proteolytic activity begins declining after the fourth day from imbibition (at 20°C) when as much as 90% of the protein has been degraded (Hara *et al*, 1976b). Reilly *et al* (1978) also found that about 88% of the globulin in *Cucurbita moschata* was degraded after four days (at 30°C). This also agrees with the ultrasturctural evidence which shows that by the fourth day, protein bodies have become a series of vacuoles containing small amounts of residual protein (Lott and Vollmer, 1973a). Nelson (1932), using stained paraffin sections of *Cucurbita maxima* found that the initial mobilization of storage reserves was from the provascular areas while reserves in the palisade mesophyll cells farthest from the provascular areas were mobilized last. More recently, Davies and Chapman (1981) found essentially the same pattern in germinating *Cucumis sativus* seeds.

During initial lipid degradation the triglycerides are cleaved by lipases and then the degradation is continued via the glyoxylate cycle (Bewley and Black, 1985). Lipases were not detectable in dry *Cucumis* seeds and probably arose from *de novo* synthesis following imbibition (Huang and Moreau, 1978). As degradation proceeded lipid bodies decreased in size and disappeared; this was paralleled by the appearance of glyoxysomes (Wanner *et al*, 1982). Davies and Chapman (1979a) found that about 75% of the lipid reserve had been degraded by the seventh day after imbibition.

None of the authors mentioned above have extended their studies to the mobilization of mineral reserves in cucurbit embryos. The greater part of the work on mobilization of minerals has been carried out on cereals

and legumes. The results presented in Chapter 12 show the fate of the minerals in cucurbit cotyledons during various germination regimes.

CONCLUSION

This chapter has shown that cucurbits have been studied from a number of aspects but that little information is available on mineral metabolism in these species. The studies undertaken for this thesis endeavour to fill in some of the knowledge about cucurbit embryo mineral reserves. The emphasis has been on Ca and it will be shown that this element shows a number of differences from the storage patterns of Mg, K and P. The differences are present in both the two parent species studied and their reciprocal hybrids and are evident in both the dry embryos and seedlings.

Chapter 2

STANDARDIZATION OF THE ATOMIC ABSORPTION PROCEDURE FOR ANALYSIS OF CALCIUM IN CUCURBIT EMBRYOS.

INTRODUCTION

Atomic absorption spectroscopy has been used for Ca analysis in a wide range of biological and non-biological samples. The procedure requires only relatively inexpensive instrumentation and has the advantage of being able to run many samples in a short time. It is easy to learn and does not involve specialized technical assistance. While the analysis itself is quite rapid the overall procedure can be time-consuming because of the need to work with samples in fairly dilute solution. To achieve this the organic matrix of plant materials must be removed. Procedures for the destruction of the organic matrix are many and varied (Gorsuch, 1970) and contribute to the problems and errors of the whole analytical procedure. In Ca analysis of cucurbit embryos what appeared to be analytical problems were eventually traced to problems arising during the ashing of the embryo samples. In this chapter only the standardized procedure and the experiments leading to the standardization are presented. Problems arising out of the analytical steps are discussed in Chapter 4 and problems arising from the destruction of the organic matrix are dealt with in Chapter 5.

The principles of AAS have been discussed by Allen (1974), Peters et al (1974), Robinson (1975) and Walsh (1982) and its application to plant tissues has been outlined by Isaac (1980). The liquid samples to be

measured are aspirated into the instrument where the solution is atomized and the fine droplets are passed into a 1500-3000°C flame. At these temperatures the droplets dry and the Ca and other ions are converted to ground state atoms which then absorb the light of a wavelength characteristic for the desired element. The maximum production of atoms depends on flame temperature, flame composition, height of the flame with respect to the radiation beam, speed of sample aspiration and droplet size. The degree to which the above factors can be adjusted by the analyst varies with the instrument used. The flame types most commonly used for Ca analysis are air-acetylene and nitrous oxide-acetylene. Anything that interferes with the production of the ground state atoms causes a lower level of Ca to be measured than is actually present in the sample. Both kinds of flames are subject to factors that interfere with production of ground state atoms.

The main problems in AAS arise from spectral interferences, chemical interferences, ionization interferences and also from matrix effects. Spectral interferences, in which other elements absorb at wavelengths close to that of the desired element, are not present in Ca analysis. Chemical interferences occur when Ca is bound into compounds which are resistant to degradation in the flame permitting the Ca to pass through the flame without its absorbance being recorded. Chemical interferences are very critical in Ca determinations using air-acetylene flame and solutions containing phosphate ions. The use of lanthanum, which preferentially binds with phosphate ions leaving Ca free for conversion to the atomic state, has minimized this interference (Yofe and Finkelstein, 1958). Nitrous oxide-acetylene flame has a higher temperature than an

air-acetylene flame so that the calcium phosphate formed can be degraded again without causing any interference. This hotter flame, on the other hand, ionizes the atoms. The ions do not absorb light and thus pass through the flame undetected. To overcome ionization interference in Ca analysis an excess of a more easily ionizable element such as K or Na is added to the solutions being measured. The analysis of Ca using the nitrous oxide-acetylene flame was tried, but as will be shown in Chapter 4, the problems encountered with Ca analysis in cucurbits were not resolved by the hotter flame. The air-acetylene flame was more commonly used for Ca analysis locally and was much more convenient to use. Although Robinson (1975) strongly favoured the nitrous oxide-acetylene flame, past studies of plant tissue have been carried out about equally using both flames. Matrix effects, which cause values to be lower than the actual level of Ca present, can be overcome by dilution of the sample being aspirated. The presence of a matrix effect in cucurbit samples will be shown in Chapter 4.

Phosphorus is the main interferent, especially with seed samples which have a considerable amount of P present as phytin (Lott, 1984). Allen (1974) reported that anions such as silicates and sulphates interfere in Ca analysis but concluded that the interference could be prevented by lanthanum. Allen mentioned aluminum and iron as interfering agents but Robinson (1975) found that cationic interferences were generally absent in AAS.

The procedure eventually accepted for Ca analysis of cucurbit embryos involved the dry ashing of the embryo tissues, an acid treatment of the ash, extraction of the ash into dilute hydrochloric acid containing

lanthanum, dilution to overcome matrix effects and analysis in the atomic absorption spectrophotometer using an air-acetylene flame. The method was applied to the analysis of C. maxima and C. andreana embryos and their reciprocal hybrids and the procedure was standardized to suit the three-fold difference in Ca level between the two parent embryos.

MATERIALS AND METHODS

Atomic Absorption Procedure :

Spectrophotometers: Two spectrophotometers (Perkin Elmer 603 AAS and Varian AAS 1275) were used during the study. The Perkin Elmer instrument was used in the initial standardization of the technique but was replaced by the more recent and more flexible Varian instrument for the bulk of the work. The two instruments produced essentially the same results and each was used according to the manufacturer's instructions. The lamp current for the hollow cathode Ca lamp varied from 3.5 to 4.0 mA set at 422.7 nm with a spectral band pass of 0.5 nm. The acetylene (Purified, Medigas Ltd.) to air ratio was adjusted to give an oxidizing flame. The aspiration rate of the sample and other variable parameters on the instrument were adjusted to give the highest stable readings for the standard solutions.

Standards: Inorganic standards were prepared in 5% HCl from Ca carbonate and also from the dilution of BDH 1000 ppm Ca^{2+} stock solution of $Ca(NO_3)_2$. Double distilled or deionized-distilled water was used for all solutions. While the standard curves were identical, the more convenient prepared solution was used for most of the work. The cucurbit samples were also compared to Ca levels in the standard

reference materials of Tomato Leaves (SRM 1573) and Wheat Flour (SRM 1567) purchased from the National Bureau of Standards, Washington, DC. Lanthanum was routinely added to all standard solutions in the same concentration as used with the ash solutions.

Use of Lanthanum as a Releasing Agent from Phosphate Interference: The need for lanthanum was demonstrated by adding lanthanum ions to ash solutions of individually ashed embryos of C. maxima . Various trials were made using ash solutions of both C. maxima and C. andreana embryos and solutions of La^{3+} ranging from 0.1-1.0%. Lanthanum solutions were prepared either from lanthanum chloride (BDH) or lanthanum oxide (Sigma Chemical Co.). The solution of 0.5% La^{3+} in 5% HCl will be referred to as La/HCl and has been used in all analyses of cucurbit embryos.

Acid Treatment of the Ash: The cucurbit ash was given a double acid treatment prior to extraction into La/HCl. First a solution of 1:2 V/V nitric acid (BDH Aristar) and deionized distilled water was added to the crucibles containing the ash (about 1.5 ml per 0.25 g initial embryo weight). The crucibles were heated on a hot plate to evaporate the acid. Then, about the same volume of 1:1 V/V HCl was added and heated to dryness. This procedure was adapted from Gorsuch (1970). Blank crucibles were acid treated and then ashed as controls with each batch of embryo samples. To determine the minimum amount of acid needed and to find whether an excess of acid affected the measured concentration of Ca, 0.45 g samples of C. maxima embryos were ashed and acid treated with volumes of acids ranging from 0.25 to 4.0 ml.

Test of the Accuracy and Sensitivity of the Atomic Absorption Analysis of Ca in Cucurbit Embryos: To determine whether the procedure

was sensitive enough to detect small differences in Ca levels but accurate enough to give good agreement between duplicate samples, eight batches of five embryos were ground and pooled. Five samples of 0.15 g were weighed from each batch, ashed at 550°C and analyzed in La/HCl. Analysis of variance using a one factor random effects model was carried out on the results.

Stability of the La/HCl Ash Solutions: The La/HCl ash solutions of C. maxima and C. andreana were measured on several consecutive days and also after several weeks of storage in the refrigerator to determine the extent of instrumental variation and the stability of the ash solutions.

Sample Dilution as Related to the Ratio of Ca in C. maxima and C. andreana Embryos: To determine whether the two cucurbit species were reacting in the same manner to dilution and measurement in the flame, small (0.05 g) and large (0.45 g) embryo samples of each species were ashed and analyzed at several dilutions. The ratio of Ca levels between the two species was compared at the different dilutions.

Standardization of Sample Preparation :

Seed Coat Removal: The seeds of C. maxima cv. Warted Hubbard were purchased from Stokes Seed, Ltd., St. Catharines, Ontario. The C. andreana seeds were obtained from Dr. W.P. Bemis, University of Arizona, Tucson. Cucurbit seeds have two seed coats and only the outer one is easily removed in the dry state. The thin, membranous inner seed coat had to be peeled off the embryos after a soaking in water. To ensure that there was no major loss of Ca during the soaking, several batches of embryos were soaked for one to 24 hours with and without the seed coats

and the solutions were analyzed for Ca. The inner seed coats of some embryos were cut off with a razor blade so that Ca levels in soaked and unsoaked embryos could be compared.

Grinding of Embryo Tissue: The cucurbit embryos were ground with a porcelain pestle and mortar. Other grinding procedures such as grinding in acetone and ball-milling in liquid nitrogen were also tried and are discussed in Chapter 4. While grinding was used to obtain a homogeneous sample of pooled embryos from which subsamples were weighed out for ashing, the procedure worked equally well on whole embryos. Equal numbers of samples consisting of individual embryos and pooled, ground embryo samples of matching weights were ashed and analyzed and the means were compared by a t-test.

Moisture Content of Cucurbit Embryos: Cucurbit embryos were dried in the intact state and as ground tissues at temperatures ranging from 50 to 130°C for 1 to 24 hours. The effect of drying at various sample sizes was also compared. Drying of NBS Wheat Flour standard at the recommended minimum sample size of 0.4 g at 85°C for 24 hours was compared to drying at the same times and temperatures used for the cucurbit embryos. The moisture content of the embryos was calculated using the formula suggested by Allen (1974) in which the loss in weight on drying is divided by the initial sample weight and multiplied by 100. The moisture contents or dry weights were generally obtained on separate samples from those being ashed and analyzed.

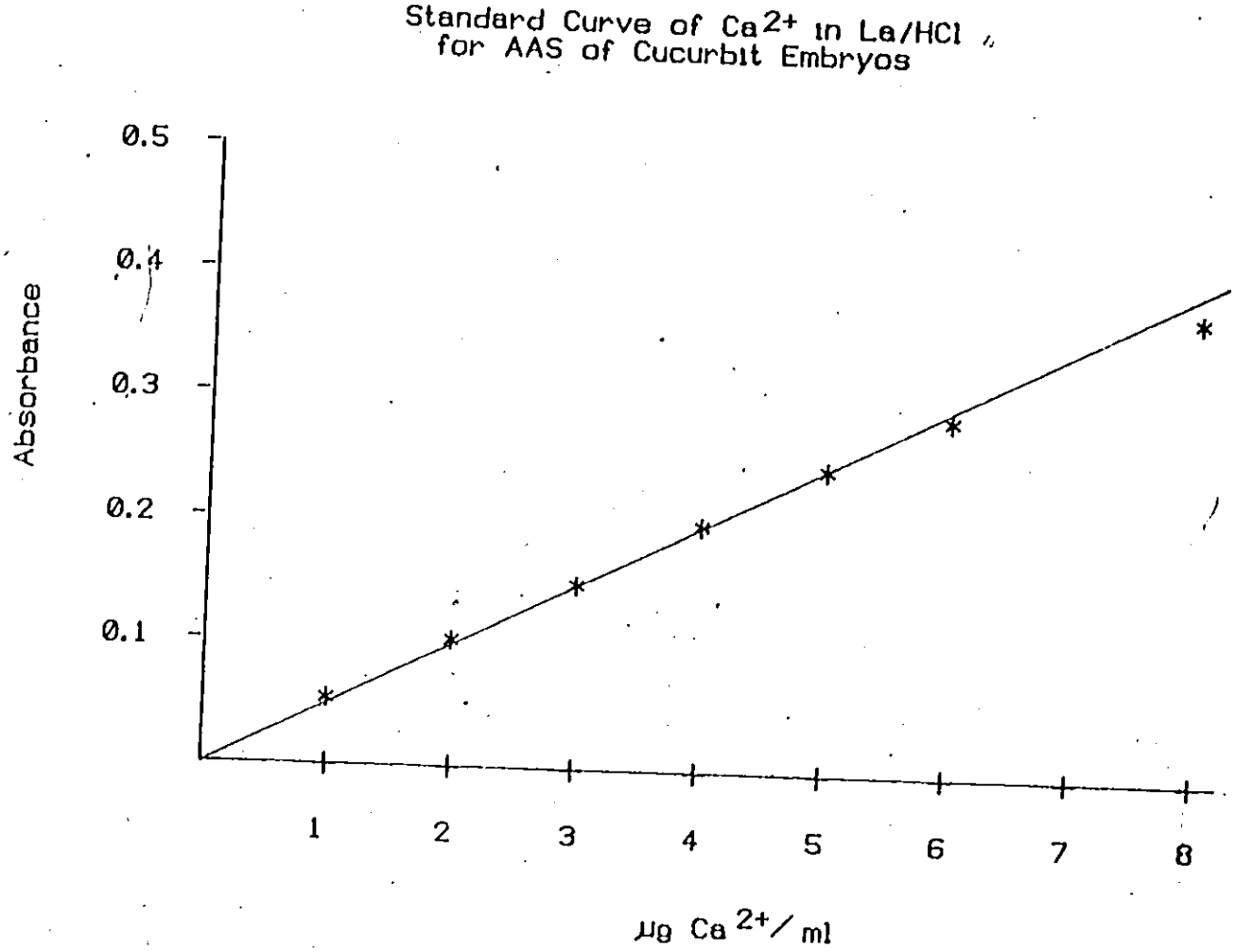
Determination of Sample Size: Forty-five randomly selected C. maxima embryos were ashed and analyzed individually. The Ca levels obtained for the individual embryos were then averaged in batches of 5,

15, 20, 25 and 35 values to determine how many embryos needed to be pooled to eliminate the embryo-to-embryo variation. C. andreana embryos were also ashed singly and in batches of 2, 3, 5, 10 and 20 embryos.

Destruction of the Organic Matrix: The embryo samples (intact or ground) were weighed into porcelain crucibles of 17 ml capacity. The crucibles were then placed on the periphery of a hot plate with a central temperature of 465°C and moved centrally to increasingly hotter areas as smoking ceased at each of the lower temperatures. In the final heating the crucibles were lightly covered with aluminum foil to complete the charring of the sample. The charred samples were ashed in a Blue M Electric Muffle furnace at 550°C for four hours. Ashing times of 1/2 to 24 hours were tested at the 550°C ashing temperature using three sample sizes of ground C. maxima embryos. Experimentation with ashing at different temperatures is discussed in Chapters 4 and 5. If the ash samples were to be weighed for percent ash determination the crucibles were cooled in a desiccator.

Defatting of Cucurbit Embryos and Determination of Ca in the Oil: The two procedures most commonly used to extract crude lipids from embryos use chloroform and methanol 2:1 V/V (McGibbon and Williams, 1981; Nakayama et al , 1981) and hexane (Bair and Snyder, 1980; Bemis et al , 1977; Nakayama et al , 1981). Both procedures were tried but the hexane gave a clear solution which was easier to decant. Oil extraction was performed on both ground and intact embryos. In the intact state the embryos were extracted with three lots of Ca-free hexane overnight for three consecutive days. In the ground state the extraction was shorter but required filtering instead of decanting. The extracts were pooled and

Figure 2 : 1


Standard Curve of Ca2+ in La/HCl
for AAS of Cucurbit Embryos
Absorbance
0.5
0.4
0.3
0.2
0.1
1
2
3
4
5
6
7
8
µg Ca 2+/ ml

taken to dryness before weighing. The samples were transferred to the crucibles and charred prior to ashing and analysis by the same procedure as used on the embryos.

RESULTS AND DISCUSSION

Atomic Absorpion Procedure :

Figure 2:1 shows a typical standard curve for the diluted BDH Ca solution which is linear up to 6 µg/ml Ca^{2+}. Samples with absorbance readings higher than 0.3 were generally diluted in La/HCl and re-read. As will be shown later *C. maxima* embryos had low levels of Ca compared to levels of minerals such as P, K and Mg so that, if the Ca levels were large enough to produce high absorbance readings, the solutions were concentrated enough to show a matrix effect. Dilution of such samples produced higher Ca levels than measured in the initial samples. It was found that 0.01 g of unashed *C. maxima* embryo tissues per ml of final ash solution in La/HCl was an adequate dilution to keep the absorbance readings of most of the samples analyzed within the 1-5 µg/ml portion of the standard curve. The higher Ca levels of the *C. andreana* embryos required higher dilutions in the first place so that dilution to avoid the matrix effect was less of a problem. The concentration of 5% for hydrochloric acid was chosen after trials with 1%, 5% and 10% solutions and was a compromise between higher signal depression at 10% and lower ash solubility at 1%. The analysis of both the Tomato Leaves and Wheat Flour Standards gave Ca levels within the certified range (Alvarez, 1980; Gladney *et al*, 1984).

In Table 2:1 each sample represents the ash solution of single

TABLE 2:1

Effect of Lanthanum on the Absorbance in Air-Acetylene Flame of Dilute HCl Extracts of *C. maxima* Embryo Ash.

Sample	Absorbance		
	No La^{3+}	+ La^{3+}	% Increase
1	0.107	0.374	71.4
2	0.107	0.305	64.9
3	0.177	0.582	69.6
4	0.034	0.180	81.1
5	0.084	0.295	71.6
6	0.082	0.293	72.0
7	0.204	0.612	66.7
8	0.192	0.570	66.3
9	0.175	0.580	69.8

TABLE 2:2

Comparison of Ca Levels in La/HCl Ash Extracts of *C. maxima* Embryos After Ash Treatment with Various Volumes of Dilute HNO_3 and HCl.

Vol. of Acids (ml)	Wt. Embryo Samples (g) (Ashed 550°C)	µg Ca^{2+}/ g Embryo (Mean ± SD; N=3)
0	0.45	216.5 ± 15.3
0.25	0.45	238.8 ± 4.8
0.5	0.45	233.1 ± 8.7
1.0	0.45	223.8 ± 3.5
2.0	0.45	233.0 ± 0.2
3.0	0.45	231.4 ± 9.5
4.0	0.45	240.6 ± 13.2

embryos hence the large sample-to-sample variation in absorbance. When lanthanum was added the absorbance increased an average of 70.4%. The average increase for C. andreana samples was 25%. Thus the depression of Ca absorption was more pronounced in the C. maxima embryo ash. For C. maxima a 0.1% lanthanum solution did not release all the Ca from the phosphate depression but 1% had no more effect than a 0.5% solution. The 0.5% or 5000 ppm La^{3+} was suitable for both species and was chosen for routine analysis. This is within the concentration recommended in the literature (Allen, 1974; Isaac, 1980).

The double acid treatment of the ash proposed by Gorsuch (1970) was found necessary for the full extraction of Ca from cucurbit embryo ash. The acid treatment after 550°C ashing was not as essential as after ashing at higher temperatures but was used as a precaution since the temperature variation within a muffle furnace could expose some crucibles to the higher temperatures. The Ca binding which occurred at the higher temperatures is discussed in Chapter 5. Table 2:2 shows that acid volumes of 0.25 to 4.0 ml produced essentially the same Ca levels thus showing that an excess of the acids had no adverse effects on the analysis.

Table 2:3A shows that the mean Ca levels for the eight batches of pooled embryos had acceptably low coefficients of variation indicating good agreement among the five subsamples of each batch. Analysis of variance (Table 2:3B) showed that, at the very high confidence level of P=0.001, there was significantly more variation between batches than within the batches. Thus, the technique was suitable for detecting differences in Ca level between embryos from different sources.

Repeat analysis of the same solutions showed that there was little

TABLE 2:3

A Test of the Sensitivity and Accuracy of the AAS for Measurement of Ca in *C. maxima* Embryos.

A.

Batch #	µg Calcium / g Embryo	c.v.
1	233.9 ± 6.8	2.9%
2	219.8 ± 8.0	3.6%
3	275.5 ± 11.8	4.3%
4	246.9 ± 10.7	4.3%
5	238.0 ± 16.1	6.8%
6	228.5 ± 15.0	6.6%
7	222.1 ± 4.1	1.8%
8	219.6 ± 7.0	3.2%

B

Sources of Variance	Sum of Squares	Mean Square	$F_{(7,32)}$
Between Batches	12,340.8	1,763.0	15.4***
Within Batches	3,674.0	114.8	(P = 0.001)
Totals	16,014.8		

TABLE 2:4

Comparison of Ca Levels in *C. maxima* and *C. andreana* Embryos Ashed in Two Sample Sizes and Analyzed at Various Dilutions.

Sample Size (g)	Vol. La/HCl (ml)	µg Ca^{2+}/g Embryo, N=2		B/A
		C. maxima (A)	*C. andreana* (B)	
0.05	5	203.9	661.7	3.2
0.05	10	236.1	646.8	2.7
0.45	25	215.2	624.0	2.9
0.45	50	230.5	655.7	2.8
0.45	100	228.0	660.3	2.9

fluctuation in the absorbance day to day that was not accounted for by similar fluctuation in the absorbance of the standards. On the whole there was remarkable consistency in the absorbance readings over many months and many batches of standards. The ash solutions themselves were also stable giving virtually identical readings after weeks of storage. Thus there was no problem with either instrumental or sample instability.

In the initial analyses the ratio of Ca per gram of C. andreana embryos to Ca per gram of C. maxima embryos varied from 2 to 10. The ratio became consistent at about 3 when the ash treatment, sample-size effect (Chapter 4) and matrix effect problems were recognized and eliminated. Neutron activation analysis (Chapter 3) confirmed the three-fold difference in Ca levels between the two cucurbit species. Table 2:4 shows that there was considerable leeway in the dilutions at which the two species could be compared. The small sample weights of 0.05 g were generally avoided for reasons described in Chapter 4 except for the measurement of some of the embryo axes which did not have sufficient tissue for larger sample sizes. In Chapter 3 it will be shown that the axes have so much more Ca present that the problems related to sample size were minimized. The 25 ml dilution (Table 2:4) shows a slightly lower Ca level for both species due to the matrix effect since the dilutions were not adequate. The good agreement between levels in both species at both 50 and 100 ml dilution shows that over-dilution was not a problem as long as measureable absorbance readings were produced.

Standardization of Sample Preparation :

In addition to variation in Ca level from changes in instrumental parameters and in the concentrations of the solutions being measured,

TABLE 2:5

Comparison of Mean Ca Levels in Individually Ashed Embryos and Subsamples of Pooled, Ground *C. maxima* Embryos.

Measurements Made	Whole Embryos	Subsamples of Ground Embryos
No. of Samples	27	27
Range of Sample Weight (g)	0.14 - 0.27	0.14 - 0.27
Range of μg Ca^{2+} per Sample	19.9 - 89.4	32.6 - 68.5
Largest Ca Level / Smallest Ca Level	4.5	2.1
μg Ca^{2+}/g Embryo (Mean ± SD)	231.0 ± 80.3[a]	249.8 ± 21.0[a]
c.v.	34.8%	8.4%

[a] - the means are not different at P=0.05

other steps in the preparation of the embryos had an effect on the measured Ca level. The cucurbit embryos have an inner seed coat which contained more Ca than the embryo (Chapter 10, Table 10:11) and therefore had to be removed. This seed coat was, however, so closely adherent to the embryo in the dry state that it was difficult to remove without soaking (Brown, 1940). Experiments with soaking the embryos with and without the testas showed that little to no Ca was leaking from the embryos during the immersion in water. Less than 1% of the Ca per embryo was lost in one hour of soaking and little further loss occurred after two or more hours of soaking. The Ca levels in razor-peeled and soaked embryos were identical indicating no loss of Ca as a result of the soaking. Results in Chapter 12 show that the leakage of elements from the embryos was much less than from the outer seed coats and that losses of Ca were very small compared to the losses of K. Experiments by Loomis and Smith (1980) with imbibing cabbage seeds also showed a negligible loss in Ca and Mg but a high loss of K during 1 to 16 hours of soaking.

When grinding plant tissues for mineral analysis, the aim was to produce a sample of uniform consistency and of small particle size and if these criteria were not met, there could be variations in mineral levels of subsamples taken from the ground tissues (Nelson and Boodley, 1965; Smith et al , 1968). The efficacy of the grinding procedure is often judged by the size of the mesh through which the bulk of the ground sample could pass (Allen, 1974) but oily tissues, such as ground cucurbit embryos, were too sticky to be tested in this manner. Johnson and Collins (1973) discussed the problems of grinding oily seed tissues and concluded that low temperature grinding under liquid nitrogen was best for such

TABLE 2:6

Determination of the Moisture Content of Cucurbit Embryos and NSB Wheat Flour Standard.

Drying Procedure Temp. (°C)	Time (hr.)	% Moisture (N = 2) *C. maxima* 0.15 g Sample Ground, Pooled Embryos	*C. maxima* 2 Intact Embryos	*C. andreana* 10 Intact Embryos	NSB Wheat Flour Std. 0.4 g
50°C	1	- 0.6	3.5	- 8.7	1.3
	2	- 0.4	3.6	- 9.1	-0.5
	24	3.9	4.8	- 4.5	1.7
85°C	1	4.0	5.0	3.8	5.6
	2	4.4	5.8	7.5	5.4
	24	4.7	6.2	7.7	5.4
105°C	1	4.7	4.8	5.0	6.0
	2	4.8	5.1	5.7	6.3
	3	5.0	5.1	5.5	6.3
	24	4.8	5.1	4.7	6.1
130°C	1	5.0	5.5	3.9	6.2
	2	5.3	5.7	4.9	6.1
	3	5.1	5.7	4.7	6.0
	24	-	5.9	-	-

tissues. In Chapter 4 the use of such a grinding procedure is reported for the cucurbit tissues but the procedure was not found superior to pestle and mortar grinding for this study.

Since the AAS was required for the analysis of single embryos as well as subsamples of ground mixtures, the method was tested on both types of samples (Table 2:5). The range of embryo sizes was matched by similar ground sample sizes to eliminate any bias from sample size. The ranges and ratios of Ca levels showed the embryo-to-embryo variation that was present and also showed that variation could be reduced by pooling. The remaining variation was due to the analytical procedure. The t-test (MTB) showed that the means were not significantly different for the two groups. Thus grinding was not essential except to obtain representative Ca levels on pooled mixtures of many embryos.

The high oil content of the embryos caused problems with dry weight determinations in the form of weight losses due to volatilization and weight gains due to oxidation. Table 2:6 shows some of the commonly used drying times and temperatures (Allen, 1974) tested on both the cucurbit embryos and NBS Wheat Flour standard. Temperatures of 40°C or less produce "air-dried" samples. The low temperature of 50°C gave erratic results with cucurbit embryos. Overnight drying at 85°C is recommended for the NBS Wheat Flour standard. The cucurbit embryos showed only a small difference between the 2 and 24 hour dry weights at this temperature. The most uniform results for the moisture contents of the three kinds of cucurbit samples were obtained at 105°C. Roberts and Roberts (1972) recommended 130°C for drying the embryos of Cucurbita sp., but at 130°C the embryos and the Wheat Flour standard samples

TABLE 2:7

Variation in Ca Level in Individually Ashed *C. maxima* Embryos and the Reduction of This Variation by Pooling of the Embryos.

No. Pooled* Per Batch	No of Batches Analyzed	$\mu g\ Ca^{2+}/g$ Embryo		c.v.
		Mean ± S.D.	Range of Means	
1	45	220.3 ± 84.0	91.5 - 408.9	38.1
5	9	220.3 ± 44.4	136.2 - 228.5	20.2
15	3	220.3 ± 14.0	204.0 - 230.1	6.4
20	4	218.4 ± 4.8	213.6 - 225.0	2.2
25	3	216.7 ± 5.2	211.4 - 221.7	2.5
35	2	220.3 ± 3.3	218.0 - 222.6	1.5

* 45 embryos were analyzed individually; the Ca levels were pooled in the indicated batches.

TABLE 2:8

Measurement of Ca in Intact Embryos of *C. andreana*.

No. Embryos Ashed Per Crucible	No. of Samples Analyzed	Av. Wt. per Sample	$\mu g\ Ca^{2+}/g$ Embryo (Mean ± S.D.)	c.v.
1	50	0.013	695.5 ± 129.5	18.6
2	8	0.027	715.6 ± 83.6	11.7
3	8	0.039	696.2 ± 83.7	18.0
5	8	0.066	704.5 ± 61.9	8.8
10	8	0.133	697.2 ± 31.2	4.5
20	8	0.264	701.8 ± 37.9	5.4

turned increasingly brown indicating some charring. Thus 2 hours at 105°C were selected as the drying time and temperature for the two cucurbit species. The dry weights of various batches of ground embryos from the stock C. maxima and C. andreana seeds ranged between 4 and 5% and were remarkably constant over the time of the study. When dry weights of embryos from the fruits grown for this study were compared there was no difference in the dry weights of the two parent species and the hybrids. The field grown C. maxima fruits had mean moisture contents of 4.8 ± 0.6% (N=70). The greenhouse fruits had 4.9 ± 0.6% moisture (N=56) and the growth chamber specimens had 4.5 ± 0.8% moisture (N=63). Although dry weights were determined for each specimen analyzed, most mineral levels are reported for the air-dried weights.

With the considerable embryo-to-embryo variation in Ca levels the accuracy of results depended not only on homogeneous grinding of the pooled embryos but also on the selection of an adequate number of embryos to eliminate the effect of the variation. Table 2:7 shows that both the range of Ca levels produced and the coefficients of variation decreased as more embryos were pooled. The difference between 20 and 25 embryos was not marked so that the embryo-to-embryo variation was eliminated when 20 embryos were pooled. To ensure the elimination of the variation at least 25 embryos were routinely pooled from the stock seeds.

The results in Table 2:8 show that the small C. andreana embryos could also be analyzed individually in the intact state because of their high Ca content. As more embryos were ashed together the standard deviations and the coefficients of variation decreased. The mean values were remarkably consistent for both individual and pooled measurements.

TABLE 2:9

Variation in Ashing Times at 550°C on the Measured Ca Levels Levels in C. maxima Embryos.

Sample Size	μg Ca^{2+}/g Embryo (Mean + S.D.) for Various Ashing Times					
	1/2 Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.
0.05 g	265.6 ± 9.3	257.3 ± 3.0	248.6 ± 4.1	269.6 ± 9.9	282.8 ± 9.1	262.6 ± 11.1
0.25 g	254.0 ± 10.9	261.1 ± 8.8	262.9 ± 8.2	268.6 ± 10.4	274.6 ± 12.9	269.4 ± 13.9
1.0 g	234.4 ± 6.8	257.4 ± 3.6	262.5 ± 5.2	257.5 ± 2.2	259.6 ± 3.0	259.5 ± 6.1

The problems related to analysis of very small dry-ashed embryo samples (Chapter 4) did not appear to be present when intact C. andreana embryos were analyzed probably because of the minimal contact between the embryo and the crucible compared to that of a ground sample of the same size. A hundred or more of the C. andreana embryos were required to give an adequate amount of ground sample so that the embryo-to-embryo variation was no problem with these smaller embryos.

The described dry ashing procedure was selected after considerable experimentation with various dry ashing techniques and, as well, with plasma ashing and wet ashing (Chapters 4 and 5). The charring (Gorsuch, 1970) or pre-ashing (Sansoni and Panday, 1983) at lower temperatures was necessary to prevent ignition of the samples in the muffle furnace and was preferable to the alternative of raising the furnace temperature slowly. The final product from charring was glossy and brittle and did not smoke or burst into flame at temperatures up to 450°C. Charring on the hot plate was more satisfactory for the cucurbit samples than charring in a sand bath.

The selected temperature of 550°C was in a range where the Ca binding (Chapter 5) was minimal but the ash was no longer brittle and hydrophobic and so was easier to disperse. Ashes from the lower temperatures also tended to foam and splatter with acid treatment. Ashing at temperatures above 550° was avoided because of the frequency of visible damage to the crucible glaze. Table 2:9 shows that the length of ashing time could be varied and even overnight ashing (results not shown) produced Ca levels similar to shorter ashing periods. The ashing of the one gram sample for 1/2 hour produced a lower Ca level probably due to

TABLE 2:10

Percent Ash in Cucurbit Embryos and in NBS Standards After Dry Ashing at 550°C.

Specimens Ashed	No. of Samples	Sample Size (g)	% Ash (Mean ± S.D.)
C. maxima Embryos	24	0.05	4.2 ± 0.4
"	19	0.25	4.2 ± 0.3
"	6	1.0	4.3 ± 0.1
C. maxima Defatted Embryos	9	0.05 - 0.25	7.0 ± 0.4
C. andreana Embryos	15	0.05 - 1.0	3.9 ± 0.6
NBS Wheat Flour	32	0.05 - 1.0	0.8 - 0.5
NBS Tomato Leaves	9	0.06 - 1.0	18.3 ± 1.4

TABLE 2:11

Oil Contents of Embryos Containing High and Low Ca Levels.

Type of Embryos	μg Ca^{2+}/g Embryo	% Oil
Large (0.26 g) Stock C. maxima Embryos (N = 24)	156.9 ± 5.0	36.4 ± 1.1
Small (0.14 g) Stock C. maxima Embryos (N = 24)	216.1 ± 1.1	42.3 ± 0.9
Low* Ca Embryos from Fruit # 1 (N = 50)	89.8 ± 5.9	49.1 ± 1.0
High Ca Embryos from Fruit # 19 (N = 50)	235.3 ± 8.5	48.4 ± 0.3
Low** Ca Embryos from 5 Fruits (N = 50 per Fruit)	146.0 ± 6.6	48.8 ± 3.2
High Ca Embryos from 5 Fruits (N = 50 per Fruit)	338.1 ± 58.2	49.3 ± 1.8

*
**from Figure 8:3
derived from total C. maxima crop (Chapter 8)

incomplete ashing. One of the 0.05 gram samples had a higher Ca level than the rest of the samples suggesting the presence of the Ca level enchancement discussed in Chapter 4. As already mentioned such small sample sizes were avoided in routine embryo analysis. Four hours was chosen as a convenient ashing which produced clean crucibles and ash that dispersed easily. As will be explained in Chapters 4 and 5, the ash from 550°C was black, mainly insoluble and contained residual carbon.

Table 2:10 shows the percent ash in the cucurbit embryos and NBS standards. The leafy type tissue (NBS Tomato Leaves) had the highest percent ash while the starchy type tissue (NBS Wheat Flour) had the lowest ash content. Hinton (1959) found that the ash content of the wheat embryo was 4-5% and hence was similar to cucurbit embryos but the endosperm ash content was about 0.6% and thus was similar to the Wheat Flour standard. The cucurbits were fairly constant in having about 4% ash and this percent was obtained from ashing samples varying from 0.05 to 1.0 g in weight. The defatted embryos had a higher ash content and this was consistent with a loss of 40-45% oil which was essentially free of minerals.

The extraction and analysis of oil was included in the standardization of the procedure for several reasons. One reason was the tendency of the oil to creep up the sides and occasionally over the edges of the crucibles on charring. It was essential to know whether any Ca was being lost as a result of oil loss. Secondly the variation in the oil content could affect the measured Ca level. Extraction of oil from ground cucurbit tissues showed small amounts of Ca present in the oil perhaps due to some particulate matter from the damaged cells. When the extraction was carried out on intact embryos little to no Ca was found in the oil.

TABLE 2:12

Outline of the Procedure for the Analysis of Ca in Cucurbit Embryos and NBS Wheat Flour Standard by AAS.

Sample Preparation:

The seed coats were removed from seeds to obtain the embryos which were pooled and ground in a pestle and mortar. Subsamples of the ground embryo mixture or of the NBS Wheat Flour Standard were weighed into porcelain crucibles, charred on a hot plate and ashed in a muffle furnace at 550°C for 4 hours. The ash was heated with diluted Aristar nitric acid (1:2 v/v HNO_3:H_2O), taken to dryness and then heated with 50% HCl, also to dryness. Cucurbit samples of 0.2 - 0.25 g required about 1.5 ml of each acid.

Analytical Procedure:

The acid treated ash residue was taken up in 5% HCl containing 0.5% La^{3+} (called La/HCl). C. maxima samples of 0.2 - 0.25 g required 25 ml La/HCl to give a dilution of 0.01 g embryo tissue per ml HCl. C. andreana samples of 0.15 g required dilution to 50 ml with La/HCl. Samples of axis tissue of 0.05 g or less required dilution to 25 ml. NBS Wheat Flour Standard samples of 0.4 - 0.5 g were diluted to 50 ml. All the samples required centrifugation or filtration through ashless filter paper to remove the insoluble portion of the ash.

The supernatant was aspirated into the spectrophotometer adjusted to give the maximum, stable absorbance for Ca^{2+} in La/HCl standards of 1-5 ppm in an air-acetylene flame. Ca levels of the samples being measured were obtained from the standard curves.

The stock C. maxima embryos contained 36.7 to 38.5% oil while the stock C. andreana embryos had 40.5 to 42.1% oil. Various categories of embryos with high and low Ca content had their oil content measured and related to the Ca level. Some of the results are summarized in Table 2:11. In each case shown the oil content was more similar than the Ca level. In the one case (Stokes seeds) where there was a difference in the oil content, the difference served to accentuate rather than decrease the difference in Ca level. Hence it appears that the Ca level differences in results presented in the subsequent chapters were not due to any major differences in the oil content of the embryos being analyzed.

Table 2:12 summarizes the steps involved in tissue preparation and analytical procedure for Ca analysis. This procedure has been standardized against both organic and inorganic standards and with the outlined precautions is free of interferences arising during the analytical steps. No major problems arising from the steps in tissue preparation were detected. The method has been used in all subsequent chapters where Ca results from AAS are presented.

Chapter 3

MEASUREMENT OF MINERALS IN CUCURBIT EMBRYOS BY NEUTRON ACTIVATION ANALYSIS.

INTRODUCTION

Neutron activation analysis (NAA) is a technique well suited for the analysis of plant tissues. The advantages of NAA are the high sensitivity of the method, which allows one to detect 10^{-6} to 10^{-12}g of an element, the non-destructive nature of the procedure and the ability to analyze many elements simultaneously (Wang et al , 1975). Most samples, including biological ones, can be measured without destroying the organic matrix. Under the irradiation conditions used in this study samples, were unchanged in appearance and could be re-run or otherwise re-used after they were no longer radioactive. However, the procedure requires costly equipment and specialized irradiation facilities. Although not a complex technique, it can be hazardous if used by untrained personnel.

The principles of NAA have been outlined by Corliss (1964), Heydorn (1984), Hoffman (1980) and Wang et al (1975). The application of the procedure to a variety of plant samples has been described by Naidenov and Raikov (1980). Their analyses by NAA were compared to analyses by a number of laboratories using various other techniques and the agreement was generally good. In the NAA procedure, samples containing the elements to be measured are bombarded by neutrons resulting in the production of radioactive isotopes within the samples (Corliss, 1964; Hoffman, 1980).

The radioactive species then decay emitting gamma-rays which have energies characteristic of the element from which they were derived and which are emitted in amounts directly proportional to the elemental concentration present (Corliss, 1964; Hoffman, 1980). While neutrons can be derived from electrostatic particle accelerators or isotopic sources, nuclear reactors are considered to be the best sources of neutrons (Corliss, 1964).

The high neutron fluxes available at reactors increase the sensitivity of the analysis. Sensitivity is also increased by lengthening the irradiation and counting periods. The required irradiation time varies with the type of element being measured and the half-life of the isotope produced (Corliss, 1964). Since the decay of the radioactive species begins immediately on their formation, the measurement of the sample has to take the rate of decay of the various elements into account (Wang et al , 1975). The number of radioactive nuclei formed depends on the flux of neutrons to which the sample was exposed (Corliss, 1964). The flux is measured as the number of neutrons passing through a square centimeter per second. The nuclear cross-sections of the elements are also important since elements with smaller cross-sections take longer periods of bombardment to produce the same number of radioactive molecules (Corliss, 1964). Thermal neutrons, also called slow neutrons, are the most commonly used activation particles because they are the main species of neutrons present in the reactor flux and because they can react with nuclei of most of the elements in the periodic table (Corliss, 1964). The elements C, N and O, which are present in the highest amounts in biological tissues, are barely activated by the thermal neutrons hence do not cause interference in the measurement of elements present in trace amounts. The epithermal

and the fast neutrons are found in smaller concentrations within the reactor flux and cause more complex nuclear reactions with emission of not only gamma-rays but also neutrons, protons and alpha particles (Corliss, 1964). Heydorn (1984) cautions that there are major differences in the design and operation conditions of most research reactors so that the neutron flux is variable and conditions of analysis for the same sample would vary for each reactor. Irradiation times and subsequent counting conditions can be controlled by the analyst. Variations in the neutron flux can not be controlled by the analyst (Heydorn, 1984). To compensate for flux variations, flux monitors must be run along with the samples.

The samples to be irradiated are enclosed in small polyethylene vials and sent into the reactor for irradiation within pneumatic tubes in containers called "rabbits" (Corliss, 1964). For many biological samples irradiation time is measured in seconds although for some samples longer irradiation times are needed (Wang et al , 1975). The irradiated sample is then counted, generally by computerized equipment called a multichannel analyzer (Corliss, 1964; Heydorn, 1984). Scintillation counters were used to count the gamma-rays in the past but various high resolution solid state detectors are now more commonly used (Corliss, 1964; Heydorn, 1984). These detectors, including the high purity germanium detector used for the cucurbit samples, have a greater resolution of the gamma-ray energies but a lower efficiency than scintillation detectors (Heydorn, 1984).

MATERIALS AND METHODS

Sample Preparation :

Most of the analyses were carried out on pestle and mortar ground

embryo tissues which were weighed into polyethylene vials 1 cm. in diameter. With embryos from most cucurbit fruits there was sufficient sample to perform AAS and NAA analysis on the same ground embryo mixtures. The sample weights generally ranged from 0.15 to 0.25 g but samples as small as 0.01 g could be analyzed. For the germination experiments described in Chapter 12, seedling tissues were ground and analyzed by the same procedure as the embryo samples. Liquid samples (in 5 ml vials) required an additional step of acidification with ultra pure HNO_3 (Ultrex) to make the samples contain 1% acid in order to minimize adsorption of the elements to the surfaces of the vials. Duplicate or triplicate vials were prepared for each solid or liquid sample to be analyzed. Vials containing NBS Citrus Leaves (SRM 1572), Tomato Leaves (SRM 1573) and Wheat Flour (SRM 1567) were prepared as organic standards. Liquid samples were also compared to NBS Trace Elements in Water (SRM 1643b). Vanadium flux monitors (Atomic Absorption Standard) were used for the calibration of the neutron flux.

Irradiation of the Samples for the Analysis of Mg, K and Ca :

Sealed vials containing samples of embryo tissue or organic standards were placed in "rabbit" carriers and sent into the reactor for 120 seconds. The samples were activated by thermal neutrons with a flux of about 5×10^{12} neutrons per cm^2 per second. After irradiation the samples were transferred into pre-weighed, unirradiated vials and were ready for counting. Liquid samples were irradiated for 180 seconds.

Instrumental Analysis :

Vials containing solid samples were placed one cm from the snout of an Aptec lithium drifted germanium detector (8% efficient) and were

counted for 10 minutes. Delay times were in the range of 90 to 200 seconds and were selected as needed for the sample size being analyzed. The detector was coupled to a series 40 Canberra multi-channel analyzer and a pile-up rejection unit. The dead times were selected to not exceed 25%. Resolution of the counting apparatus was 2.1 keV at the 1332 keV cobalt peak. Mineral levels derived from the data analysis were expressed as weighted means and standard errors taking into account various sources of error. The liquid samples were placed 2.5 cm from the snout of an Aptec hyperpure germanium detector with 28% efficiency. Resolution of this counting apparatus, when coupled to a Series 90 analyzer, was 1.85keV at the 1332 keV cobalt peak. The selected delay time was three minutes and the counting time was 10 minutes.

Procedure for Phosphorus Analysis :

Since Al and P produce the same irradiation product of ^{28}Al, the determination of P in samples that may contain both elements had to be made using two separate irradiations. The first irradiation was made with thermal neutrons as already described for the other elements with additional data being recorded by the analyzer for the ^{28}Al. The second irradiation was with the faster epithermal neutrons. To achieve differential irradiation with the faster neutrons within the flux which was made up predominantly of thermal neutrons, the samples were irradiated in cadmium-lined "rabbits" for 20 sec. The cadmium shielded the samples from bombardment by thermal neutrons. The samples were then transferred into unirradiated vials and were counted for 300 sec. after a delay time of 80 to 150 sec. The concentrations of Al and P were separated mathematically using the results of both irradiations and the formulas

described by Gatschke and Gawlik (1980). No phosphorus analyses were possible in the liquid samples with the current system. Ultrapure phosphorus was used as an additional standard for P analysis.

RESULTS AND DISCUSSION

Initial standardization of the NAA procedure was made on embryos of the *C. maxima* and *C. andreana* seed stocks described in Chapter 7. For a pooled embryo mixture that gave Ca levels by AAS of 200 ± 10 µg per gram embryo, the NAA gave very similar Ca levels of 195 ± 13 µg per gram embryo based on the irradiation of 10 separate samples. Comparison of the Ca levels in *C. maxima* and *C. andreana* embryos showed that the species had a three-fold difference in Ca level by both methods. The NAA and AAS analysis of the embryos of 9 non-cucurbit species and 12 different cucurbit species are presented in Chapter 6. With the exception of two results with larger, unexplained discrepancies, the Ca levels for the other 19 species were within 7% of each other. Table 3:1 shows the mean Ca values for embryos from parent and hybrid fruits analyzed by both methods. The agreement between the two different, multi-step procedures is about as good as could be expected and the mean Ca levels for the two procedures were not significantly different by a t-test (MTB). Table 3:2 shows that when axes and cotyledons were compared separately the cotyledon Ca levels agreed well but axes tended to be higher by NAA although the correlation coefficients between the two methods, for both axes and cotyledons, were at least 0.97. Mean Ca levels by the two methods were significantly different only for *C. maxima* and *C. maxima* x *C. andreana* . Due to the problems with the analysis of embryo samples of less than 0.5 g (Chapter

TABLE 3:1

Ca Levels in the Parent and Hybrid Embryos by AAS and NAA on the Same Pooled Ground Embryo Mixtures.

Type of Embryos	AAS				NAA				% Diff.
	μg Ca^{2+}/g Embryo				μg Ca^{2+}/g Embryo				
	N	Mean	SD	SE	N	Mean	SD	SE	
C. maxima	82	232.5	57.5	6.3	22	240.0	80.0	17.0	+3.2
C. maxima x *C. andreana*	12	356.0	104.0	29.7	12	350.0	110.0	31.4	-1.7
C. andreana x *C. maxima*	46	589.0	153.0	22.5	27	560.0	160.0	30.8	-4.9
C. andreana	35	651.8	87.9	14.9	16	690.0	90.0	22.5	+5.9

TABLE 3:2

Ca Levels in Axes and Cotyledons of Parent and Hybrid Embryos as Measured by AAS and NAA.

Type of Embryos	μg Ca^{2+}/Axis		% Diff.	μg Ca^{2+}/Cotyl. Pair		% Diff.
	AAS	NAA		AAS	NAA	
C. maxima	1,544.0 N = 79	1,850.0 N = 17	+19.8%	202.7 N = 79	197.0 N = 17	-2.8%
C. maxima x *C. andreana*	1,627.0 N = 11	1,980.0 N = 8	+21.7%	321.0 N = 11	337.0 N = 8	+5.0%
C. andreana x *C. maxima*	1,442.0 N = 33	1,480.0 N = 15	+2.6%	521.0 N = 33	507.0 N = 15	-2.5%
C. andreana	1,627.0 N = 21	1,870.0 N = 9	+14.9%	615.6 N = 21	613.0 N = 9	-0.4%

4) It had been expected that the Ca levels for the axes derived from the AAS analysis might be too high. However, since Ca levels in the axes measured by NAA were actually higher, sample-size effect from AAS analysis must have been minimal. The reason for the discrepancy is not known but may be related to the differences in the sample sizes for the two types of tissues. The small size of the axis relative to the cotyledons makes this a problem that is not easy to overcome (Chapter 11). Fortunately the difference in the two methods does not invalidate any of the comparisons made on the axes in Chapter 11 since the higher NAA results only accentuate the differences already found by AAS.

The matrix in which the elements were measured was a problem in the AAS analysis and K was the source of part of the problem (Chapter 4). It has been reported that in NAA the nuclear reactions produced by irradiation were completely independent of the atom's chemical associations (Corliss, 1964). We were also assured by the reactor staff involved in the standardization of the procedure that no matrix effects seemed to be present with the procedure being used. The overall error for the NAA procedure, as reported by Dr. S. Landsberger, was 5-8%. Some of the potential errors arose from the variations in the neutron flux but this could generally be controlled by the appropriate use of standards and flux monitors. Detectors were also subject to errors (Heydorn, 1984). Some of the errors could be caused by improperly selected parameters by the analyst. For example, the selected delay times must be appropriate for the counting capabilities of the analyzer used. The geometry of the sample being counted, the distance from the detector and the counting time could also contribute to the error (Heydorn, 1984). The calibration of the NAA

procedure for use with cucurbit samples included the adjustment of all of the above parameters.

No gamma emitters, which produced interference in the measurement of Mg, K or Ca, were detected, but aluminum and silicon have both been noted as interfering with P analysis (Gatschke and Gawlik, 1980; Wang et al , 1975). Embryo tissues were found to contain little to no Si but very low and variable amounts of Al were found present. While P concentrations ranged from 1 to 2%, the Al amounts were generally well below 100 ppm. Nevertheless for accurate P analysis it was necessary to carry out the two irradiations. The bombardment by the thermal neutrons without the cadmium shield caused the Al to become radioactive via the reaction $^{27}Al(n,\gamma)^{28}Al$. The epithermal neutrons passing through the cadmium shield caused the P to become radioactive via the reaction $^{31}P(n,\alpha)^{28}Al$. The difference in the P/Al levels between the two methods of irradiation when used in the appropriate mathematical formulas gave P concentrations.

The importance of neutron activation analysis to the study of cucurbit embryos was two-fold. The procedure was needed to solve the sample-size effect problem discussed in Chapter 4 and the Ca binding which will be explained in Chapter 5. The NAA also had the potential to contribute information on the variation in Ca level as related to the variation in the other three main storage minerals. The results in Chapters 10 to 12 attest to the usefulness of the procedure.

Chapter 4.

A POSSIBLE ENHANCEMENT OF MEASURED CALCIUM IN SMALL SAMPLES OF DRY-ASHED *CUCURBITA* EMBRYOS AS DETERMINED BY AAS.

INTRODUCTION

One of the qualifications in the application of AAS to Ca analysis of cucurbit embryo tissues, as outlined in Chapter 2, involved the avoidance of sample sizes below 0.05 g. Such small samples persistently produced higher Ca levels than larger samples derived from the same pooled embryo mixtures. The problem in the initial standardization of the procedure was to determine whether the high or low Ca levels represented the actual amount of Ca present in the embryo tissue. Considerable time and effort was also expended trying to discover the origin of the discrepancy and on finding a solution to the problem. The existence of the problem was discovered as a result of an attempt to conserve the small *C. andreana* seeds which were not as easy to obtain in bulk as the large *C. maxima* seeds. Instead of using these rarer seeds, comparably small samples of a ground pooled mixture of embryos from the large seeds were used in the standardization of the analytical procedure for Ca analysis. Had both species, with their different Ca concentrations been used instead of two sample sizes of the same species, the problem may have been masked. While samples of small weight could have been expected to give more variable results, the consistently higher Ca levels in the smaller samples were hard to explain. Attempts to find the source of the discrepancy, called the "sample-size effect", led to the conclusion that the problem

arose during the charring and ashing steps. A possible reason for the apparent enchancement of Ca levels in small samples, based on problems discussed in the literature, is presented.

MATERIALS AND METHODS

Sample Preparation :

Embryos were obtained from the same C. maxima and C. andreana seed stocks referred to in Chapter 2. Wheat Flour (SRM 1567), Tomato Leaves (SBM 1573) and Orchard Leaves (SRM 1571) standard reference materials were obtained from the National Bureau of Standards, Washington, DC. The outer and inner seed coats were removed from the embryos as described in Chapter 2. The embryos were air dried and dry weights were measured on separate samples by heating for two hours at 105°C. Unless otherwise specified all Ca levels reported are based on the air-dry weights. In most cases embryos were ground with a pestle and mortar. A minimum of 25 embryos were pooled for the large seeds of C. maxima but several hundred embryos were pooled for the smaller C. andreana seeds.

Ashing Procedures :

Embryo samples and empty crucibles (as blanks) were charred and dry ashed in a muffle furnace as described in Chapter 2. Ashing temperatures of 500, 550 and 700°C were used and ashing times were varied as indicated in the tables. The wet ashing procedure, adapted from Gorsuch (1970) and Zasoski and Burau (1977), was carried out in 30 ml Kjehldahl flasks and heating apparatus. The 0.2 g embryo samples were first digested with 15 ml of 1:2 V/V nitric acid to water and then with concentrated nitric acid. This last step was repeated with a few ml of

nitric acid at a time until the samples no longer charred after being refluxed and distilled to dryness. The final digestion was with 5 ml concentrated nitric acid and 2 ml of 70% perchloric acid. This mixture was refluxed for about an hour but not permitted to boil dry. The NBS Wheat Flour and Tomato Leaves standards were digested in the same manner. The digests were transferred to volumetric flasks, diluted to required volumes with La/HCl and analyzed by AAS using appropriate blanks and standards.

Carbon content of the ash was determined in a WR12 Carbon Determinator and a LECO Induction Furnace (LECO Corporation, St. Joseph's, Michigan). Plasma ashing (Gleit and Holland, 1962; Gleit, 1963) was carried out at 100°C in a PLASMOD plasma instrument (Legal Corporation, Richmond, California). The plasma-ashing times generally approached 30 hours and were determined by the samples reaching an adequate loss on ignition (LOI) of 95-96%.

Analytical Procedure :

The ash from dry and plasma ashing was treated with dilute nitric and hydrochloric acids as described in Chapter 2. The treated ash was taken up in La/HCl and analyzed by AAS using the standard conditions outlined in Table 2:12 or by the special conditions outlined in specific tables. In the case of Ca analysis by AAS via the standard addition method subsamples of the ash solutions were analyzed with 2, 5 and 8 ppm Ca^{2+} added as $Ca(NO_3)_2$. For EDTA extraction the ash was heated with 0.001 M EDTA and adjusted to pH 11 with 1 M NaOH (7.5 ml EDTA solution plus 4.5 ml NaOH for the 0.25 g samples). The precipitate formed was centrifuged and the supernatant containing the Ca was diluted to the appropriate volume with La/HCl. Standards were prepared with and without

EDTA and NaOH.

Neutron activation analyses for Mg, K, Na, Ca and P were carried out on unashed embryos and on ash from the muffle furnace ashing by the procedures outlined in Chapter 3.

RESULTS

Inadequate compensation for the interference effects, described in Chapter 2, was initially considered as a possible reason for the sample size effect. Of the potential chemical interferents (P, Al, S and Si) in analysis of Ca AAS (Isaac, 1980; Allen, 1974), only P was present in the cucurbit ash in significant amounts. The P produced a 60-80% depression in Ca absorption but this depression appeared to be adequately counteracted by lanthanum at the optimum concentration of 0.5% (5000 ppm) at both sample sizes (Allen, 1974; Yofe and Finkelstein, 1958).

Robinson (1975) and Isaac (1980) define the matrix effect somewhat differently but in both cases the presence of the effect is detected by and removed by dilution of the sample being measured. Cucurbit samples had a matrix effect which showed up as an increase in measured Ca level at all dilutions up to 100-fold dilution of the original embryo sample. Harnly et al (1982) in their multi-element analysis found a need to dilute some of the NBS Standards 100-fold to obtain the expected Ca levels.

Using the outlined assay conditions (Table 2:12) NBS Wheat Flour standard and both cucurbit species produced adequately precise and repeatable results on samples of 0.25 g and larger. When very small samples were ashed and analyzed the measured Ca level was persistently higher. Table 4:1 shows the measured calcium level in 0.05 and 0.25 g

TABLE 4:1

Sample-size Effect Produced in Different Samples Ashed at 550°C for 16 Hours and Measured by AAS.

Type of Sample	µg Calcium/g Sample*		Sample-Size Effect
	0.05g Samples	0.25g Samples	
C. maxima Embryos	258.1 ± 30.9**	202.7 ± 7.1	27.3
C. andreana Embryos	515.4 ± 27.1	474.7 ± 9.2	8.5
NBS Wheat Flour	208.0 ± 4.6	186.9 ± 4.0	11.3

* Air-dry weights: cucurbit embryos 4.3% and NBS Standard 6.1% moisture
**N = 6, Mean ± SD

TABLE 4:2

Degree of Sample-size Effect Between Ca Levels of Small (0.05g) and Large (0.25g) Samples of Pooled C. maxima Embryos Ashed at 550°C for 16 Hours and Measured by AAS.

Instrument	Flame	Sample-Size Effect
Perkin Elmer AAS, Model 603	Air/C_2H_2	15.8%
	N_2O/C_2H_2	21.8%
Perkin Elmer AAS, Model 703	Air/C_2H_2	15.9%
Varian AA-1275, with BC*	Air/C_2H_2	10.8%
Varian AA-1275, without BC	Air/C_2H_2	11.2%

*BC - background correction
N = 6 for each size. One set of samples were used for all Perkin Elmer measurements. New samples were ashed for analysis with the Varian spectrophotometer.

samples. The sample-size effect was most prominent in Cucurbita maxima but varied from 5 to 30% with 10 to 15% representing the most commonly recorded difference. Cucurbita andreana occasionally produced a large sample-size effect but NBS Wheat Flour samples never exceeded 12%. All large samples gave essentially the same Ca levels suggesting that the high concentrations of the small samples were anomalous.

A systematic error responsible for the anomaly was looked for in the glassware, balance and spectrophotometer. Table 4:2 shows that sample-size effect persisted on several instruments, with or without background correction and in both nitrous-oxide/acetylene and air/acetylene flames.

Because of the matrix effect, dilution was initially a problem. Table 4:3 shows the Ca levels in C. maxima embryos ashed at three sample sizes and measured at several dilutions of the ash in La/HCl. The 0.25 g sample measured at 1:40 dilution and the 1.0 g sample measured at 1:50 dilution produced lower Ca levels than the same sample sizes measured at higher dilutions of the ash. Beyond 1:100 dilution no additional increase in measured Ca level occurred indicating that the matrix effect had been overcome. When all sample sizes were measured at adequate dilution the 0.05 g samples still showed a higher measured Ca level than the larger samples indicating that the sample-size effect was still present.

Spiking the solutions with calcium carbonate standards showed a good recovery of the added Ca and a shift in degree but not an elimination of the sample-size effect. Measuring Ca by the standard addition method gave variable results but no consistent removal of the sample-size effect. Hosking et al (1979) and Smets (1980) do not recommend the use of the

TABLE 4:3

Effect of Measuring Ca in the Same Ash Samples at Various Dilutions on the Sample-size Effect in Pooled *C. maxima* Embryos Ashed Overnight at 500°C.

Wt. Embryo Sample (g)	Initial Volume (ml)	Type of Dilution	Actual Dilution wt/vol*	Measured Ca Level µg Ca^{2+}/g Embryo (Mean ± SD N = 3)
0.5	5	--	1:100	237.6 ± 20.2
	5	1:1	1:200	240.6 ± 18.8
0.25	10	--	1:40	186.3 ± 9.6
	10	1:1	1:80	208.3 ± 8.5
	10	1:5	1:200	210.5 ± 4.3
1.00	50	--	1:50	191.1 ± 4.3
	50	1:1	1:100	205.9 ± 4.9
	50	1:4	1:200	209.5 ± 2.6

*All dilutions in La/HCl

TABLE 4:4

Calcium Levels in Embryo Samples of Different Sizes That Were Ashed at 550°C Overnight and Analyzed With and Without Subsequent Digestion in Perchloric Acid.

Wt. Embryo Sample (g)	µg Ca^{2+}/g *C. maxima* Embryos No $HClO_4$	With $HClO_4$
0.05	258.1 ± 30.9	150.8 ± 5.3
0.25	196.9 ± 10.1	196.9 ± 2.5
1.00	208.4 ± 4.1	203.7 ± 3.1

*N = 3, Mean ± SD
Perchloric acid digestion: 2.5 ml HNO_3 + 1 ml $HClO_4$ were added to embryo samples and heated until 1 ml remained. Standards were processed in a similar manner. Measurements were carried out by the standard procedure in La/HCl.

standard addition in systems with prominent interference effects since such interferences have been found to vary non-linearly over the analytical range.

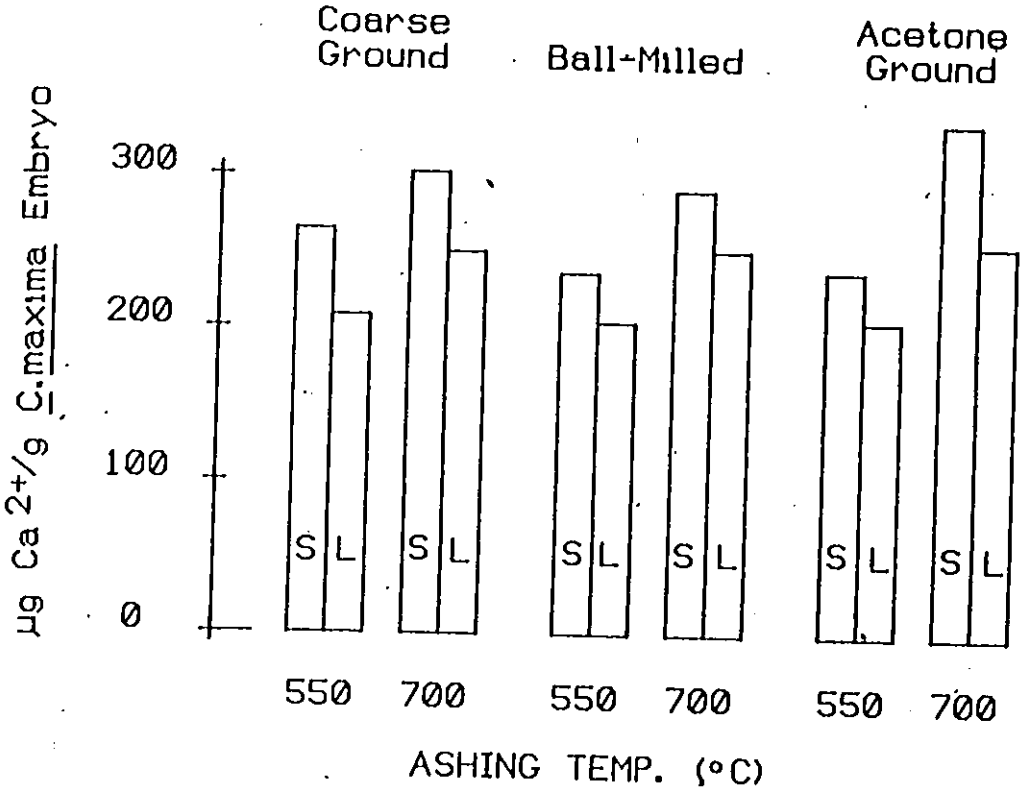
Assuming that the higher Ca level in the smallest sample is due to the genuinely higher Ca level in these samples one can explain the origin of this Ca in one of two ways. The additional Ca may have been acquired as a contaminant or may have been released from the insoluble portion of the ash more completely than from the residue of the large samples.

Contamination of the sample has been reported for most grinding methods by Hood et al (1944). The pestle and mortar grinding caused least contamination and the problem for Ca in all methods was less severe than for the trace metals. In grinding of the cucurbits a large batch of embryos (25 to 300) were ground so that when small and large subsamples were removed from the pooled mixture, contaminants should have been evenly distributed. Contamination can also arise from crucible walls during ashing but blank crucibles and ashed BDH $Ca(NO_3)_2$ standard solution did not indicate any pick-up of Ca from this source. Glassware can contribute Ca to solutions but using plastic vials gave no difference in Ca level and still produced a 13.1% sample-size effect. Aluminum can be picked up from muffle furnace walls (Isaac and Jones, Jr., 1972) but this has not been reported by others for Ca and was not observed in this work.

Minerals are not homogeneously distributed throughout plant tissues and in the case of cucurbit embryos there is considerably more Ca in the small axis than in the large cotyledons. Nelson and Boodley (1965) and Smith et al (1968) found that ground plant materials with non-uniform particle size could produce higher or lower Ca levels at different sample

Figure 4 : 1

Degree of Sample-size Effect Between Measured Ca in Small (0.05 g) and Large (0.25 g) Samples of C. maxima Embryos Ground by Several Methods and Ashed at 500 and 700°C.



sizes depending on the distribution of particles with high or low Ca content. To determine whether finer particle size could eliminate the sample-size effect, cucurbit samples were ground in several ways and also pressed through various-sized screens. Analysis of three such preparations is shown in Figure 4:1. The coarse ground samples had particle size of 1 mm or less but the preparation was fairly loose and flaky. Ball-milling under liquid nitrogen produced a sticky paste because of the high oil content but the particle size was more uniform and finer. Grinding in acetone extracted the oil and permitted the rest of the embryo to be ground to a fine powder in a pestle and mortar. Acetone was then evaporated leaving the oil on the sample. After 550°C ashing the Ca levels were essentially the same in all 3 preparations (Figure 1) and the sample-size effect was present in all. Thus it appears that particle size was not the cause of the discrepancy.

To test whether the higher Ca levels were due to a more complete release of Ca from the smaller samples a more rigorous ashing was tried. More thorough ashing occurs at higher temperatures in more finely divided samples (Gorsuch, 1970). Each of the three preparations in Figure 4:1 was also ashed at 700°C. While the ash at 550°C is black, ash produced at 700°C is more soluble, light gray and carbon-free. The results showed the persistence of the sample-size effect plus the additional problem of still higher Ca levels as a result of the higher ashing temperature.

Since the lighter more soluble ash gave a higher Ca level it suggested that the large samples may not have been recording the total available Ca. Occlusion of minerals in carbonaceous ash has been

TABLE 4:5

Effect of EDTA Extraction on the Measured Ca Level of Small and Large C. maxima Embryo Samples Ashed at 550 and 700°C.

Wt. Embryo Sample (g)	Ashing Temp (°C)	µg Ca^{2+}/g Embryo	
		No EDTA Extr.	With EDTA Extr.
0.05	550	231.6 ± 3.7*	192.8 ± 13.6
0.50	550	212.3 ± 6.8	197.9 ± 12.6
0.05	700	299.1 ± 18.3	251.9 ± 28.4
0.50	700	232.3 ± 6.2	226.0 ± 14.7

*N = 3, Mean ± SD

TABLE 4:6

Degree of Sample-size Effect for Various Sample Weights.

	Sample Wt. (g)	µg Ca^{2+}/g Sample		
		C. maxima Embryos	C. andreana Embryos	Wheat Flour NSB Std.
Samples With Enhancement	0.025	257.8	784.2	206.7
Samples Without Enhancement	0.05	236.0	714.2	190.6
	0.10	240.5	728.3	185.4
	0.25	213.2	710.5	189.2
	0.50	238.8	711.5	202.7
	1.00	230.9	691.7	193.4
Average of All Samples Without Enhancement		231.9	709.4	193.4
% Enhancement		11.2	10.5	7.5

recognized as a problem by a number of authors (Middleton Stuckey, 1953; Wichmann, 1942b). However, this view was contradicted by the failure of other methods to produce the higher Ca levels. Wet- and plasma-ashed samples analyzed by AAS and results from NAA of unashed embryo samples produced levels in good agreement with the large sample Ca values.

Another possibility was that the higher Ca level in the small sample sizes was due to an enhancement rather than due to an actual increase in Ca. Atomic absorption analysis has been shown to be subject to both negative and positive interferences (Allen, 1974; Gorsuch, 1970; Robinson, 1975). Work with perchloric acid and EDTA seemed to support the view that an enhancement of the Ca level was being seen.

In an effort to increase the measured Ca in the large samples, ash from the furnace was subjected to a digestion in perchloric acid. No visible improvement in ash colour and solubility resulted from this treatment. Table 4:4 shows that perchloric acid digestion of the ash had no effect on the measured Ca level of the 0.25 g and 1.0 g embryo samples. The 0.05 g samples which without perchloric acid had a higher measured Ca level, produced a much lower Ca level when perchloric acid digestion was used. Not only was the sample-size effect removed but an additional depression in Ca level was produced suggesting that the original high Ca level was due to an enhancement effect. A number of authors (Allen, 1975; Baker and Johnson, 1954; Dinnin, 1960) advise the use of one or two percent perchloric acid in the samples to ensure a full releasing effect by lanthanum but this does not seem to be the effect seen with cucurbits.

Another possibility considered was that some residual depressive effect by P might be present in the larger samples. To counteract that,

TABLE 4:7

Measured Ca Level in Samples of *C. maxima* Embryos Ashed at 500°C and Analyzed With and Without the Addition of Potassium Chloride Solution.

Wt. Embryo Samples (g)	µg Ca^{2+}/g Embryo	
	No added K^+	10,000 ppm K^+
0.03	209.2	185.4
0.05	179.6	177.8
0.25	184.3	178.8

N = 2
K^+ added as 0.2 ml of 10% KCl to ash solution in La/HCl

TABLE 4:8

Addition of Potassium to *C. maxima* Embryo Samples Before and After Ashing for 4 Hours at 550°C.

Addition of K^+	Time of Addition of K^+	µg Ca^{2+}/g Embryo Untreated Ash	Treated Ash
no K^+	--	182.8	174.2
as KCl	After Ashing*	256.5	255.6
as KCl	Before Ashing**	216.5	186.0
as KOH	After Ashing	213.8	182.0
as KOH	Before Ashing	Erratic	Erratic

* ash +KCl soln. taken to dryness and then La/HCl added
** embryos +KCl ashed together then La/HCl added
Wt. of embryo samples was 0.25 g, N = 2
10,000 ppm K^+ added as 2 ml of 1% KCl in H_2O or as 2 ml of 1% KOH in H_2O

the Ca was extracted from the ash by EDTA leaving the insoluble P component in the residue. In Table 4:5 the Ca levels produced without EDTA extraction have a sample-size effect after ashing at both 550°C and 700°C. In addition the measured Ca levels are higher after ashing at 700°C. The effect of EDTA extraction was to eliminate the sample-size effect produced after 550°C ashing by lowering the Ca level of the 0.05 g samples. The discrepancy in the measured Ca levels from 550°C and 700°C ashing remained but the degree of the sample-size effect was reduced for the 700°C ashing. In repeated trials either the temperature or the sample-size effect were removed but rarely both. The trend was always toward lowering the high Ca levels.

The higher Ca level after ashing at higher temperatures persisted throughout many experiments and then spontaneously disappeared when charring was carried out on a different hot plate. The purchase of a larger hot plate capable of producing a temperature of 465°C at its centre permitted all embryo samples to be charred more slowly and more thoroughly. From this point on, lower but similar Ca levels were obtained after ashing at all temperatures in the 400 to 700°C range. Simultaneously there was a change in the expression of the sample-size effect as seen in Table 4:6 where the 0.05 g samples no longer showed the sample-size effect seen in Table 4:1. Sample-size effect had shifted to still smaller samples but has persisted in this form.

Assuming the higher Ca level in the small samples arose from an enhancement of the Ca signal in the flame, possible sources of the enhancement would be K and Na. Several authors including David (1959) have found enhancements in Ca measuring systems related to these elements. NBS

TABLE 4:9

Evidence for the Interaction Between Porcelain Crucibles and Potassium Hydroxide Ashed at 550°C as Detected By High Absorbance Readings in AAS.

No. of Crucibles	Crucible Glaze Condition	Vol. KOH* Added (ml)	Hours of Ashing	Absorbance ± SD
2	Undamaged	0	0	0.004 ± 0.000
2	Undamaged	0	4	0.011 ± 0.001
2	Undamaged	2	0	0.020 ± 0.006
6	Undamaged	2	4	0.295 ± 0.044
6	Damaged	2	4	0.236 ± 0.038

* 2 ml of 1% KOH (10,000 ppm K^+) added to crucible, taken to dryness on a hot plate, ashed as indicated and analyzed in 2.5 ml La/HCl. A 2 ppm Ca^{2+} standard gave an absorbance of 0.105. The results are for untreated samples but acid treated KOH samples produced the same results.

Wheat Flour standard (Gladney et al ,1984) has 1360 ppm K and only 8 ppm Na and this is characteristic of most plant tissues. Thus, if enhancement was occuring in the ash samples, K was more likely to be involved than Na.

Addition of K to cucurbit samples produced rather variable results. Two types of results are shown in Tables 4:7 and 4:8. In Table 4:7 addition of K to ash solutions in La/HCl eliminated the sample-size effect in the 0.03 g samples without affecting the Ca level of the larger sample sizes. In Table 4:8 the large sample sizes showed enhanced Ca levels when K was added either to the embryo tissues before ashing or to the solid ash. In various experiments the measured Ca levels varied with the type of K salt used, with the presence or absence of ash treatment and with the ashing temperature. The Ca levels in samples without K (Table 4:8) were in good agreement with the 175.0 $\pm$ 7.0 ug Ca^{2+}/g embryo obtained by NAA on the same batch of pooled embryos. In Table 4:8 ashing of embryos with KOH produced very high and erratic results. This appeared to be due to KOH/crucible interaction since KOH alone ashed in blank crucibles produced a strong Ca signal where no Ca should have been present (Table 4:9). Ashed KCl produced the expected low blanks.

To determine whether or not there were any actual differences between the ashes which showed the enhanced Ca levels and ashes which gave the lower measured Ca levels, the ashes were analyzed by NAA (Table 4:10). In Part A the K was essentially the same for the two kinds of ashes. The small sample ash had somewhat more Mg and Na. There was 8.9% more Ca in the small sample ash but carbon analysis on separate samples showed that there was only 7.5% carbon in this ash as compared to the 11% in the large sample ash. Taking the carbon content difference into account reduced the

TABLE 4:10

Analysis of *C. maxima* Ash from Muffle Furnace Ashing by NAA.

	Type of Ash	%K	%Na	%Mg	%Ca	%P
A	0.01 g Sample Ash From 550°C	16.4 ± 1.0*	0.048 ± 0.002	9.6 ± 0.6	0.45 ± 0.03	21.3 ± 4.3
	0.5 g Sample Ash From 550°C	16.4 ± 2.1	0.038 ± 0.001	9.0 ± 0.5	0.41 ± 0.02	25.2 ± 0.3
B	0.25 g Sample Ash From 500°C With 11% Carbon**	15.7 ± 1.5	0.043 ± 0.004	12.2 ± 0.8	0.65 ± 0.05	30.0 ± 1.2
	0.25 g Sample Ash From 700°C With 0% Carbon	14.5 ± 0.9	0.446 ± 0.015	12.8 ± 0.8	0.64 ± 0.05	32.1 ± 1.1

* N = 3, Mean ± SD

** the values have been corrected to correspond to 0% carbon

difference to only 5%, which could not account for all of the 10-15% difference in the Ca level. The P concentration was lower in the small sample ash but there was a high sample-to-sample variation in these values. The combination of these differences in the minerals of the ashes shows that Ca could be measured in a different mineral matrix for the two ashes. In Part B the ashes compared in Figure 1 were analyzed. While the 700°C ash showed a higher Ca level by atomic absorption analysis there appeared to be no corresponding difference in Ca level in the ash. Some K had been lost with the higher temperature ashing but there was an unexpected gain in Na. Since the overall Na level was so low compared to the K level it was uncertain whether the 10-fold increase could have accounted for the enhanced Ca levels measured in this ash. The increase in Na did indicate an interaction of the sample with the crucible wall. Blank crucibles never indicated any leaching of Ca from the crucible walls.

DISCUSSION

Examination of various factors which could have resulted in genuine increases in Ca levels in the small embryo samples produced negative results leading to the conclusion that an enhancement of Ca level was being observed. Elimination of the high Ca levels in small samples by the addition of perchloric acid, EDTA and in some instances K also suggested that the Ca levels were enhanced. The agreement between Ca measured by NAA and the large sample Ca levels gave further support to the suggestion that the lower Ca levels were the correct ones.

A literature survey supported the idea that enhancement of Ca level could occur. While depressive effects of one mineral on the

absorption of another occupy the bulk of the reports there are persistent observations of errors arising from enhancements. Unfortunately the term enhancement is used differently by different authors. For example, K and Na are said to enhance the Ca level in nitrous-oxide/acetylene flame (Amos and Willis, 1966; Manning, and Capacho-Delgado, 1966). Since the alkali metals in this case act as ionization suppressants the measured Ca level is increased to the true value not really enhanced beyond this level. Many reports deal with simple solutions and report enhancements in absorbance without relating this to the actual Ca concentration of the sample. Instrumental conditions, flame types and parameters are so varied that it is difficult to compare one report to another let alone to work done on contemporary instruments. The following summarizes reports which specifically implicate K and Na in enhancements.

Margoshes and Vallee (1956) reported that Na and K could increase or decrease Ca emission in oxygen/hydrogen flame. David (1959) using air/acetylene flame found that low concentrations of K and Na enhance Ca absorbance while higher amounts overcome the enhancement. West (1964) found that aliphatic acids and their Na and K salts caused an enhancement of Ca flame emission and suggested that this might be due to the dispersion of Ca among more volatile Na and K salts. Dickson and Johnson (1966) reported that cations such as Na or K could enhance, depress or have no effect on Ca absorbance depending on the particular system and concentration. Ramakrishna et al (1968) found in their simple chemical systems that alkali metals La, Na and K enhanced Ca absorbance. Magill and Svehla (1974) using a calcium chloride solution in 1% HCl also found a small, 3 to 5%, enhancement of Ca in an air/acetylene flame.

While K could explain the enhancements, P is also present in significant amounts in embryo tissues (Cosgrove, 1966) and may be implicated. Dippel et al (1954) and West et al (1973 a,b) showed that under certain conditions phosphate could depress or enhance Ca absorbance. Embryo P is mainly in the form of phytic acid salts and when Ca nitrate and Na phytate were ashed together both depression and enhancement was produced depending on the ashing temperature (Chapter 5). Whether this can be related to the complex embryo tissues is not known.

Mechanisms that can explain how enhancement occurs all deal with droplet size. It is now generally accepted (Long and Boss, 1982 a,b; Smets, 1980; Smith and Browner, 1984) that most interferences such as that of P on Ca are condensed phase interferences. The reaction between the analyte and the interfering element takes place as the aerosol droplet vaporizes in the flame. The smaller the droplet size the less the extent of the interference. Alkemade (1966) explained how both depression of the absorption signal and enhancement could occur. As the solvent evaporates the analyte remains in a solid matrix where it has either reacted with an interferent or has been occluded in other elements which had comprised the original solution. The relative volatility of the matrix or of the compound formed by the analyte determines the rate at which ground state Ca atoms can be produced. This rate can be higher or lower than that of the Ca in the standards.

Rocchiccioli and Townshend (1968) found that droplets of different size produced free Ca atoms at a different rate and that extent of P interference varied with droplet size. A larger proportion of small droplets would give a higher Ca absorbance. West et al (1973 a,b) report

that P can enhance Ca absorbance in nitrous-oxide flame via a lateral diffusion interference effect where the analyte becomes concentrated at the centre of the flame. Long and Boss (1982 a,b) found that small uniform droplet size eliminated positive interferences by elements capable of aerosol ionic redistribution (AIR). Lanthanum and perhaps Na or K can cause the analyte to be shifted to smaller droplets in a system with non-uniform droplet production which then travel faster through the flame giving a more intense Ca signal. Fassel and Becker (1969) and Tyson (1984) have stressed that the expression of interference effects is strongly dependent on the instrument and conditions under which it is used and this may apply to the expression of the sample-size effect.

The results with addition of K to cucurbit embryos at various stages in the analysis resulted both in the elimination of enhancement and in the generation of an enhancement in the measured Ca level. While the examples of enhancements referred to from the literature were all manifested by reactions taking place in the atomic absorption flame, the results with cucurbit embryos showed that reactions taking place during charring and ashing could affect the measured Ca not by actual changes in Ca level but rather by changes in other mineral levels. Presumably changes in the aerosol droplet size and in the stability of such droplets could be affected leading to a different expression of Ca relative to that of the larger samples. The sample-size effect was strongest in C. maxima which had a very low Ca concentration compared to that of K and P.

Increases in ash Na and abnormally high blanks with ashed KOH testify to the interactions between samples and crucibles. Attacks on silica crucible walls by phosphoric acid and alkali hydroxides and

carbonates have been reported by Gorsuch (1970). Lambert (1976) stated that silica crucibles were attacked by alkali compounds in the 400-700°C ashing range. Meyer and Meyer (1976) found more problems with porcelain than silica crucibles. Liver ashed in porcelain crucibles gave higher and more variable Ca levels than samples ashed in silica or platinum crucibles. As in this study with cucurbits, blank crucibles showed no Ca being picked up from the walls. Thus, the higher measured Ca levels must have resulted from the interaction of the liver with the crucible walls during ashing. Based on NBS Bovine Liver standard (Gladney et al ,1984) liver has a high concentration of P, K and Na and a low level of Ca. Except for the Na its mineral levels are similar to those of cucurbit embryos.

The small cucurbit samples have a large surface area in contact with the crucible walls and during charring or ashing such samples are better aerated than larger samples. Since the degree to which the enhancement was expressed proved to be influenced by a change in the charring procedure, it was possible that K in the embryos might more readily form KOH under some conditions. Differences in the amount of KOH formed could lead to differences in the extent of sample-crucible interaction. Fortunately this interaction became negligible with larger sample sizes which all gave the same measured Ca level.

Thus, while the sensitivity of the technique permitted the measurement of Ca in very small embryo samples, the results were erroneous as a result of the charring and ashing steps. The ashing procedure most acceptable for larger sample sizes could not be used with the small samples due to the interaction of elements other than the Ca being

measured with the porcelain crucible walls. The problems encountered with the ashing of cucurbit tissues emphasize the need to define the reactions which occur during ashing of complex tissues and to consider these reactions as potential sources of interferences in AAS.

Chapter 5.

EASE OF EXTRACTION OF CALCIUM FROM ASH OF *CUCURBITA MAXIMA* AND *CUCURBITA ANDREANA* EMBRYOS.

INTRODUCTION

Atomic absorption procedures for mineral analysis require the destruction of the organic matrix of the sample. The ease with which the matrix can be destroyed depends on the composition of the sample. The handbook of analytical procedures accepted by the Association of Official Analytical Chemists recognizes the need for various ashing methods for different food and agricultural products (Horwitz, 1975). Materials containing oils and proteins are generally highly resistant to any form of oxidation or ashing (Gorsuch, 1970). Most plant tissues are less troublesome to ash than animal tissues but this does not apply to seed tissues (Middleton and Stuckey, 1953). The high oil and protein content of cucurbit embryos makes their tissues quite resistant to ashing.

Gorsuch (1970), Lambert (1976) and Sansoni and Panday (1983) have compared various ashing procedures. Ashing is divided into wet ashing, in which chemical oxidation is used, and dry ashing using heat. For ashing of plant tissues the latter is traditionally done in a muffle furnace at temperatures ranging from 400 to 600°C. A more recently developed dry ashing technique (plasma ashing) is done at about 100°C in a stream of activated oxygen (Gleit, 1963; Gleit and Holland, 1962).

Most literature on dry ashing suggests that the ash should be

white or at least light gray, but cucurbit ash was black after both 500 and 550°C ashing. Ash treatments varying from moistening with water and re-ashing (Wichmann, 1940; Iverson, 1972) to heating with concentrated nitric acid (Gorsuch, 1959) are recommended to obtain a light-coloured ash. Another suggestion is ashing at higher temperature, particularly for a mineral like Ca which is not volatilized during 700°C ashing (Isaac and Jones, Jr., 1972). After 700°C ashing, cucurbit samples produced a light gray ash but Ca was less easily extracted from this ash than from the lower temperature black ash. NBS Wheat Flour and Tomato Leaves standards showed no change in ash characteristics at higher ashing temperatures.

MATERIALS AND METHODS

The embryos were derived from the same seed stocks as used in the previous chapters and procedures for seed coat removal, drying, grinding and charring have been described in Chapter 2. Methods for dry ashing, plasma ashing, wet ashing and carbon analysis were outlined in Chapter 4. The defatting procedure was described in Chapter 2. The ash from the muffle furnace ashing was either left untreated or was treated with dilute or concentrated acids (Gorsuch, 1959, 1970). The volumes of all the acids used for ash treatment were 1 and 1.5 ml for the 0.15 and 0.25 g sample sizes respectively. Procedure for AAS was summarized in Table 2:12.

RESULTS

Table 5:1 shows the Ca levels obtained by AAS of wet- and dry-ashed Cucurbita maxima embryos. Although the three methods gave

TABLE 5:1

Analysis of Ca in *C. maxima* Embryos by AAS Following Different Methods of Ashing.

Sample Weight* (g)	Number Ashed	Ashing Method	% Ash	µg Ca^{2+}/g Embryo
0.3	3	Wet Ashing $HNO_3/HClO_4$	—	181.6 ± 9.7**
1.0	9	Plasma Ashing 30 hrs. at 100°C	5.7 ± 0.4	224.4 ± 20.7
0.25	30	Muffle Furnace 4 hrs. at 550°C	4.3 ± 0.6	220.3 ± 13.8

* All samples from the same batch of 100 pooled embryos.
** Mean ± SD

TABLE 5:2

A Comparison of Ash Treatments on Calcium Nitrate and *C. maxima* Embryos Ashed Separately at 650°C for 4 Hours.

Ash Treatment	µg Ca^{2+}*/g Embryo	µg Ca^{2+} Recovered from Added 30 µg Ca^{2+}**
No Treatment	117.3 ± 5.8***	22.2 ± 0.4
Dil. HNO_3, 50% HCl	183.8 ± 4.5	27.8 ± 0.5
50% HCl, Dil. HNO_3	193.1 ± 1.8	29.7 ± 0.0
Dil. HNO_3	185.3 ± 1.2	28.6 ± 0.8
50% HCl	196.6 ± 2.5	28.1 ± 0.9
Conc. HNO_3	139.3 ± 3.2	28.4 ± 0.3
Conc. HNO_3, Ashing	90.8 ± 1.3	32.6 ± 2.5

* N = 2, 0.25 g samples, expected Ca^{2+} = 200 ± 10 µg/g embryo
** 30 µg Ca^{2+} added as $Ca(NO_3)_2$ in H_2O, N=2
*** Mean ± SD

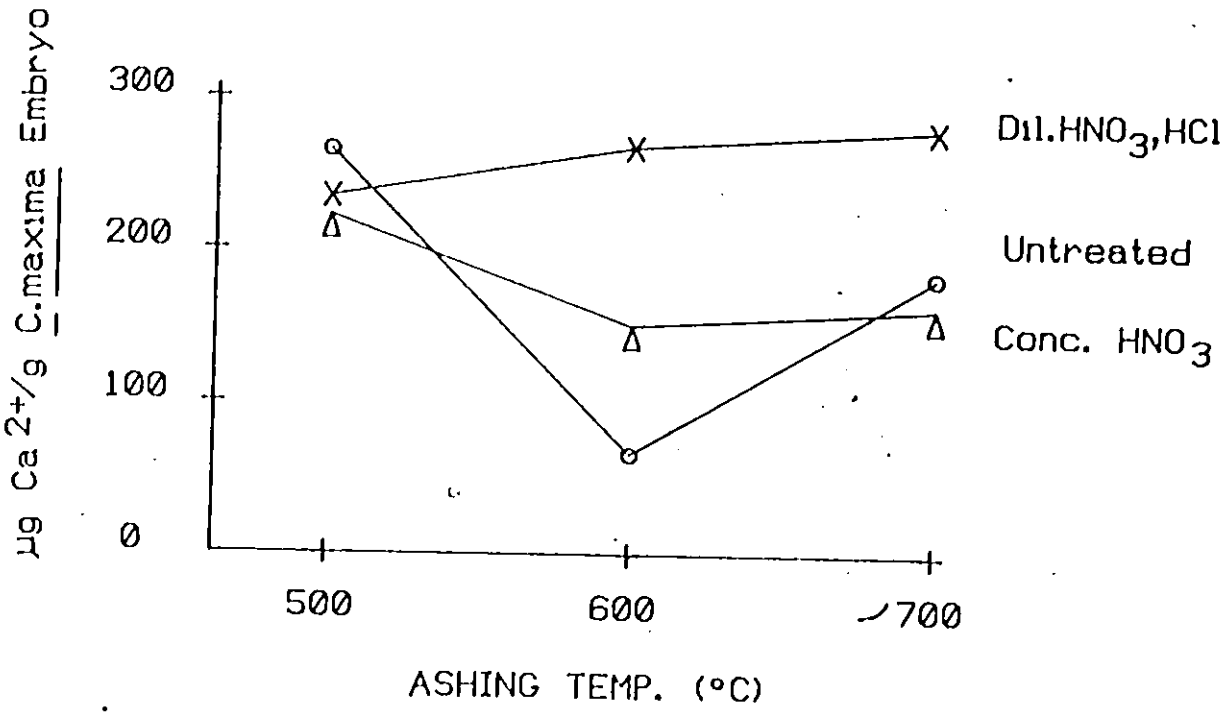
results in the expected range, the Ca levels from wet ashing were low compared to the dry-ashed samples. In addition wet ashing was very time-consuming since the high oil content necessitated a prolonged digestion with nitric acid prior to addition of perchloric acid. While the light-coloured, fairly soluble ash produced by plasma ashing gave comparable results to the muffle furnace ashed samples this procedure was also too slow. Cucurbit embryo samples required about 30 hours of plasma ashing to oxidize a sufficient amount of the organic matter. These limitations led to the selection of muffle furnace ashing as a rapid and convenient procedure for ashing a large number of embryo samples. This method, however, was not free of problems.

The results in Table 5:1 for the furnace ashed samples were obtained after the black ash from 550°C (containing 11% carbon) was treated with dilute nitric and hydrochloric acids (Gorsuch, 1970). When ashed at 700°C, cucurbit embryos produced a light gray and carbon-free ash which was more soluble in dilute hydrochloric acid. The question was whether this gray ash still needed the double acid treatment to ensure complete extraction of Ca. To test this, cucurbit embryos were ashed at 3 temperatures and analyzed with and without ash treatments of concentrated nitric acid or dilute nitric and hydrochloric acids.

Figure 5:1 shows that the untreated gray, carbon-free ash from 700°C ashing gave a lower measured Ca level than the untreated ash from 500°C. The lowest measured Ca levels were obtained from the untreated ash produced at 600°C. The Ca levels for all 3 temperatures were more comparable after the double acid treatment but concentrated nitric acid treatment only partly counteracted the reduction in Ca level

Figure 5 : 1

Variation In Measured Ca Concentration in C. maxima Embryo Ash Produced at Several Temperatures and Analyzed in Treated and Untreated States.



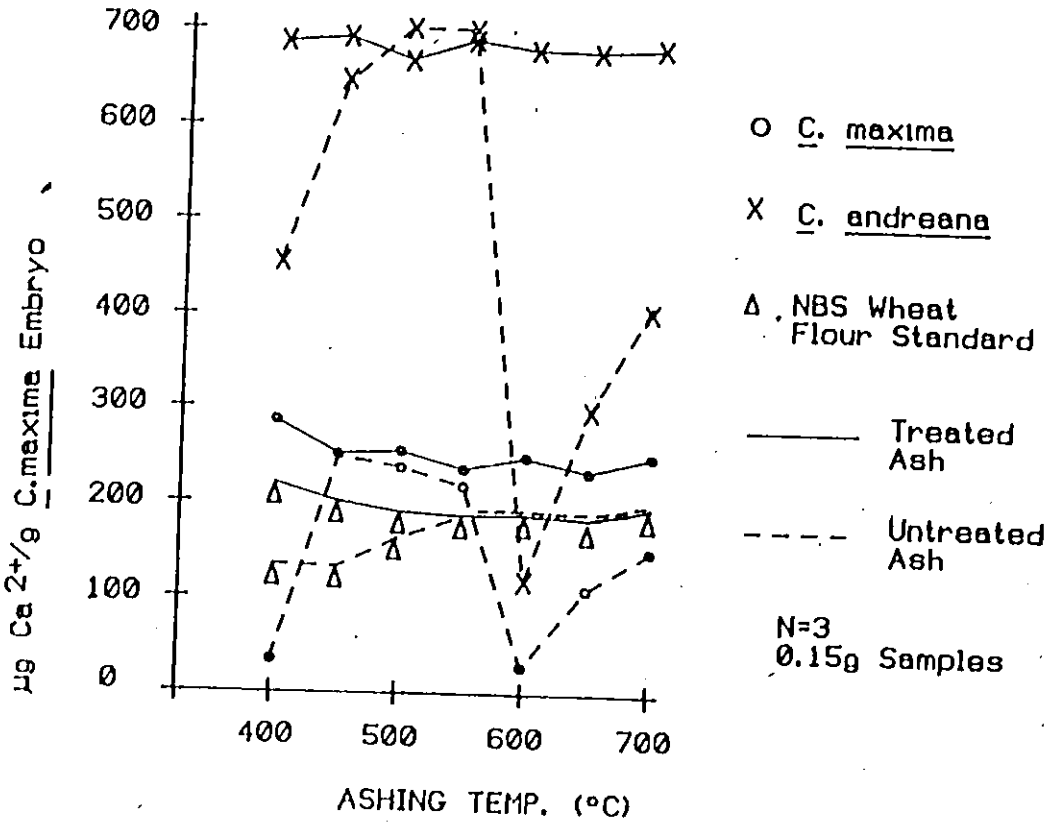
following ashing at either 600 or 700°C.

Table 5:2 gives more evidence for the ineffectiveness of concentrated nitric acid as ash treatment of cucurbit samples. If the samples were given a short period of ashing, following the addition of the acid, the binding was increased even more. Dilute acids were effective both singly or in combination. To see if ashing at 650°C also affected the solubility of a simple Ca salt, the $Ca(NO_3)_2$ standard was ashed and then analyzed without treatment and with the same treatments as applied to the cucurbit samples. The results in Table 5:2 show that only 22.2 µg Ca were detected in the untreated state out of the 30 µg added and a visible residue attested to the fact that ashing had converted Ca into less soluble salts. The insolubility of the Ca in the inorganic salt was counteracted equally well by concentrated nitric and the dilute acids.

To determine at what temperature or how abruptly the change in the ash occurred, samples were ashed at 7 temperatures. This time both cucurbit species and NBS Wheat Flour standard were analyzed but only the double acid treatment was used. The results in Figure 5:2 show that while the two cucurbit species had different Ca levels, both showed a similar response to ash treatment and temperature. When treated, all samples produced virtually the same Ca level at all 7 temperatures. In the untreated state the Ca levels were low after 400°C ashing because the ash was hard, brittle and hydrophobic making extraction more difficult. Untreated samples from temperatures of 450, 500 and 550°C gave similar Ca levels to those of treated samples. There was a marked drop in measured Ca level at 600°C. The slight increase in Ca at 700°C still registered only about one-half of the total Ca present in the samples.

Figure 5 : 2

Calcium Extracted from Treated and Untreated Ash Produced at Various Temperatures.



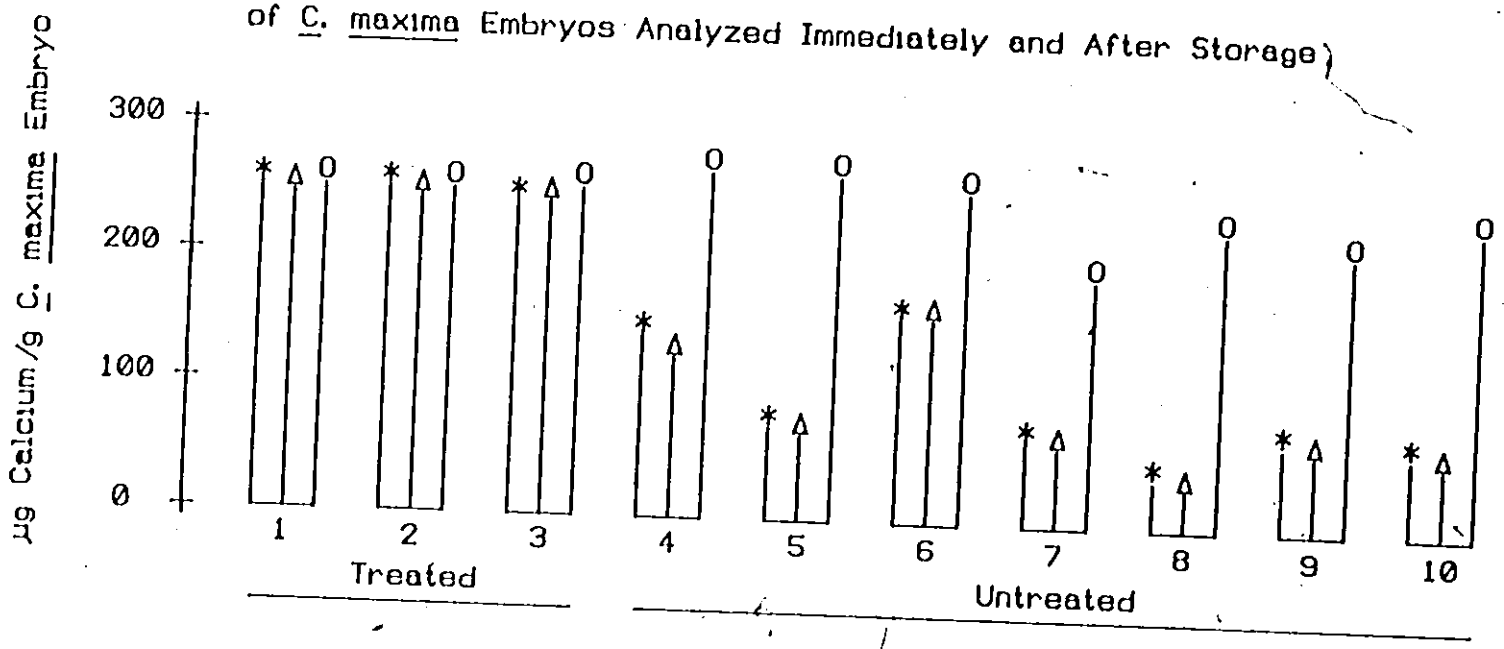
Wheat Flour standard showed a completely different response to the ashing temperatures tested. Both 400 and 450°C and perhaps even 500°C gave lower Ca levels without acid treatment but no treatment seemed necessary at the higher temperatures.

One of the main differences between wheat flour and cucurbit seed tissues is that wheat flour is high in starch and low in oil whereas cucurbit tissues are high in oil and low in starch. To determine whether or not oil produced the observed differences in Ca binding, embryo samples and wheat flour were defatted in hexane and the experiment was repeated. While measured Ca levels were higher in both cucurbit tissues, since the oil contained little to no Ca, defatted embryo tissue still bound the Ca but defatted wheat flour again showed no effect.

While the acid treatment immediately counteracted the Ca binding, storage of samples produced the same effect over several days. In Figure 5:3 the lines with the stars represent the initial Ca measurements. Triangles are for measurements made two days later. The insoluble ash residue had been removed from these samples by centrifugation. The Ca levels measured two days apart are essentially the same with untreated samples still giving very low Ca levels. The circles represent samples which had been stored for 2 days without centrifuging so that the ash solution and insoluble residue remained together. When centrifuged and analyzed these samples showed much higher Ca levels than in the untreated samples indicating that a slow release of Ca from the insoluble portion of the ash had taken place. Whether the Ca is itself present as a less soluble salt or is trapped in a matrix of less soluble salts is not known but the term bound will be used for convenience.

Figure 5 : 3

Measured Ca Levels in Treated and Untreated Ash Extracts of C. maxima Embryos Analyzed Immediately and After Storage



Sample size = 1 g, ashed at 650° C for 4 hours
Ash extracted into 100 ml La/HCl
A 2 ml aliquot was centrifuged and measured by AAS (--- *)
The sample was stored for 2 days and re-measured (--- Δ).
After 2 days standing in contact with the insoluble residue a second 2 ml aliquot was centrifuged and analyzed (--- 0).

Table 5:3 shows that the change in the ease of extraction of Ca is independent of the length of time the sample is ashed. Table 5:4 shows that sample sizes between 0.075 g and 1.0 g have about 80% lower measured Ca level in the untreated state after 600°C ashing and about a 50% lower Ca after 700°C ashing as compared to treated samples. Thus the response for the various sample sizes is essentially the same. However the results for 0.025 g and 0.05 g samples from 600°C ashing are anomalous. This anomaly is related to the overall problem with sample/crucible interaction manifested in the small sample sizes as an enhancement of Ca level. The problem has been discussed in the Chapter 4.

While all embryos contain phytic acid (Maga, 1982) the total P levels vary. Cucurbits were found to contain about 1% P per gram fresh embryo (Lott *et al*, 1978) while NBS Wheat Flour standard contains only about 0.14% (Gladney *et al*, 1982). To test the involvement of phosphorus in the change in Ca solubility at 600°C ashing, several experiments were performed. Calcium phytate obtained from corn (Sigma Chemical Company) was ashed and analyzed with and without treatment. The treated phytate ash measured 0.197 $\pm$ 0.001 g Ca^{2+}/g sample after 650°C ashing but only 0.179 $\pm$ 0.006 g Ca^{2+}/g sample if the ash was left untreated. Thus there was a difference of 9.3% but this was much less than the percent binding seen with cucurbit embryos. Since the phytic acid Ca was measured in milligrams rather than micrograms like the cucurbit Ca, multiple dilutions were required and could have released some of the Ca from the bound state. To see if a binding effect could be produced in NBS Wheat Flour standard by the addition of phytic acid a 5% solution of Ca free sodium phytate (Sigma Chemical Company) was added to wheat flour

TABLE 5:3

The Effect of Time and Temperature of Ashing on the Ca Level of Treated and Untreated Ash of 0.15 g Samples of C. maxima Embryos.

Ash Treatment	µg Ca^{2+}/g Embryo At Various Ashing Conditions					
	500°C		600°C		700°C	
	4 hrs.	16 hrs.	4 hrs.	16 hrs.	4 hrs.	16 hrs.
None	236.0 ± 8.3*	265.1 ±23.8	74.5 ±25.4	65.9 ±13.0	166.0 ±14.3	182.7 ±23.2
Dil. HNO_3 50% HCl	259.7 ±26.7	234.6 ±12.5	280.1 ±22.4	267.1 ±22.4	260.4 ±10.9	279.7 ±17.5

* N=3, Mean ± SD

TABLE 5:4

Measured Ca Concentration in Treated and Untreated C. maxima Embryo Ash After Ashing of Various Sample Sizes at 600 and 700°C.

Sample Weight (g)	µg Calcium/g Embryo					
	600°C			700°C		
	Untreated	Treated	% Diff.	Untreated	Treated	% Diff.
0.025	214.3	252.2	15.0	104.9	207.9	49.5
0.05	149.2	205.2	27.2	39.4	210.7	81.3
0.075	43.7	249.3	82.5	53.5	209.6	74.5
0.10	39.9	200.6	80.1	101.8	215.2	52.7
0.15	47.4	210.0	77.4	106.8	238.4	55.2
0.25	54.9	228.9	76.0	123.2	220.2	44.1
0.50	34.6	217.9	84.1	101.1	205.0	50.7
1.00	56.8	216.1	73.7	125.9	219.9	42.7

Each result represents a single ashing.

samples, dried and then ashed. Table 5:5 shows that the P added as phytate had no effect on untreated ash Ca level. Wheat germ which naturally contains more P did not produce the binding. NBS Tomato Leaves standard also was unaffected by the ashing temperature.

The involvement of K in the binding effect was also considered. Variable and often contradictory results obtained when embryo samples were ashed together with K salts led to the abandonment of such experiments. Instead, calcium nitrate was ashed with and without added P in the form of sodium phytate and K in the form of KCl or KOH. Table 5:6 represents the results with K added as KOH and shows that there were several interactions even among these simple salts. These interactions were different in ashed and unashed samples and also varied with ashing temperature and ash treatment. Unashed calcium nitrate yielded acceptable Ca levels with and without acid treatment. Ashing, even at 500°C reduced the solubility of Ca. In this simple system the amount of phytic acid added swamped the lanthanum present thus giving a lower Ca level. When more lanthanum was added the Ca level increased, but the much more marked depression in Ca level in ashed samples was not counteracted at all by added lanthanum. Acid treatment had no effect on ashed or unashed samples except to eliminate the unexpected enhancement of Ca level in untreated 650°C samples. This enhanced level was produced several times but its cause is unknown. Potassium did not affect the unashed samples when the Ca levels were compared to standards containing the equivalent amount of K. When K was ashed with calcium nitrate the solubility of Ca was decreased to a lesser degree and the depression caused by P was also slightly less severe. Potassium hydroxide blanks, when ashed, gave very highly enhanced

TABLE 5:5

Ashing of NBS Wheat Flour Standard at 650°C With and Without Added Sodium Phytate and Analysis of the Ash for Ca in Untreated and Treated States.

P Content in µg			µg Ca^{2+}/g NBS Wheat Flour Std.	
Present*	Added**	Total	Untreated	Treated
690	0	690	187.4 ± 0.2***	184.5 ± 1.0
690	≃ 425	≃ 1115	192.8 ± 1.0	193.5 ± 1.7
690	≃ 850	≃ 1540	197.3 ± 4.0	188.6 ± 5.0

* P in 0.5 g Standard
** Added as 5% Solution of Na phytate in H_2O
*** Mean ± SD

TABLE 5:6

Interactions of Ca, K and P During Ashing of Calcium Nitrate, Sodium Phytate and Potassium Hydroxide.

Components Added	Treatment of Residue	µg Ca^{2+} Recovered from 30 µg Ca^{2+}		
		Unashed	500°C Ash	650°C Ash
$Ca(NO_3)_2$	None	29.2 ± 0.1*	20.5 ± 1.9	17.9 ± 0.6
	HNO_3/HCl	30.1 ± 0.4	29.7 ± 0.2	28.9 ± 0.3
$Ca(NO_3)_2$ Na Phytate	None	23.0 ± 0.0	4.2 ± 0.1	33.5 ± 2.9
	HNO_3/HCl	23.0 ± 1.7	2.1 ± 0.9	6.9 ± 3.0
$Ca(NO_3)_2$ KOH	None	27.4 ± 0.3	25.2 ± 0.8	24.5 ± 5.4
	HNO_3/HCl	29.0 ± 0.5	28.8 ± 0.2	27.3 ± 1.3
$Ca(NO_3)_2$ Na Phytate KOH	None	22.1 ± 0.0	7.2 ± 1.3	26.9 ± 3.0
	HNO_3/HCl	22.2 ± 0.1	8.0 ± 2.2	13.8 ± 2.6

* Mean ± SD, N=3 for all samples

** 30 µg Ca^{2+} added in H_2O, dried and ashed for 4 hours
Unashed samples - dried on hot plate, then La/HCl added
850 µg P added as Na phytate in H_2O
12,500 µg K^+ added to each sample as KOH in H_2O
All analyzed in La/HCl by the standard procedure

Ca levels as was discussed in the previous chapter but the ashing of KOH and sodium phytate reduced the blanks to more acceptable levels.

DISCUSSION

A number of recent comparative studies, mainly on leafy plant tissue have concluded that for elements like Ca either wet or dry ashing give adequate results (Isaac and Johnson, 1975; Haynes, 1980; Prasad and Spiers, 1978; Watson, 1981). Giron (1973) concluded that dry ashing was better for plant materials and for cucurbit embryos this was found to be the case. While adjustment of ashing parameters might have speeded up the low temperature plasma ashing the procedure was still limited by the small chamber size so that muffle furnace ashing was the best option.

Liver is stated to be very difficult to ash completely (Middleton and Stuckey, 1954; Munter et al , 1979) and the black chars produced by ashing liver at lower temperatures resemble cucurbit ash. Of the procedures used to achieve more complete oxidation, namely wetting the ash with water and re-ashing (Wichmann, 1940, 1942b) or using salts as ashing aids (Gorsuch, 1970; Wichmann, 1940) or treating the ash with acids (Allen, 1974; Gorsuch, 1970) only the latter was suitable for ash from cucurbit embryos. Oxidation with nitric acid was found to release Ca occluded in the carbon residue of the ash (Clegg et al ,1981; Labanauskas and Handy, 1975) but with cucurbit embryos the lower Ca levels were found in the carbon-free ash. Gorsuch (1970) claimed that concentrated nitric acid led to the formation of compounds which resisted further oxidation and recommended acid treatment with dilute nitric acid followed by hydrochloric acid. The results in this chapter testify to the greater

effectiveness of this dilute acid treatment.

When concentrated nitric acid was used on the insoluble residue produced from ashing $Ca(NO_3)_2$ at 650°C the Ca was converted back into the soluble form. The $Ca(NO_3)_2$, on ashing, most likely produced $CaCO_3$ and CaO which may have reverted to the nitrate with the nitric acid treatment. In ashing of biological materials Ca reacts with P to form a variety of insoluble phosphates (Wichmann, 1953). Nitric acid in concentrated form appeared to be ineffective in converting all the Ca to the soluble form in Cucurbita maxima embryo ash produced at 650°C. There were also several reports in the literature of low Ca levels when concentrated nitric acid treatment of the ash was used. Hoover (1976) ashing mineral mix feeds concluded that refractory compounds were being formed at 550°C ashing. The ash was treated by HCl followed by concentrated HNO_3 and their Ca levels were 50% lower than expected. Menden et al (1977) ashing rat pups at 400°C had trouble producing a clean, carbon free ash and so tried several types of aqua regia (HNO_3/HCl) treatments. Their lowest Ca levels were obtained on samples that had concentrated nitric acid as the last step of the ash treatment. Munter et al (1979) ashing liver at 485°C, found aqua regia ineffective and also found that concentrated nitric acid gave lower results than those from wet ashed samples. Their results after HNO_3 were low for other minerals as well as Ca.

The cucurbit results showed that there was an abrupt change in the ease with which Ca could be extracted from the ash at 600°C ashing temperature. Wichmann (1953) studying ashing of both inorganic salts and food products concluded that there was a change in the ash at temperatures

of 575-600°C leading to the formation of less soluble products. This suggests that similar changes might be occurring in the cucurbit ash. The slow but nearly complete release of the bound Ca on standing suggests less soluble rather than insoluble compounds containing Ca were formed.

Occlusion in residual carbon in the ash has already been mentioned as a cause of lowered mineral levels. Middleton and Stuckey (1954) suggested that insoluble metaphosphates can cause extraction problems. Gorsuch (1970) mentioned the trapping of iron by condensed phosphates formed by heat from simple phosphates. Aluminates and silicates are implicated in mineral retention in ash (Allen, 1974). Cucurbit embryos are high in P but have little Al and Si.

The results with the ashing of Ca phytate indicated that P was involved in the Ca binding. The lack of binding in NBS Wheat Flour standard with added phytate showed that a mere addition of P was not sufficient and that certain other conditions had to be met. Because of the depressive effect of P on Ca absorption, the system was not flexible enough to experiment with larger amounts of phytate.

Wichmann (1940, 1942b, 1953) found that ashes with an alkaline balance were more soluble. The alkaline balance was caused by high amounts of K and Na which on ashing yielded carbonates and oxides. As K was reported to be lost at higher ashing temperatures (Isaac and Jones, Jr., 1972) the loss could be associated with a decrease in Ca solubility. However, as mentioned in the results, it was found that ashing of added K and embryos produced variable results. This was not unexpected in view of the problems described in the previous chapter with enhancement of Ca levels by K. Results with ashed Ca nitrate showed that the reduction in

measured Ca was much more marked when P was present. The severity of the Ca binding was reduced when K was present during ashing. Thus the interaction of one mineral with another was modified by a third mineral and the interactions appeared to produce both depressions and enhancements in measured Ca levels. The potential for such interactions is even greater in the complex embryo tissues. Differences in the mineral complements may explain the differences in the ashes of cucurbits and the two NBS standards tested.

The role of K and P in decreasing the ease with which Ca can be extracted from carbon-free ash of cucurbits and the occurrence of the binding in oily seeds other than cucurbits will be examined in the following chapter.

Chapter 6.

VARIATION IN EXTRACTION OF CALCIUM FROM DRY-ASHED EMBRYOS OF CUCURBITS AND OTHER LIPID-RICH SEEDS.

INTRODUCTION

While light-coloured, carbon-free ash is generally considered desirable (Gorsuch, 1970; Lambert, 1976) Ca was less easily extracted from Cucurbita maxima ash produced at 650 or 700°C than from the black ash produced at 500°C (Chapter 5). Ashing temperatures of 600°C or higher resulted in a lowered solubility of Ca in the ash of both C. maxima and C. andreana embryos either due to the formation of less soluble salts or due to the binding of Ca to less soluble ash components. Ca levels within the ash of leafy material (NBS Tomato Leaves) or starchy material (NBS Wheat Flour) showed no change at different ashing temperatures from 500 to 650°C. A number of different cucurbit species were ashed at various temperatures and analyzed with or without ash treatment to find out whether the Ca binding was a general property of cucurbit embryos or limited to the two species initially analyzed. Also, to discover whether the decrease in Ca availability with increases in ashing temperature was characteristic of cucurbit species or present in all oily, protein-rich embryos, seeds of a number of different species were analyzed for Ca by AAS. In order to determine whether the binding of Ca was affected by the relative concentrations of the other three storage minerals, Mg, K and P (Lott, 1980), the embryos were also analyzed by NAA.

This latter procedure was carried out on unashed tissues so that problems related to ashing were avoided.

MATERIALS AND METHODS

Preparation of Samples :

To obtain the embryos from all species of cucurbit seeds the outer seed coats were removed in the dry state and the inner seed coats were peeled off after brief soaking in water. Seed coats and any inner layers not belonging to embryo tissue were also removed in the dry state from all other seeds except the castor bean where both endosperm and embryo were analyzed together. The names of the embryos analyzed are listed in Table 1. All the embryos were pooled in numbers commensurate with embryo sizes to give an adequate amount of embryo mixture for about 20 samples of 0.15 g. The embryos were pestle and mortar ground. Dry weights on separate samples were obtained after two hours heating at 105°C. Air-dried weights were used in all tables.

Analytical Procedures :

The dry ashing procedure has been described in Chapter 2. Embryos of all species were charred prior to ashing. Ashing temperatures of 500, 600, 650 and 700°C were used as indicated in the tables but ashing time was unvaried at 4 hours. The pH of the ash was determined in a hot water extract of the ash as described by Wichmann (1953). Ash referred to as treated was digested in dilute nitric acid followed by hydrochloric acid as derived from Gorsuch (1970) and outlined in Chapter 2. The AAS procedure has also been described in Chapter 2 and summarized in Table 2:12. Preliminary analysis of a few samples of each of the embryos

TABLE 6:1

Embryos Analyzed by AAS and NAA.

Scientific Name	Common Name
Cucurbit Species	
Apodanthera undulata	--
Benicasa hispida	Wax gourd
Citrullus colocynthis	Bitter watermelon
Citrullus vulgaris	Watermelon
Cucumis sativus	Cucumber
Cucurbita andreana	Bitter gourd
Cucurbita foetidissima	Buffalo gourd
Cucurbita maxima	Hubbard squash
Echinocystis lobata	Wild cucumber
Luffa cylindrica	Loofah
Sicyos angulatus	Bur cucumber
Trichosanthes dieniensis	Snake gourd
Non-cucurbit Species	
Anacardium occidentale	Cashew
Pistacea vera	Pistachio
Helianthus annuus	Sunflower
Ricinus communis	Castor bean
Juglans regia	Walnut
Bertholletia excelsa	Brazil nut
Arachis hypogaea	Peanut
Glycine max	Soybean
Macadamia integrifolia	Macadamia nut

established the volume of La/HCl required for adequate dilution of the ash of different embryos. The NAA was performed on the same ground samples as used for AAS but the samples were not ashed. The details of the NAA method are given in Chapter 3.

RESULTS

Table 6:2 shows the Ca analysis of 12 cucurbit species. These belong to 9 genera in the Family Cucurbitaceae and include both tropical and temperate species. Each value represents a mean of triplicate or pentuplicate ashings but the standard deviations were omitted to simplify the table. However, the low coefficients of variation for three of the columns indicate that the results were in close agreement. After 500°C ashing the Ca levels were essentially the same in treated or untreated ash. This ash was uniformly black and mainly insoluble. After ashing at 650°C untreated ash had considerably lower Ca levels which were also more variable as indicated by the higher average coefficient of variation. Acid treatment of this ash resulted in Ca levels comparable to those obtained after 500°C ashing. Thus, for all cucurbit species tested, Ca was less easily extracted from the more soluble, light-coloured ash produced at 650°C. Table 6:3 shows the percent of the total Ca that was bound in an insoluble form hence not measured in untreated ash from 650°C. The average reduction was close to 50% of the available Ca but the degree varied even for the same species measured on different occasions.

When non-cucurbit species were ashed (Table 6:4) it was apparent that most of these embryos ashed more completely and produced the expected

TABLE 6:2

Calcium Concentration in Treated and Untreated Ash of Embryos of the Family Cucurbitaceae as Measured by AAS.

Species N = 5 for * N=3	µg Ca^{2+}/g Embryo			
	500°C Ashing		650°C Ashing	
	Untreated	Treated	Untreated	Treated
Apodanthera undulata	486.4	532.1	283.8	529.0
Benicasa hispida	413.7	412.4	228.6	416.4
Citrullus colocynthis	600.8	570.5	214.9	605.4
Citrullus vulgaris	432.4	428.3	370.7	428.0
Cucumis sativus	703.2	712.6	389.2	706.0
Cucurbita andreana	639.8	655.2	360.5	658.8
Cucurbita foetidissima	1001.9	989.5	408.5	1004.5
Cucurbita maxima	177.6	191.3	66.8	196.1
Echinocystis lobata	403.6	417.3	260.9	406.6
Luffa cylindrica	843.0	831.8	375.9	812.0
Sicyos angulatus	176.4	190.6	94.1	192.3
Trichosanthes dieniensis	532.6	538.1	354.6	541.3
Ave. c.v.	2.8%	2.0%	9.0%	3.0%

TABLE 6:3

Percent of Ca Bound in Untreated Cucurbit Embryo Ash Produced at 650°C as Compared to the Ash Produced at 500°C.

Cucurbit Species	% Ca Bound
Apodanthera undulata	45.0
Benicasa hispida	45.1
Citrullus colocynthis	63.7
Citrullus vulgaris	13.7
Cucumis sativus	44.9
Cucurbita andreana	44.1
Cucurbita foetidissima	59.1
Cucurbita maxima	64.5
Echinocystis lobata	36.2
Luffa cylindrica	54.7
Sicyos angulatus	49.5
Trichosanthes dieniensis	34.0
Average % Binding	46.2

light-coloured ash even at 500°C. However even within these species there was some variation in ash colour. Pistachio, peanut and Brazil nut produced a speckled ash while castor bean and sunflower ashes were uniformly black. The lighter ashes were consistently more soluble than the darker ashes. All of the ashes lightened further in colour and increased in solubility with higher temperature ashing. The type of ash produced was not related to the oil and protein content. Soybean, which had more protein than oil and Macadamia nut, which had more oil than protein, produced the same ash colour. Of the non-cucurbit species tested only castor bean showed a change in Ca availability in untreated ash when the temperature of ashing was raised from 500 to 650°C. The percent of Ca bound was 46% which was in the same range as for cucurbits. Double acid treatment of the castor bean ash counteracted the binding. Although the sunflower embryos produced a black ash, no change in Ca level was found in the untreated 650°C ash.

To ensure that the differences between cucurbit and non-cucurbit species were not the result of differences in embryo preparation, namely the soaking required to remove the inner seed coat from the cucurbits, razor-peeled cucurbit embryos were prepared. Unsoaked embryos still produced the expected drop in Ca level in untreated ash from 650°C so that soaking had no effect on the character of the ash.

Table 6:5 shows that the soft, lighter-coloured ash produced an alkaline extract in hot water while the black brittle ash was closer to neutral pH. Castor bean which did show the binding also had a less alkaline ash pH than the other non-cucurbit species. There was no distinctive change in the pH in the ash produced at 700°C but the

TABLE 6:4

Ashing Characteristics of Oily Embryos.

Family Name	Common Name	% Oil/ Protein*	Ash Colour 500°C	Ash Colour 650°C	Calcium Binding at 600°C
Anacardiaceae	Cashew	47/12	gray	white	-
	Pistachio	50/20	gray to black	gray to white	-
Compositae	Sunflower	28/30	black	gray	-
Cucurbitaceae	Squash	45/35	black	gray	+
Euphorbiaceae	Castor bean**	50/18	black	black to white	+
Juglandaceae	Walnut	35/42	gray	white	-
Lecythidaceae	Brazil nut	67/17	black to gray	gray to white	-
Leguminosae	Peanut	49/27	black to white	gray to white	-
	Soybean	21/40	gray	white	-
Proteaceae	Macadamia nut	77/ 9	gray	white	-

* from Vaughan (1970)

** Embryo plus endosperm

TABLE 6:5

Ash Colour, Consistency and pH After 500 and 700°C Ashing.

Species Ashed	Ash Colour 500°C	Ash Consistency 500°C	pH of H_2O Extract of Ash 500°C	700°C
Peanut	white-gray	soft	9.8	9.6
Soybean	light gray	soft	10.6	11.7
Cashew	light gray	soft	10.2	9.7
Castor bean	black	brittle	7.8	8.6
Trichosanthes	black	brittle	7.1	7.2
C. maxima	black	brittle	7.6	7.4
C. andreana	black	brittle	7.2	7.2

N = 2, after 700°C all ashes were lighter in colour and softer.

difference between those that release Ca easily and those species that need ash treatment was maintained. The change in Ca availability at higher temperature ashing was thus not reflected in a change in ash pH.

To explain the differences in the ash after 650°C ashing, all the embryos analyzed in the ashed state by AAS were also analyzed as unashed samples by NAA. Table 6:6 shows the concentration of K, Ca, Mg and P in the embryos. These four elements represent the bulk of the minerals stored as phytin in the electron dense globoid crystals within the protein bodies of the embryo tissue (Lott, 1980; Maga, 1982). Comparing the Ca levels obtained by AAS with NAA results showed that in most cases the results agreed within 7% but in the case of Brazil nut and castor bean the discrepancies were larger. Since these analyses were designed for Cucurbita species it was possible that some conditions such as dilutions used in AAS were not ideal for these two species. The difference after 650°C ashing remained for the castor bean despite the discrepancy. Table 6:6 shows that within both cucurbit and non-cucurbit species there was considerable species-to-species variation in all four elements so that overall differences were not readily apparent. Table 6:7 shows the means and ranges for the mineral values shown in Table 6:6. The means for Ca and Mg are somewhat different for the cucurbits and non-cucurbits but the ranges for both minerals overlap considerably. The mean values for K are almost identical but an even greater variation is seen in the ranges. However, the means for P are quite different and the range overlap is small. The cucurbits which produced the ash from which Ca was less easily extracted contained much more P.

The ratios in Table 6:8 show more clearly the differences

TABLE 6:6

Mineral Analysis in Fresh Embryo Tissue by NAA and Analysis of Ca in Ash by AAS.

Species	AAS	NAA			
	%Ca	%Ca	%Mg	%K	%P
Cashew	0.042 ± 0.003	0.044 ± 0.002	0.22 ± 0.01	0.56 ± 0.06	0.45 ± 0.01
Pistachio	0.091 ± 0.002	0.085 ± 0.004	0.10 ± 0.01	0.69 ± 0.03	0.65 ± 0.14
Sunflower	0.098 ± 0.001	0.101 ± 0.005	0.41 ± 0.03	0.72 ± 0.04	0.80 ± 0.03
Castor bean	0.020 ± 0.001	0.016 ± 0.001	0.36 ± 0.01	0.47 ± 0.02	0.71 ± 0.06
Walnut	0.087 ± 0.008	0.081 ± 0.004	0.15 ± 0.01	0.41 ± 0.02	0.41 ± 0.01
Brazil nut	0.191 ± 0.009	0.132 ± 0.016	0.24 ± 0.02	0.54 ± 0.05	0.53 ± 0.02
Peanut	0.035 ± 0.001	0.033 ± 0.001	0.20 ± 0.01	0.68 ± 0.03	0.44 ± 0.06
Soybean	0.116 ± 0.002	0.109 ± 0.004	0.25 ± 0.01	2.05 ± 0.09	0.89 ± 0.03
Macadamia nut	0.051 ± 0.001	0.051 ± 0.002	0.10 ± 0.01	0.29 ± 0.01	0.17 ± 0.03
Apodanthera	0.052 ± 0.001	0.054 ± 0.002	0.39 ± 0.02	0.61 ± 0.03	0.97 ± 0.10
Benicasa	0.042 ± 0.006	0.040 ± 0.002	0.41 ± 0.02	0.82 ± 0.04	1.55 ± 0.09
Citrullus vulgaris	0.043 ± 0.001	0.041 ± 0.002	0.44 ± 0.03	0.81 ± 0.04	1.03 ± 0.08
C. colocynthis	0.059 ± 0.005	0.066 ± 0.004	0.54 ± 0.05	0.61 ± 0.04	1.35 ± 0.24
Cucumis sativus	0.071 ± 0.003	0.064 ± 0.005	0.55 ± 0.04	0.82 ± 0.08	1.51 ± 0.04
Cucurbita andreana	0.065 ± 0.001	0.065 ± 0.003	0.51 ± 0.03	0.61 ± 0.03	1.33 ± 0.04
C. foetidissima	0.100 ± 0.002	0.103 ± 0.007	0.56 ± 0.04	0.84 ± 0.07	1.22 ± 0.06
C. maxima	0.019 ± 0.001	0.018 ± 0.001	0.39 ± 0.02	0.62 ± 0.03	1.03 ± 0.01
Echinocystis	0.041 ± 0.001	0.041 ± 0.002	0.52 ± 0.03	0.95 ± 0.04	1.22 ± 0.07
Luffa	0.083 ± 0.002	0.080 ± 0.004	0.50 ± 0.03	0.67 ± 0.03	0.94 ± 0.07
Sicyos	0.019 ± 0.001	0.018 ± 0.001	0.59 ± 0.03	1.08 ± 0.05	1.25 ± 0.02
Trichosanthes	0.054 ± 0.002	0.052 ± 0.002	0.27 ± 0.02	0.42 ± 0.02	0.68 ± 0.03

N=3, Mean ± SE

between those species that bind Ca and those that do not. The ratios for leafy plant tissue in the form of NBS Tomato Leaves standard have been added for comparison. The ratios that appear to be different are the K/P and total cation to P. In all embryos that did not bind Ca in the ash the K/P ratio was higher than in the cucurbits embryos. The total cation content was also higher relative to P in the non-cucurbit species. These are the initial differences in unashed tissues but whether there is also a differential loss of K at 700°C is not known at present.

DISCUSSION

The explanation of the differences in ash characteristics and the change in the ease with which Ca can be extracted from the ash may lie in the differences in the organic matrix, the mineral matrix or an interaction of the two. However, which predominates is at present difficult to assess for, as Panday and Sansoni (1983) point out, there is little information available on the chemical reaction mechanisms of ashing methods. Michotte et al (1976) studied the structure of compounds formed during plasma ashing but comparable information for dry ashing products is minimal.

Middleton and Stuckey (1953) discussed how various tissue components ash. The carbon residue from carbohydrates burned off readily at fairly low temperatures. Fats required gradual heating to drive off the existing volatile components, as well as those being formed during decomposition, to avoid a violent ignition of the sample. However with gradual heating fats eventually oxidized completely leaving no residue.

TABLE 6:7

NAA of Elements Stored in Embryos.

Species	%Ca	%Mg	%K	%P
Mean and Range for 9 Non-cucurbits	0.072 (0.016-0.132)	0.23 (0.10-0.41)	0.71 (0.29-2.05)	0.56 (0.17-0.80)
Mean and Range for 12 Cucurbits	0.054 (0.018-0.103)	0.47 (0.27-0.59)	0.74 (0.42-1.08)	1.17 (0.68-1.55)

TABLE 6:8

Mineral Ratios in Various Species Analyzed by NAA.

Species	Mg/K	Ca/K	$\frac{Ca+Mg}{P}$	Mg/P	K/P	Ca/P	$\frac{Mg+K+Ca}{P}$
Castor bean	0.76	0.03	0.79	0.50	0.66	0.02	1.19
Ave. for 12 Cucurbits	0.65 ±0.12	0.08 ±0.04	0.73 ±0.15	0.41 ±0.07	0.64 ±0.13	0.05 ±0.02	1.10 ±0.18
Ave. for 8 Non-cucurbits	0.33 ±0.15	0.13 ±0.07	0.46 ±0.19	0.41 ±0.14	1.35 ±0.48	0.16 ±0.08	1.92 ±0.50
NBS Wheat Flour	0.30	0.14	0.44	0.30	0.99	0.14	1.42
NBS Tomato Leaves	0.16	0.67	0.83	2.06	13.11	8.82	24.00

Proteins ashed with varied ease depending on both their amino acid composition and on additional components such as P or other minerals. They state that some animal proteins were apparently more difficult to decompose because of their high lysine, tyrosine and tryptophan content. When proteins decomposed at about 350°C they left a black residue resembling carbon and this residue needed much higher temperatures to decompose further.

Since all the cucurbit embryos tested here had between 25 and 35% protein their black ash could be the result of protein ashing. However the embryos also all contained phytic acid which produced a black ash after 550°C ashing (Chapter 5). The non-cucurbit embryos had varying amounts of protein and the content was not correlated with ash colour. Since the binding of Ca was prominent in the carbon-free ash, inorganic reactions appeared to be involved but the extent to which such reactions take place may be governed by the organic components present during ashing. Gorsuch (1970) emphasized that the organic material chars may be quite reactive and have the potential for the expression of a number of catalytic steps. Wichmann (1940, 1942a,b, 1943, 1953) studied the interaction of salts within the ash of various foods. The solid state reactions between ash components varied with the initial constituents of the organic matter and with the ashing temperature and resulted in ashes of varied solubility. Wichmann was not concerned with the mineral composition of the ash. His aim was to produce a representative ash weight for the various food products. Ash weights would vary with the type of salt that predominated and were dependent on sample composition and on temperature. His main work was done on ashing of various calcium phosphates with sodium or potassium

carbonates and he used this to define the potential products of ashing from 400 to 700°C. His final work was on ashing of milk which, like seed tissues, was high in P. Wichmann found that the composition of the ash varied with the temperature and the carbonate to phosphate ratio in the samples. The K and Na in the samples formed soluble carbonates and oxides while Mg and Ca formed insoluble phosphates. Between these components there then occurred a neutralization reaction in the solid state without fusion taking place. If there was little carbonate compared to phosphate, mixed insoluble salts such as $CaKPO_4$ were formed and alkali metals entered the insoluble portion of the ash. As the carbonate level rose relative to phosphate the main products were insoluble hydroxyapatites ($Ca_3(PO_4)_2.Ca(OH)_2$) and trisodium phosphates (Na_3PO_4). If there was a large excess of carbonate over phosphate the excess carbonates degraded the apatites into pentoxides thus making the P component of the ash more soluble. The above reactions increased with increasing temperature. Harper et al (1982) ashing rice hulls and lucerne leaves and stems concluded that hydroxyapatite may be formed in the ashes of these tissues provided sufficient Ca and P were present.

Cornes (1944) stated that plant tissues naturally contained enough Ca and Mg to convert all the phosphoric acid into calcium and magnesium phosphates while the K and Na ended up as soluble chlorides and carbonates. He also pointed out that the above did not hold for storage tissues such as seeds where both Mg and Ca were in lower concentration relative to the alkali content. Ashing such tissues led to the formation of insoluble double phosphates such as $CaKPO_4$. He did not discuss

differences within seed tissues themselves.

Ash high in carbonate would have an alkaline balance while that high in P would not be alkaline. The non-cucurbit species had alkaline ash and had a high level of K relative to P. Cucurbits and castor bean had a neutral ash pH and a higher level of P than K. The change in the Ca solubility or binding occurred at 600°C. Wichmann (1953) found changes in the ash composition at temperatures 575-600°C. Beyond this temperature the equilibrium shifted towards the formation of more of the insoluble products such as the hydroxyspatites. In cucurbit ash the shift towards less soluble products was also noted at this temperature. The acid treatment converted the Ca into more soluble forms. Insoluble pyro- or metaphosphates can be converted to orthophosphates by dilute mineral acids (Wichmann, 1940).

Thus the comparison of mineral components of the cucurbit and non-cucurbit embryos explained the differences seen in the ease with which Ca could be extracted from the ash.

Chapter 7.

CHARACTERSTICS OF THE FRUITS AND SEEDS OF *C. MAXIMA* CV. WARTED HUBBARD AND *C. ANDREANA*.

INTRODUCTION

The vegetative growth of *C. maxima* and *C. andreana* is very similar except that the latter has leaves with white spots between the veins (Whitaker, 1951). The plants have a strong tap root and a stem consisting of 3-8 prostrate branches (Bemis *et al*, 1970; Whitaker and Davis, 1962). Both species are monoecious with large, yellow flowers that in pistillate form have an inferior ovary (Esau, 1977; Whitaker and Davis, 1962). Although cucurbits are naturally bee pollinated (Bushnell, 1920) the flowers in this study were pollinated by hand. Pistillate flowers are receptive throughout their 24 hour open period and can be successfully pollinated under a wide variety of conditions. A large percent of the pollinated flowers fail to set fruit (Bushnell, 1920). The fruits of *Cucurbita* are classified as inferior berries of pepos (Whitaker and Davis, 1962). They are fleshy, fruits consisting of more than 90% water but less than 5% sugar (Coombe, 1976). Instead of the thick, edible, pericarp of *C. maxima*, *C. andreana* fruits have a hard durable outer layer with thin, stringy and bitter flesh (Whitaker, 1951). The growth pattern of fleshy fruits have been described by Crane (1964), Coombe (1976) and Nitsch (1953). Cucurbit fruit flesh is made up of the ovary and adherent receptacle and the growth pattern follows a simple sigmoid curve (Coombe, 1976). The weight of the fruit at maturity is determined by the

cell number, cell volume and cell density (Coombe, 1976). The balance of these factors as well as the initial size of the ovary produces small or large cucurbit fruits (Sinnott, 1939).

Seed growth consists of a short lag phase, a period of linear increase in dry weight and a period of decreasing growth rate leading to cessation of growth at maturity (Egli, 1981). Both growth rate and duration of growth are correlated with seed size but an even stronger correlation exists between seed size and cell number (Egli et al , 1981; Guldan and Brun, 1985). The number of cells per embryo is established early with most of the division being completed during the lag phase (Egli et al , 1981; Guldan and Brun, 1985). Davies (1977) studying various species in the genus Vicia found that the small-seeded species consistently had fewer cells in their cotyledons than the large-seeded species. The difference in cell number varied 140-fold while the individual cell size varied only about 4.5-fold thus indicating that cell number was the main cause of the differences in seed size. Egli et al (1981) found that growth conditions and environmental stresses could modify seed size via changes in cell number, growth rate and the duration of growth, but that genetic differences in seed size within the soybean cultivars being studied were stronger than environmental influences.

MATERIALS AND METHODS

Source of Seeds :

The initial seed stocks of C. maxima cv. Warted Hubbard were purchased from Stokes Seeds Ltd., St. Catharines, Ontario. Cucurbita andreana seeds were obtained form Dr. W.P. Bemis, University of Arizona,

Tucson. These seeds will be referred to as stocks in this and subsequent chapters.

Growth in Field Plots :

Randomly selected seeds of the above stocks were planted in field plots with about 2.5 x 3.0 metres of space allotted to each plant. For each species twenty-nine plants were grown over a three year period. The soil was initially roto-tilled and fertilized with N-P-K (4-8-6). The plots were watered regularly and in mid-summer additional fertilizer was added. The flowers were self-pollinated by hand (Heiser, 1979) and to facilitate the selfing the plants were kept separate. Both male and female flowers were bagged the day before they opened using glassine pollination bags of 5x3x9 inches with a 2 inch gusset (#535 from Lawson Bags, Northfield, IL). After pollination the pistillate flowers were re-bagged for another day. Flowers were pollinated from the first week of July until mid-August. The fruit were harvested about 8 weeks after pollination.

Growth in the Greenhouse :

The plants were grown on benches in about 100 litres of soil per plant. Natural lights were supplemented with fluorescent lights as necessary to maintain an average light level of 415 $\mu Es^{-1}m^{-2}$. The fruits were harvested at about 8 weeks.

Growth in the Growth Chambers :

Two plants were grown per 15 square foot chamber (Conviron Controlled Environments, Model E15) in large nursery tubs. The day/night cycle was set at 8/16 hours to ensure flower production by C. andreana . The the day/night temperature was 25/18°C. The average light intensity was 300 $\mu Es^{-1}m^{-2}$ provided by a combination of fluorescent and

incandescent bulbs. Watering was with 1/4 strength Hoagland's solution (Hoagland and Arnon, 1950). Plants were regularly supplemented with 4-8-6 fertilizer solution. Fruits were harvested at 8-12 weeks.

Post-harvest Fruit and Seed Preparation :

The fruits were generally weighed and measured immediately after harvesting and then stored for about four weeks. The fruits were cracked open to avoid cutting the seeds and the seeds were placed on drying trays for about 4 weeks to air-dry. The dried seeds were counted, weighed and measured. Ten seeds per fruit were germinated in vermiculite as a test of physiological maturity. The embryos were obtained by removing the two seed coats as described in Chapter 2. The embryos consisted of a small root-shoot axis and two large, flat cotyledons. The measurements were compared and correlated using the Minitab (MTB) statistical program (Ryan et al , 1985).

RESULTS

While each of the two cucurbit species were relatively easy to grow, the optimum conditions for the two species were not the same. Cucurbita maxima grew best in the field plots where a plant was able to produce several large fruits of up to 10 kg each. In the greenhouse and growth chambers the fruit weight rarely surpassed 2 kg and with a few exceptions only one fruit was produced per plant. Cucurbita andreana also grew well in the field plots but its flower production was delayed compared to C. maxima . The best flower production occurred in late August by which time it was too late to produce mature fruits. Even then abortion rate for pollinated flowers remained high. In the growth chambers

TABLE 7:1

Fruit and Seed Parameters of *C. maxima* and *C. andreana* Grown in the Field, the Greenhouse, and Growth Chambers.

Parameters Measured	Species *	Size Range	Largest / Smallest	Mean ± SD	c.v. %	Mean A/B
Fruit Weight (kg)	A	0.5–10.4	20.8	4.9±3.0	61.2	
	B	0.05–0.28	5.6	0.11±0.05	45.5	44.5
Fruit Circumf. (cm)	A	34.0–105.0	3.1	69.0±18.4	26.7	
	B	14.5–25.0	1.7	19.0±2.6	13.7	3.6
Fruit Length (cm)	A	15.0–43.0	2.9	30.2±8.3	27.5	
	B	5.0–12.0	2.4	7.5±1.6	21.3	4.0
Fruit Width (cm)	A	10.5–33.0	3.1	20.8±5.3	25.5	
	B	4.5–8.0	1.8	5.9±0.9	15.3	3.5
No. of Seeds per Fruit	A	54–622	11.5	290±132	45.5	
	B	94–461	4.9	229±93	40.6	1.3
Total Seed Wt. (g)	A	9.0–156.2	17.4	76.0±39.8	52.4	
	B	0.9–10.5	11.7	4.3±2.2	51.2	17.6
Ave. Wt. per Seed (g)	A	0.13–0.37	2.8	0.25±0.05	20.0	
	B	0.008–0.027	3.3	0.019±0.005	26.3	13.2

* A – *C. maxima*, N=82
B – *C. andreana*, N=35

TABLE 7:2

Fruit and Seed Parameters of *C. maxima* Grown in the Field and in the Greenhouse and Growth Chambers.

Parameters Measured	Location *	Size Range	Largest / Smallest	Mean ± SD	c.v. %	mean F/G
Fruit Weight (kg)	F	3.0–10.4	3.5	6.6±1.9	28.8	
	G	0.5–2.7	5.4	1.3±0.5	38.8	5.1
Fruit Circumf. (cm)	F	62.0–105.0	1.7	80.0±10.1	12.6	
	G	34.0–58.0	1.7	45.8±6.0	13.1	1.7
Fruit Length (cm)	F	26.0–43.0	1.7	34.9±5.0	14.3	
	G	15.0–30.0	2.0	20.4±3.8	18.6	1.7
Fruit Width (cm)	F	17.0–33.0	1.9	23.9±3.3	13.8	
	G	10.5–20.0	1.9	14.5±2.0	13.8	1.6
No. of Seeds per Fruit	F	100–622	6.2	352±109	31.0	
	G	54–320	5.9	165±69	41.8	2.1
Total Seed Wt. (g)	F	30.9–156.2	5.0	95.5±31.6	33.1	
	G	9.0–91.8	10.1	34.8±17.7	50.9	2.7
Ave. Wt. per Seed (g)	F	0.21–0.37	1.8	0.27±0.04	14.8	
	G	0.13–0.31	2.4	0.21±0.05	23.8	1.3

* F – Field, N=55
G – Greenhouse and growth chambers, N=27

and greenhouse C. andreana grew well and sustained multiple fruits per plant. Random abortion of pollinated flowers resulted in an irregular number of fruits per plant with both species.

C. maxima seeds usually had 100% germination in germination trials, but a few batches of C. andreana seeds did not germinate on first try. It was concluded that these smaller seeds were more stringent in their germination requirements and were especially sensitive to moisture conditions. A small percentage of the C. maxima seeds in some fruits had completely normal seed coats but no embryos. Such empty seed coats were discarded since their occurrence has been reported for other cucurbits (Hume and Lovell, 1981; Kihara, 1951; Kwack and Fujieda, 1985; Wong, 1941) and since they were found to have no effect on any of the measured fruit and seed parameters.

Table 7:1 shows the fruit and seed parameters of all the C. maxima and C. andreana fruits. There were more C. maxima fruits as a result of the successful field growth of this species. The size ranges show that all fruit parameters were smaller for C. andreana fruits. The ratio of largest to smallest within each fruit and seed parameter showed a wider range of measurements for the C. maxima species. The coefficients of variation showed that fruit weight, seed number and total seed weight were the most variable parameters in both species. The average seed weight varied less as did the fruit circumference, length and width. The two species differed most markedly in their fruit weights with a 45-fold difference in the mean weights. The other fruit size parameters differed only 4-fold. The parameter with the smallest difference between the two species was the number of seeds per fruit but within each species there

TABLE 7:3

Variation in Fruit Weight and Seed Yield in C. maxima (A) and C. andreana (B) Grown at Three Growth Locations.

Growth Location	N	Fruit Wt. (kg)	Seed No.	Total Seed Wt. (g)	Ave. Wt. per Seed (g)
(A) Field	55	6.6 ± 1.9	352 ± 109	95.5 ± 31.6	0.27 ± 0.04
Greenhouse	20	1.2 ± 0.5	145 ± 61	29.1 ± 13.0	0.21 ± 0.04
Growth Chambers	7	1.5 ± 0.5	217 ± 64	50.2 ± 20.4	0.24 ± 0.07
(B) Field	1	0.28	319	7.6	0.024
Greenhouse	16	0.08 ± 0.03	174 ± 62	2.8 ± 1.1	0.017 ± 0.005
Growth Chambers	18	0.134 ± 0.04	274 ± 90	5.5 ± 2.0	0.020 ± 0.005

was considerable variation in seed number.

The very broad range of fruit weights within C. maxima shown in Table 7:1 was due to the difference in the weights of the fruits grown at the outdoor and indoor locations. Table 7:2 shows the fruit and seed parameters of the C. maxima fruits segregated by the growth location. The lowest weight of the field grown fruits (3.0 kg) was higher than the highest weight of the fruits (2.7 kg) produced at the indoor locations. The difference between the mean fruit weights was 5-fold while all the other parameters varied less. The average seed weight had the smallest difference. At each location fruit weight, the number of seeds per fruit and the total seed weight were the most variable parameters. In Table 7:3 the fruit weights and seed yield are shown for each of the three locations for both species. The largest fruits and best seed yields were obtained in the field plots. Of the two indoor locations the growth chambers produced slightly larger specimens. Fruit size showed the greatest difference between locations; seed size remained much more constant.

The correlation coefficients in Tables 7:4 and 7:5 show that most of the measured parameters were significantly correlated with each other. Thus, in both species, heavy fruits were also large in the other measured dimensions and had a larger seed mass as a result of an increase both in seed weight and in seed number. Only in the field grown C. maxima was the number of seeds not correlated with fruit weight and overall this group of fruit had fewer significant correlations among the compared parameters. In both tables the number of seeds was less well correlated with the other parameters and was least well correlated with the average seed weight. For all three categories of C. maxima stepwise multiple regression analysis

TABLE 7:4

Correlation Coefficients for Fruit and Seed Parameters of *C. maxima* and *C. andreana*.

	A. *C. maxima* B. *C. andreana*	Fruit Weight	Fruit Circumf.	Fruit Length	Fruit Width	No. of Seeds	Total Seed Wt.
A	Circumference	0.96**					
	Length	0.88**	0.81**				
	Width	0.95**	0.97**	0.80**			
	No. of Seeds	0.63**	0.66**	0.56**	0.63**		
	Total Seed Wt.	0.73**	0.77**	0.61**	0.72**	0.93**	
	Av. Wt. per Seed	0.63**	0.70**	0.55**	0.65**	0.36*	0.63**
B	Circumference	0.91**					
	Length	0.93**	0.82**				
	Width	0.78**	0.80**	0.75**			
	No. of Seeds	0.60**	0.51**	0.53**	0.42**		
	Total Seed Wt.	0.84**	0.75**	0.79**	0.67**	0.86**	
	Av. Wt. per Seed	0.59**	0.65**	0.67**	0.64**	0.04	0.52**

* significant at 0.05
** significant at < 0.01

TABLE 7:5

Correlation Coefficients for Fruit and Seed Parameters of Indoor and Outdoor *C. maxima*.

	F. Field plots G. Greenhouse and Growth Chambers	Fruit Weight	Fruit Circumf.	Fruit Length	Fruit Width	No. of Seeds	Total Seed Wt.
F	Circumference	0.84**					
	Length	0.62**	0.24				
	Width	0.82**	0.90**	0.29*			
	No. of Seeds	0.11	0.19	-0.06	0.15		
	Total Seed Wt.	0.27*	0.41**	-0.04	0.29*	0.89**	
	Av. Wt. per Seed	0.37**	0.54**	0.04	0.36**	-0.06	0.39**
G	Circumference	0.95**					
	Length	0.69**	0.64**				
	Width	0.91**	0.94**	0.64**			
	No. of Seeds	0.37**	0.29	0.19	0.27		
	Total Seed Wt.	0.68**	0.55**	0.50**	0.55**	0.76**	
	Av. Wt. per Seed	0.66**	0.63**	0.59**	0.63**	0.08	0.61**

* significant at 0.05
** significant at < 0.01

showed that fruit circumference had the greatest significance for the prediction of the average seed weight. For C. andreana the fruit length was more significant than the circumference in predicting seed weight.

Table 7:6 shows the seed and embryo size ranges within the two seed stocks while Table 7:7 shows the values obtained for seeds produced from the stock seeds. The coefficients of variation indicated that for both species there was a higher variation in the weight of the seeds than in either length or width. The variation was essentially similar whether the measurements were taken on the seed or on the embryos. The correlation between seed and embryo weight was significant at P=0.0001 (r=0.96) and regression analysis showed that more than 80% of the variation in embryo weight could be explained by the variation in the seed weight. The correlations for seed and embryo lengths and widths were slightly lower but still highly significant. Seed weight was correlated with seed length at the 1% significance level but the correlation between the weight and width of the seed was significant only at the 5% level. Seed length and seed width were also correlated at the 5% significance level. These correlations were similar for purchased seeds and for those grown for this study. Seed coats represented a higher percent of the seed weight in C. andreana than in C. maxima . Correlation coefficients indicated that as the C. andreana seed weight increased, the weight of the seed coat increased. The opposite was true for C. maxima where the heavier seeds had less of their weight as seed coat than lighter seeds.

Table 7:8 shows the seed size variation within and between the two species. Both species had about the same level of variation with at least a 2.5-fold difference between the largest and smallest seeds and embryos.

TABLE 7:6

Variation in C. maxima and C. andreana Stock Seed and Embryo Weights, Lengths and Widths.

Species	Means ± SD and Ranges (in Parentheses) Weight (g)	Length (mm)	Width (mm)
C. maxima Seeds * N = 100	0.27 ± 0.06 c.v. 22.2 % (0.17 - 0.43)	19.9 ± 1.4 c.v. 7.0% (17.2 - 22.6)	11.1 ± 1.1 c.v. 9.9% (9.0 - 13.5)
C. maxima Embryos N = 100	0.21 ± 0.05 c.v. 23.8% (0.14 - 0.35)	17.9 ± 1.4 c.v. 7.8% (15.0 - 20.5)	9.5 ± 1.0 c.v. 10.5% (7.5 - 11.5)
C. andreana Seeds N = 100	0.019 ± 0.004 c.v. 20.6% (0.010 - 0.024)	7.0 ± 0.5 c.v. 7.1% (5.3 - 8.0)	4.1 ± 0.3 c.v. 7.3% (3.2 - 4.5)
C. andreana Embryos N = 100	0.012 ± 0.003 c.v. 26.4% (0.007 - 0.017)	6.4 ± 0.4 c.v. 6.3% (5.0 - 7.5)	3.8 ± 0.3 c.v. 7.9% (2.9 - 4.3)

* seed = embryo + seed coats (outer and inner)

% of seed wt. in seed coats: C. maxima = 22.9 ± 4.8% (outer), 1.6 ± 0.3% (inner)
C. andreana = 32.3 ± 3.3% (outer), 3.7 ± 0.8% (inner)

TABLE 7:7

Variation in Weights of Seeds and Embryos and in Lengths and Widths of Embryos of C. maxima and C. andreana Fruits Grown in Field Plots, Greenhouse and Growth Chambers.

Means ± SD and Ranges (in Brackets)

Species	Seed Wt. (g)	Embryo Wt. (g)	Embryo L. (mm)	Embryo W. (mm)	% Seed Coats
C. maxima	0.25 ± 0.05	0.19 ± 0.04	16.3 ± 1.6	8.4 ± 0.9	24.0%
N* = 82	c.v. = 20.0% (0.13 - 0.37)	c.v. = 21.1% (0.11 - 0.28)	c.v. = 9.8% (10.5 - 20.0)	c.v. = 10.7% (5.0 - 11.5)	± 3.8
C. andreana	0.019 ± 0.005	0.012 ± 0.0003	5.6 ± 0.5	3.4 ± 0.4	33.9%
N = 35	c.v. = 26.3% (0.008 - 0.027)	c.v. = 25.0% (0.007 - 0.019)	c.v. = 8.9% (4.5 - 7.2)	c.v. = 11.8% (2.0 - 4.5)	± 9.3

*N = number of fruits (60 or more seeds measured per fruit).

The seeds that were produced for this study tended to have a broader size range probably because of their varied growth locations. These ratios confirm that weight varied more than length and width. The large C. maxima seeds were 13-14 times heavier than the C. andreana seeds but their embryo weight differences were even larger because of the difference in the respective proportions of the weights in the seed coats.

The larger crop of C. maxima from field growth enabled a comparison to be made between fruits grown in the same plot in two consecutive years. There was no significant difference between the fruit weights but the seed weight difference was significant by a t-test at P=0.01 (Table 7:9A). Using both crops, a comparison was made between consecutive fruits on the same vine (Table 7:9B). A t-test was applied only to values of the first and second fruits on the vine because of the small sample size for the rest of the fruits. First fruits tended to be significantly larger (P=0.02) but produced seeds of virtually the same size.

When individual fruits of both species were opened some showed evident differences in seed size while others had a homogeneous complement of seeds. Table 7:9C shows the ratio of largest and smallest seeds from three fruits based on the measurement of 50 randomly selected seeds per fruit. The ratios for the weights were lower than the 2.5-fold differences obtained for the whole population of fruits and for the stock seeds. Smallest and largest seeds from 18 fruits were also deliberately selected for measurement. Again the ratio of the largest to smallest seeds within single fruits was 1.2 for both length and weight, hence of lesser magnitude than the overall variation.

TABLE 7:8

Comparison of Seed and Embryo Sizes of *C. maxima* and *C. andreana* Stock Seeds and Seeds Produced at the Three Growth Locations.

I. Range of Variation Within Each Species:

Ratio	*C. maxima*		*C. andreana*	
	Stock	Grown	Stock	Grown
Heaviest Seed / Lightest Seed	2.5	2.8	2.5	3.4
Heaviest Embryo / Lightest Embryo	2.5	2.5	2.6	2.7
Longest Embryo / Shortest Embryo	1.4	1.9	1.5	1.6
Widest Embryo / Narrowest Embryo	1.5	2.3	1.5	2.3

II. Comparison Between *C. maxima* and *C. andreana*:

Parameter Compared	Ratio: *C. maxima*/*C. andreana*	
	Stock	Grown
Seed Weight	14.3	13.2
Embryo Weight	17.4	15.8
Embryo Length	2.8	2.9
Embryo Width	2.9	2.5

TABLE 7:9

Some Comparisons Within the Field Grown *C. maxima* Fruits and Seeds.

A. Year to Year Differences:

Year	N	Fruit Wt. (kg)	Av. Seed Wt. (g)
1983	32	6.4 ± 2.0	0.28 ± 0.04[a]
1984	21	6.9 ± 1.6	0.25 ± 0.03[b]

B. Differences Between Position on the Vine:

Order of Fruits	N	Fruit Wt. (kg)	Av. Seed Wt. (g)
First	19	7.5 ± 1.9[a]	0.28 ± 0.05
Second	16	6.1 ± 1.4[b]	0.26 ± 0.03
Third	8	6.3 ± 2.1	0.27 ± 0.02
Fourth	5	6.2 ± 1.3	0.28 ± 0.04
Fifth	3	4.4 ± 1.1	0.22 ± 0.01
Sixth	1	9.4	0.29

C. Variation in Seeds from Single Fruits:

Ratio: Largest/Smallest

	Length	Width	Weight
Fruit # 1	1.2	1.5	1.5
Fruit # 2	1.3	1.3	1.6
Fruit # 3	1.3	1.5	1.9

a,b – values significantly different at P = 0.01

DISCUSSION

Male and female flowers are often not present at the same time on the same plant and this is a feature that can be a problem in self-pollination. Both hand-pollination and self-pollination can lead to poor fruit set and lower seed production in cucurbits (Bushnell, 1920; Mann and Robinson, 1950). These authors found that a lower percent of hand-pollinated flowers set fruit but that even with adequate bee-pollination a high proportion of pollinated flowers aborted. The cucurbit fruits have between 650 and 750 ovules based on seed counts and counts of aborted, but recognizable ovules. Snow (1982) stated that a 1:1 ration of pollen to ovule is rarely achieved. Cruden and Miller-Ward (1981) found that species with large pollen grains needed a lower pollen/ovule ratio than those with small pollen grains. Cucurbita species have very large pollen grains of about 250 µm in diameter (Echlin, 1968) but because of the large number of ovules the total number of grains needed is large. Uneven pollen deposition on the three stigmas causes mis-shapen fruits (Mann, 1943). The fruits in this study were all of regular shape and had an average seed number of 352. This was lower than the average of 478 for nine fruits that had not been selfed and had been pollinated by bees but even with bee pollination 30% of the ovules did not develop into seeds. The seed size was the same for both groups. The difference in seed number may be due to some deficiency of hand-pollination (Ba-Amer and Bemis, 1968) or to the compatibility index being 74% (Gornall and Bohm, 1984). However, Heslop-Harrison (1975) in his review of incompatibility systems did not mention any problems with cucurbits and Bushnell (1920) found no hereditary sterility in Cucurbita .

Gorchov (1985) reported that fleshy fruits of several woody plants had lower seed numbers if they were derived from self-pollination. Pet and Garretsen (1983) working with tomatoes found that seed weight was 14% higher after hand-pollination than after natural pollination. Thus at present the mechanisms that control seed number in the cucurbits remain undefined.

The ovaries are larger in C. maxima than in C. andreana and this initial difference contributes to the difference in their fruit size (Sinnott, 1939). The large C. maxima fruits are generally hollow because of the collapse of the central parenchyma tissue (Sinnott, 1939). This may account for the importance of fruit circumference in predicting seed size within this species. In C. andreana fruits the seeds remain embedded in parenchymatous tissue even at maturity and fruit length seems to be the more important parameter in predicting seed size. Because of the regulation of fruit growth by growth regulators from growing seeds (Coombe, 1976) it is expected that large number or large size of seeds would result in a larger fruit. In practice this correlation is not always present (Pet and Garretsen, 1983). Ba-Amer and Bemis (1968) did find a very close correlation between fruit weight and seed number for Cucurbita foetidissima . In the present study the fruit weight was significantly correlated with both seed weight and seed number but even more significantly with the total seed weight.

This study showed that individual cucurbit seed weights were less variable than seed number and this has been found for other species also (Cook, 1975; Mazer et al , 1986; Okusanya et al , 1981). A negative correlation between these two factors is expected as a result of a

predicted compromise in resource allocation into either more seeds or larger seeds (Harper et al ,1970). The cucurbit results varied from a small positive correlation to no correlation at all. Studies on other species have also produced variable results. Galen and Weger (1986) working with Clintonia found a negative correlation between seed weight and seed number with natural pollination but when hand-pollination was used the correlation became positive. Only some wild radish populations showed a negative correlation between seed size and seed number (Mazer et al ,1986; Stanton, 1984). Schreerens et al (1978) found no correlation between seed weight and seed number in Cucurbita foetidissima .

Ba-Amer and Bemis (1968) determined that seed weight of C. foetidissima continued to increase up to 32 days after pollination but no seed weight increase occurred during subsequent storage of the fruits. The cucurbits studied here were all older than 32 days and immaturity did not seem to be a factor in seed size variation. The marked difference in the seed size of the two species must be due to the differences in their embryo cell number and the resultant higher growth rate of the larger C. maxima embryo (Davies, 1977; Egli et al ,1981; Guldan and Brun, 1985). Larger seeds have been reported to have a higher (Fenner, 1983) and a lower (Salisbury, 1974) percent of their total weight as seed coat. The Cucurbita maxima seed was found to have a lower percent of its weight as seed coat than the C. andreana seed.

A 1.3-fold variation in seed size has been reported for both C. maxima and C. andreana (Singh and Dathan, 1972). This is lower than found for these cucurbits in this study. However, Wilson and Splittstoesser (1979) found a 1.9-fold difference in Cucurbita moschata seeds and

Scheerens et al (1978) found a 5-fold range in seed size in C. foetidissima . Three- to four-fold variations in seed size have been reported for soybeans (Egli et al ,1981; Guldan and Brun, 1985). In other legumes two-fold variation in seed size was found within a plant and a 4-fold for the whole population (Wulff, 1986). In cucurbits the within fruit seed variation was also smaller than the variation for the whole population of fruits.

As C. andreana is not a commonly studied species the characteristics of the fruits and seeds of this species have not been well documented. The results in this chapter have defined the extent of the variation in fruit and seed size within both C. andreana and C. maxima . In order to relate the variation in Ca level to seed size it was necessary to establish that seed size was a reasonably stable parameter. This was particularly important because the two species had a different preferred growth location. The results indicate that while fruit size varied considerably between locations, the seed size remained fairly constant. It was also shown that despite some variation in seed size within each species there was no overlap in seed size between the two species. The similarity in seed size parameters between stock seeds and those grown for the study confirms that growth conditions were adequate for both species at all three growth locations.

Chapter 8

VARIATION IN CALCIUM LEVEL IN *C. MAXIMA* AND *C. ANDREANA* EMBRYOS IN RELATION TO EMBRYO SIZE.

INTRODUCTION

Assimilated carbon and nitrogen account for the bulk of the structural and storage materials of growing embryos (Dure, 1975). Some of the carbon, transported mainly as stachyose in cucurbits (Richardson *et al*, 1984), forms the oil reserves which make up from 30 to 50% of the embryo weight (Jacks *et al*, 1972; Tsuyuki *et al*, 1985). Carbon together with nitrogen forms the protein reserves which account for 23 to 32% of the embryo weight (O'Kennedy *et al*, 1979; Davis, 1974). Some of the carbon is also incorporated into cell wall material, cytoplasmic components, and into the mineral reserves as phytin. The mineral component, as indicated by the ash content of the cucurbit embryos (Chapter 2), makes up less than 5% of the embryo weight. The deposition of the oil and protein reserves is similar in pattern and timing and is equivalent to the increase in embryo dry weight (Greenwood *et al*, 1984; Hocking and Pate, 1977). Greenwood *et al* (1984) found that the deposition of phytin in castor bean was somewhat slower than that of oil and protein. Hocking and Pate (1977) showed that in legumes, individual elements may accumulate faster or slower than the dry matter. Bemis *et al* (1977) studying xerophytic *Cucurbita* species found that oil and protein reached optimum levels at the same time but that the time needed to reach this

level varied with the species. No mention was made of the mineral reserves in these cucurbits.

The accumulation of mineral reserves depends first on their acquisition by the plant and subsequently on their transport to the fruits and seeds. Calcium is abundant in most soils but the amounts vary from 0.1% in acid soils to nearly 20% in calcareous soils (Hesse, 1971). Cucurbits have been called calcicole plants because of their tolerance of high soil Ca (Ingestad, 1972). The uptake of Ca from soil solution is believed to be passive and the Ca content of the root apoplast solution is in equilibrium with the external solution which is usually in the range of 1-5 mM (Clarkson and Hanson, 1980). While Ca can enter the symplast, it is actively pumped out to maintain very low cytoplasmic concentrations in the range of 10^{-8} to 10^{-6}M (Marme, 1983; Hanson, 1984). Uptake of Ca in Cucurbita pepo was restricted to the younger portions of the roots and translocation of Ca into the stele was stopped completely by the suberized endodermis (Harrison-Murray and Clarkson, 1973). Bengtsson (1982a,b) and Bengtsson and Jensen (1982, 1983) found that cucumber (Cucumis sativus) roots regulated Ca uptake independently of the transpiration stream and that most of the Ca taken up was transported to the shoot leaving only 1-4% of the total Ca in the root. The xylem acts as an ion exchange column with Ca filling the available cation exchange sites (Janova and Dvorak, 1976; Marschner, 1983; Van de Geijn and Petit, 1979); however, the transpiration rate can also affect the movement of Ca (Mengel and Kirkby, 1982). Armstrong and Kirkby (1979) found that Ca uptake into tomato roots was not affected by high humidity which decreased the transpiration rate but that the translocation of Ca to the shoots was decreased.

The vasculature of cucurbit fruits, described for C. pepo (Sinnott, 1939), consists of twenty main vascular strands running the length of the fruit and some secondary bundles which may run perpendicular to the main strands. Each seed receives nourishment via a vascular bundle in the outer seed coat (Lott, 1973). However, vascular connections usually end in the seed coat and the embryos themselves have no direct vascular link with the parent plant (Thorne, 1980, 1985). In legumes it has been found that the pod walls can act as temporary sinks for the incoming nutrients which are subsequently redistributed to the embryos (Hocking and Pate, 1977; Murray, 1980). Redistribution of minerals within fleshy fruits such as cucurbits is still poorly defined.

Calcium has been referred to as phloem-immobile but the exact reason for its immobility is being debated (Hanson, 1984; Mengel and Kirkby, 1982; Raven, 1977). Low mobility of Ca in the phloem is reflected in the low amounts of Ca in fruits and seeds (Marschner, 1983). Some authors have noted a reciprocal relationship between seed size and Ca content. Fenner (1983) found that in Compositae seeds the percent ash was lowest in the largest embryos. Taira et al (1977) reported that ash content and Ca content were higher in the smaller soybean embryos. In addition, Lott and Vollmer (1979) found that Ca was less frequently present in globoid crystals in C. maxima embryos than in corresponding regions of the smaller C. andreana embryos. In this chapter the relationship of total Ca and embryo size within C. maxima and C. andreana is explored and related to various fruit size parameters.

MATERIALS AND METHODS

Preparation of Seeds and Ca Analysis :

All procedures mentioned in this section have been described in Chapter 2. The embryos used were obtained from stock seeds and fruits grown for this study (Chapter 7). Dry weights were determined on the ground mixtures but Ca levels are reported for air-dried weights. Nine ground embryo samples of 0.25 g each were ashed for *C. maxima* fruits and five samples of 0.15 g were ashed for the *C. andreana* fruits. Hydrochloric acid extracts of the ash were analyzed for Ca by AAS.

Statistical Analysis :

Initial selection of the number of seeds to be pooled per fruit and the number of subsamples to be analyzed for Ca from the pooled mixture was determined by an experiment suggested by Dr. P. Macdonald, Bio-statistician, McMaster University. Two lots of fifty seeds were randomly selected from three *C. maxima* fruits. The embryos of one set of fifty seeds for each fruit were analyzed as individual seeds while the embryos of the other 50 seeds were pooled and ground and then five subsamples were analyzed. Fifty seeds was accepted as a practical number to process for each fruit. The number of samples that needed to be analyzed from the pooled mixture of the 50 embryos to give results with a 95% confidence interval of $\pm$ 15 µg Ca were then calculated as described in Appendix B. The required sample number for the *C. andreana* embryos was calculated from the pooled and individual embryo Ca levels obtained on stock seeds. The measured Ca levels for embryos of both cucurbit species were compared and correlated with seed size and various growth factors using the MTB.

TABLE 8:1

Determination of the Number of Samples of Ground Embryo Tissue to be Analyzed to Obtain a Representative Ca Level for Each Fruit.

A. Analysis of Embryos from 3 *C. maxima* Fruits:

	Fruit # 1	Fruit # 2	Fruit # 3
Fruit Wt. (kg)	8.5	6.8	1.3
No. of Seeds per Fruit	582	342	109
Ave. Wt. per Seed (g)	0.32	0.31	0.21
µg Ca^{2+}/g Embryo Based on 50 Pooled Embryos and 5 Subsamples Analyzed (Mean ± SD)	141.0 ± 11.1 c.v. 7.9%	229.5 ± 10.4 c.v. 4.5%	298.6 ± 16.0 c.v. 5.4%
µg Ca^{2+}/g Embryo Based on 50 Embryos Analyzed Individually	127.0 ± 19.7 c.v. 15.5%	237.9 ± 90.8 c.v. 38.2%	333.5 ± 105.4 c.v. 31.2%
No. of Subsamples Needed to Obtain Results with a 95% Confidence Interval of ± 15µg Ca	2	5	9

B. Analysis of Individually Analyzed and Pooled *C. andreana* Embryos:

µg Ca^{2+}/g Embryo Based on 50 Embryos Analyzed Individually	684.3 ± 98.2 c.v. 14.4%
µg Ca^{2+}/g Embryo Based on 50 Pooled Embryos and 5 Subsamples Analyzed	698.7 ± 13.7 c.v. 2.0%
No. of Subsamples Needed	7 (based on 50 pooled) 5 (based on 100 pooled)

RESULTS

It was determined in Chapter 2 that pooling 25 stock seeds eliminated the seed-to-seed variation in both cucurbit species. Chapter 7 showed that there was considerable variation between and within the two cucurbit species in fruit and seed parameters. It was essential to choose an adequate sample size to produce representative Ca levels for embryos from all fruits in both species. The results in Table 8:1A illustrate the range of variation present among the embryos of three C. maxima fruits that were analyzed to calculate the required sample size. The large coefficients of variation from individual embryo analyses were due to seed-to-seed variation. Smaller coefficients of variation for pooled samples reflected the variation that resulted from the analytical procedure. The difference indicated that pooling eliminated the seed-to-seed variation. Based on the calculations described in Appendix B it was found that the three fruits were different in their sample number requirements. To ensure that most of the variability was eliminated the larger sample number of 9 was selected for all analyses of C. maxima . Table 8:1B shows that for C. andreana , 50 pooled embryos require the analysis of only 7 subsamples. With these smaller embryos there was an additional problem that 50 embryos gave too small a weight of pooled sample. This need to pool more embryos enabled a smaller number of subsamples to be analyzed. Pooling 100 C. andreana embryos required analysis of 5 samples of the pooled mixture to give results that were not only representative of the Ca level of embryos from each of the fruits but were also comparable in accuracy to those obtained for C. maxima .

TABLE 8:2

Calcium Concentrations of *C. maxima* and *C. andreana* Embryos Derived from Seed Stocks and Analyzed by AAS.

Species		Ca^{2+} per Embryo (Mean ± SD)	Ca^{2+} per g Embryo (Mean ± SD)
C. maxima N = 68 *	µg Ca^{2+}	42.4 ± 13.8	228.3 ± 81.6
	c.v.	32.5%	35.7%
	Range (µg)	16.8 - 87.2	91.5 - 408.9
	Ratio: Highest/Lowest	5.2	4.5
C. andreana N = 102*	µg Ca^{2+}	9.3 ± 1.7	682.2 ± 96.8
	c.v.	18.3%	14.2%
	Range (µg)	5.6 - 13.5	446.4 - 941.1
	Ratio: Highest/Lowest	2.4	2.1
Ratio of Ca in *C. andreana* to Ca in *C. maxima*		0.2	3.0

* individual embryos analyzed.

TABLE 8:3

Calcium Concentrations of *C. maxima* and *C. andreana* Embryos Derived from Seeds of Fruits Grown in the Field, the Greenhouse and the Growth Chambers and Analyzed by AAS.

Species		Ca^{2+} per Embryo (Mean ± SD)	Ca^{2+} per g Embryo (Mean ± SD)
C. maxima N = 82 *	µg Ca^{2+}	43.3 ± 13.8	232.5 ± 57.5
	c.v.	31.6%	24.7%
	Range (µg)	17.5 - 82.8	137.3 - 441.3
	Ratio: Highest/Lowest	4.7	3.2
C. andreana N = 35*	µg Ca^{2+}	8.1 ± 2.3	651.8 ± 87.9
	c.v.	28.4%	13.4%
	Range (µg)	3.7 - 12.5	481.8 - 852.9
	Ratio: Highest/Lowest	3.4	1.8
Ratio of Ca in *C. andreana* to Ca in *C. maxima*		0.2	2.8

* for *C. maxima* - 82 fruits, 60 embryos/fruit pooled and 9 subsamples of pooled mixture ashed and analyzed/fruit

**for *C. andreana* - 35 fruits, about 100 embryos/fruit pooled and 5 subsamples analyzed/fruit

Table 8:2 shows the Ca levels in the two seed stocks. The Ca levels were calculated both on whole embryo basis and on a per gram embryo basis. As shown in Chapter 7, the embryos had about a 17-fold difference in weight and the smaller C. andreana embryos contained less Ca (Table 8:2A). However, when compared on a per gram basis the smaller embryos had three times more Ca than C. maxima embryos (Table 8:2B). Since the Ca levels were obtained by measuring single embryos, the coefficients of variation were high reflecting the embryo-to-embryo differences in Ca level. The higher coefficients of variation for the C. maxima values indicated a larger overall variation in Ca for this species as compared to C. andreana . Within each species the embryo weight varied 2.5-fold (Chapter 7). The Ca level within C. andreana embryos also varied about 2.5-fold while C. maxima embryos had a 5-fold variation. Table 8:3 provides similar information for embryos from fruits grown from the stock seeds. This time the fruit-to-fruit variation in Ca level rather than embryo-to-embryo variation is reflected in the high coefficients of variation. The Ca levels and ranges in the two tables were quite comparable.

It was shown in Chapter 7 that the growth location had a marked influence on fruit size but only a small influence on the seed size. Table 8:4 shows that the effect of growth location on the Ca level was also small. The field grown C. maxima fruit were 4.5 times heavier than the growth chamber fruits but the embryos derived from the field grown fruit were only 10% heavier than those from the growth chamber fruit. However the larger field grown embryos contained 10% less Ca than the smaller growth chamber embryos. While such differences in minerals can be due to

TABLE 8:4

Variation in Ca Levels with Growth Location in *C. maxima* and *C. andreana* Embryos.

Species	Location	N	Ca Concentration and Range µg/Embryo Mean ± SD	µg/g Embryo Mean ± SD	Embryo Wt. (g)
C. maxima	Field	55	45.5 ± 13.1 25.3 – 82.8 c.v. 28.9%	225.7 ± 54.9 137.3 – 441.3 c.v. 24.3%	0.20 ± 0.03 0.13 – 0.28 c.v. 15.0%
	Green-house	20	36.0 ± 11.4 17.5 – 60.4 c.v. 31.7%	243.4 ± 64.6 160.4 – 389.3 c.v. 26.5%	0.15 ± 0.03 0.11 – 0.22 c.v. 27.3%
	Growth Chamber	7	47.5 ± 18.5 27.0 – 76.7 c.v. 38.9%	255.5 ± 52.9 201.3 – 332.8 c.v. 20.7%	0.18 ± 0.05 0.11 – 0.24 c.v. 27.8%
C. andreana	Field	1	10.7	679.7	0.016
	Green-house	16	7.4 ± 1.6 4.7 – 9.6 c.v. 21.6%	619.3 ± 85.8 481.8 – 752.4 c.v. 13.9%	0.012 ± 0.002 0.007 – 0.017 c.v. 16.7%
	Growth Chamber	18	8.6 ± 2.6 3.7 – 12.5 c.v. 30.2%	679.1 ± 84.4 543.7 – 852.9 c.v. 12.4%	0.013 ± 0.003 0.007 – 0.019 c.v. 23.1%

TABLE 8:5

Relationship Between Ca Level and Embryo Weight in Stock *C. maxima* and *C. andreana* Embryos.

Species	Parameter Measured	Embryo Wt. and Ca Level (Mean ± SD) Randomly Selected (R)	Large	% Diff. from R	Small	% Diff. from R
C. maxima	No. of Embryos	68	29	—	32	—
	Embryo Wt. (g)	0.20 ± 0.05	0.25 ± 0.03	+25%	0.16 ± 0.02	-20%
	µg Ca^{2+}/Embryo	42.4 ± 13.8	46.5 ± 14.2	+10%	39.9 ± 11.3	- 6%
	µg Ca^{2+}/g Embryo	228.3 ± 81.6	192.1 ± 52.0	-16%	267.4 ± 81.2	+17%
C. andreana	No. of Embryos	100	27	—	34	—
	Embryo Wt. (g)	0.012 ± 0.003	0.015 ± 0.001	+25%	0.011 ± 0.001	- 8%
	µg Ca^{2+}/Embryo	9.2 ± 2.2	11.1 ± 1.7	+21%	7.3 ± 1.2	-21%
	µg Ca^{2+}/g Embryo	688.0 ± 114.0	734.9 ± 101.0	+ 7%	647.5 ± 86.6	- 6%

differences in growth conditions between the two locations, subsequent tables will show that there is a reciprocal relationship between Ca level and seed size for the larger-seeded species. The lack of field grown C. andreana fruits precluded equivalent comparisons for that species.

Table 8:5 shows the relationship between embryo size and Ca level for both species based on the analysis of individual stock seeds. In both species the larger embryos had more Ca per embryo but the results calculated per gram embryo were different for the two species. For C. maxima the embryo weight varied 20-25% from the mean weight and the Ca levels varied 16-17% with the larger embryos having a lower Ca concentration per gram embryo tissue. For C. andreana embryos the Ca level per gram embryo increased as the embryo size increased. The negative correlation between C. maxima embryo size and Ca level was confirmed in several batches of stock embryos. Figure 8:1 shows that if both weight and length were taken into account, the shorter seeds in each weight category had the higher Ca level. When correlation coefficients (r) were calculated for comparison of the per gram Ca level in C. maxima embryos with the embryo weight, length and width, all 3 size parameters were negatively correlated with the Ca level (Figure 8:2). Length was more significantly correlated than weight but, based on regression analysis, length accounted for only 24% of the variation in the embryo Ca level. When the regression included both length and weight the combined predictive value was only 28% indicating a minimal interaction between the factors. Comparable correlations were made for the per gram Ca level in C. andreana embryos and the embryo size parameters but are not shown since none of the correlations were significant.

Figure 8 : 1

C. maxima Ca Levels in Individually Ashed Embryos Compared to Embryo Weight and Length.

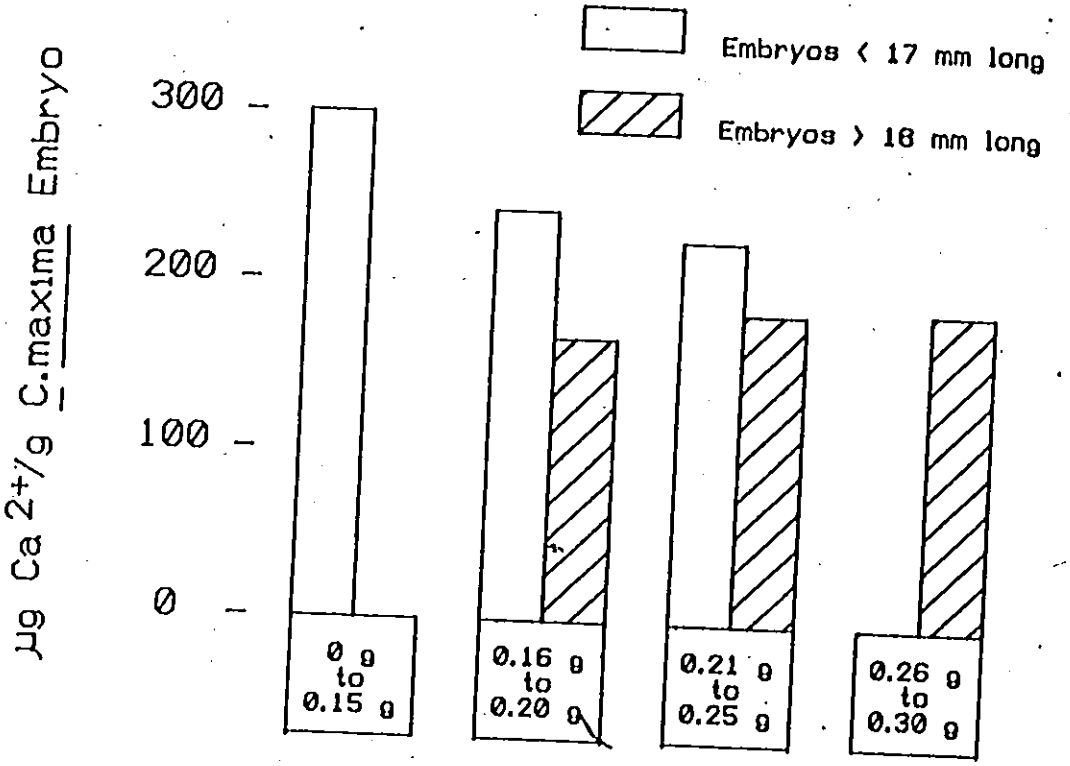
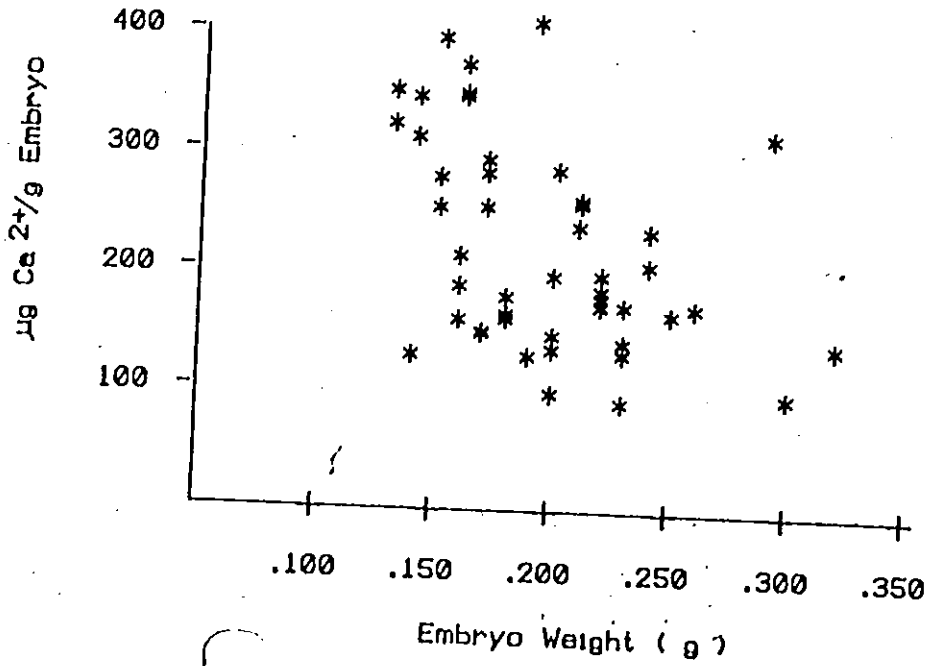


Figure 8 : 2

Variation in Ca Level With Embryo Size in *C. maxima*.



Weight vs Ca^{2+}/g Embryo, r = -0.446 (P<0.01 %)
Length vs Ca^{2+}/g Embryo, r = -0.581 (P<0.01 %)
Width vs Ca^{2+}/g Embryo, r = -0.361 (P<0.05 %)

Tables 8:6 and 8:7 present the correlation coefficients between the fruit, seed and embryo parameters and the embryo Ca levels for the embryos derived from fruits grown for this study. Most correlations for both species were positive and significant on the per embryo basis and generally not significant when correlated with the per gram embryo Ca level. Field grown C. maxima showed less significance between the size parameters and the Ca level than did indoor grown fruits. Seed number was least significantly correlated with the per embryo Ca level in both species (Table 8:6A). The Ca level per gram embryo tissue was negatively correlated with seed number in C. maxima and positively in C. andreana. While in Table 8:7 all the correlations between the per gram Ca level and embryo size measurements were negative, none of them were significant. Thus the embryos from the fruits differed from the stock embryos. The correlations indicated that the embryos with the higher Ca levels were larger in all dimensions and came from larger fruits but this correlation disappeared when the Ca levels used were calculated on a per gram embryo basis. The per gram embryo Ca level in C. maxima embryos was negatively correlated with the total seed weight suggesting that fruits with a large seed mass tended to have embryos with a lower level of Ca per gram embryo tissue.

In Chapter 7 it was shown that there was a small but significant difference in seed size among seeds produced over two consecutive years in the same field plot. Table 8:8 compares the Ca levels in these seeds. In 1983 one of the plants produced a fruit with embryos containing 441.3 μg Ca^{2+}/g embryo. This Ca level was about 100 ug higher than the level in any of the other fruits. Omitting this fruit made the Ca ranges for the

TABLE 8:6

Correlation Between Ca Levels
and Fruit and Seed Size Parameters.

A. Correlation Coefficients (r) for C. maxima and C. andreana:

Fruit and Seed Parameters	C. maxima (µg Ca^{2+}) N = 82 /Embryo	/g Embryo	C. andreana (µg Ca^{2+}) N = 35 /Embryo	/g Embryo
Fruit Weight	0.32**	-0.15	0.66**	0.26
Fruit Length	0.32**	-0.09	0.66**	0.25
Fruit Width	0.36**	-0.10	0.67**	0.10
F. Circumference	0.35**	-0.15	0.71**	0.14
Seed Number	-0.02	-0.31**	0.21	0.37*
Total Seed Wt.	0.13	-0.33**	0.50**	0.23
Av. Wt. per Seed	0.55**	-0.10	0.63**	-0.16

B. Correlation Coefficients (r) for Field and Indoor C. maxima:

Fruit and Seed Parameters	Field C. maxima (µg Ca^{2+}) N = 55 /Embryo	/g Embryo	Indoor C. maxima (µg Ca^{2+}) N = 27 /Embryo	/g Embryo
Fruit Weight	0.25	0.01	0.66**	0.20
Fruit Length	0.20	0.16	0.37**	-0.07
Fruit Width	0.27*	0.04	0.59**	0.18
F. Circumference	0.29*	-0.03	0.73**	0.27
Seed Number	-0.33*	-0.29*	-0.21	-0.06
Total Seed Wt.	-0.13	-0.32*	0.39*	-0.19
Av. Wt. per Seed	0.43**	-0.07	0.67**	-0.09

* significant at P = 0.05
** significant at P < 0.01

TABLE 8:7

Correlation Between Ca Level and Embryo Size Parameters.

Species	Embryo Measurement	For µg Ca^{2+} per Embryo		For µg Ca^{2+} per g Embryo	
		r	r^2	r	r^2
Field *C. maxima* N = 55	Length	0.15	0.4%	−0.21	3.0%
	Width	0.36**	11.2%	−0.08	—
	Weight	0.53**	26.9%	−0.08	—
Indoor *C. maxima* N = 27	Length	0.40**	12.7%	−0.19	—
	Width	0.44*	16.0%	−0.10	—
	Weight	0.60**	34.0%	−0.04	—
All *C. maxima* N = 82	Length	0.35**	11.0%	−0.12	—
	Width	0.46**	19.9%	−0.06	—
	Weight	0.62**	37.7%	−0.03	—
All *C. andreana* N = 35	Length	0.70**	46.2%	−0.03	—
	Width	0.64**	38.9%	−0.12	—
	Weight	0.88**	76.3%	−0.03	—

* significant at P = 0.05
** significant at P = 0.01 or less

r_2 = correlation coefficient
r^2 = coefficient of determination
– this coefficient was derived by regressing Ca level on size parameters and was then adjusted for the degrees of freedom

two years more comparable but also made the means significantly different at P=0.05. Considering that these fruits were grown outdoors under variable weather and moisture conditions the differences in Ca levels for the two crops were quite small.

The 1983 and 1984 crops both had plants with multiple fruits so that it was possible to determine whether there was more variation in Ca level plant-to-plant or between fruits on the same plant. The results were analyzed using analysis of variance suited to the unequal number of fruits per plant. The analysis was carried out on the combined crops of the two years but it became obvious that the plant with the 441.3 μg Ca^{2+}/g embryo was an outlier. When the outlier was included, there was very significantly more variation plant-to-plant than fruit-to-fruit with F=4.79 (48 df). Omitting the outlier the difference between plants was still greater but at a somewhat lower significance level of P=0.01 (F=3.01).

The large outdoor crop of C. maxima permitted additional comparisons in Ca level. Table 8:9 shows the measurements and Ca levels of first and second fruits on the plants. The largest difference was in fruit weight and this difference was found significant by a t-test. The second fruits had a lower total seed weight but not at a significant level. While the seed number was very variable within both first and second fruits, the means for each group were almost identical. The smaller seeds of the second fruits had embryos containing slightly more Ca than the embryos from the larger seeds of the first fruits but the difference was not significant. Since only a few plants had more than two fruits the number of third and fourth fruits was too small for significant comparison.

TABLE 8:8

Calcium Concentrations in Embryos of Fruits Grown in the Same Field Plots During Two Consecutive Years.

C. maxima		1983	1984
No. of Fruit Produced		32	21
Av. Seed Wt. (g)		0.28 ± 0.04^{b}	0.25 ± 0.03^{a}
Range in Ca Level per Year $\mu g\ Ca^{2+}/g$ Embryo	A	137.3 – 441.3	
	B	137.3 – 325.4	154.9 – 310.4
Degree of Variation	A	3.2 – fold	
	B	2.3 – fold	2.0 – fold
Mean Ca Level per Yr. $\mu g\ Ca^{2+}/g$ Embryo (Mean ± SD)	A	219.6 ± 62.6^{a} c.v. 28.5%	237.6 ± 40.9^{a} c.v. 17.2%
	B	212.4 ± 48.5^{c} c.v. 22.8%	

A based on 32 fruits
B based on 31 fruits (see text)
a,a – not significantly different
b,a – significantly different at P = 0.01
c,a – significantly different at P = 0.05

However, there appeared to be little difference in their seed size and Ca level from that of the first and second fruits. Thus position or order of the fruit on the plant did not markedly affect the Ca level of its embryos. The same analysis was not possible for the C. andreana plants because both hybrids and self-pollinated fruit were often present on the same vine and Ca levels in the parents and hybrids were not identical (Chapter 9).

The seed-to-seed variation within single fruits was also compared to the variation in Ca level between various fruits (Table 8:10). The fruits #1 to 3 had different degrees of seed-to-seed variation. Fruit #3, with a 5-fold variation, surpassed the variation fruit-to-fruit. With C. andreana the seed-to-seed variation in Ca content of embryos was very similar to the fruit-to-fruit variation and the overall degree of variation was lower than that found in C. maxima . It was also of interest to note the relationships between embryo size and Ca levels within the three fruits. Fruits #1 had no significant correlation between Ca level per gram embryo and embryo length, width or weight. Fruit #2 had a significant negative correlation with all three embryo size parameters at P=0.01 or less. Fruit #3 had significant negative correlation at P=0.01 only between the length of the embryo and the per gram Ca level. The last two fruits also had a wider range in Ca variation. Thus while the whole crop of C. maxima fruits did not produce the negative correlation between Ca level and embryo size, it appeared that such a correlation was present within individual fruits of that crop.

When seeds of small and large weight were deliberately selected from 19 individual C. maxima fruits the Ca levels of embryos of the large

TABLE 8:9

Average Ca Concentrations in Embryos from First and Second Fruits on Field Grown *C. maxima* as Related to other Fruit and Seed Parameters.

	Fruit Wt. (kg)	Total Wt. (g)	Seed Number	Av. Seed Wt. (g)	µg Ca^{2+}/g Embryo
First Fruits on Vine, N=19	7.5[a] ± 1.9	94.2[a] ±33.1	334[a] ±104	0.28[a] ±0.05	227.1[a] ±48.0
c.v.	25.3%	35.1%	31.1%	17.9%	21.1%
Second Fruits on Vine, N=16	6.1[b] ± 1.4	86.6[a] ±27.2	333[a] ±106	0.26[a] ±0.03	236.4[a] ±74.4
c.v.	23.0%	31.4%	31.8%	11.5%	31.5%
% Difference	-18.6%	- 8.1%	- 0.3%	- 7.1%	± 4.1%

[a] diff. in letters in each column denotes a difference at P = 0.05%

TABLE 8:10

Variation in Embryo Ca Level on a Seed-to-seed and Fruit-to-fruit Basis.

C. maxima	N	Range µg Ca^{2+}/g Embryo	Highest / Lowest	µg Ca^{2+}/g Embryo Mean ± SD	c.v. %
Fruit # 1[1]	50	83.5 - 182.0	2.2	127.0 ± 22.7	17.9
Fruit # 2	50	182.0 - 620.0	3.4	333.0 ± 107.0	32.1
Fruit # 3	50	114.6 - 567.4	5.0	237.9 ± 90.2[a]	37.9
Stock Seeds[2]	50	91.5 - 408.9	4.5	228.3 ± 81.6[a]	35.7
Fruits[3]	50	137.3 - 441.3	3.2	225.0 ± 55.4[a]	24.6
C. andreana					
Stock Seeds	50	446.1 - 941.1	2.1	684.3 ± 98.2[b]	14.4
Fruits	35	481.1 - 852.9	1.8	651.8 ± 87.9[b]	13.5

[1] 50 individually ashed embryos from each fruit
[2] 50 individually ashed embryos from stock seeds
[3] Ca concentrations for 50 different fruits based on pooled ground embryos

same letter names indicate means are not different at P = 0.05.

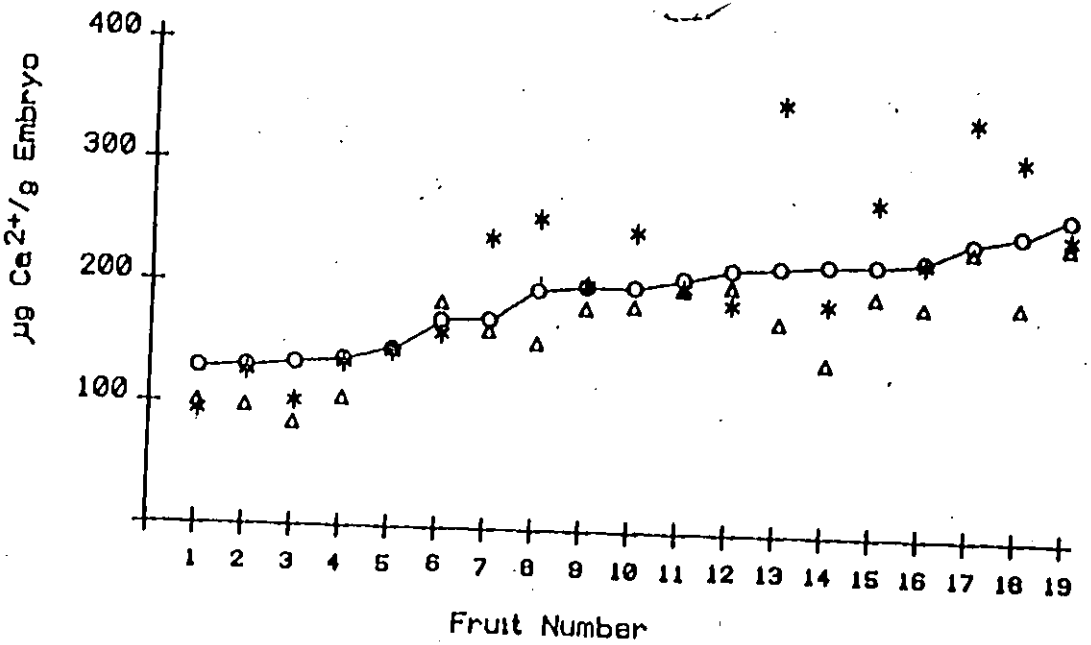
seeds tended to be lower. In Figure 8:3 the Ca levels of randomly selected embryos show about a 2-fold variation. The smallest embryos from each fruit had Ca levels higher, lower or equivalent to the levels of the randomly selected embryos. The largest embryos were more consistent in having lower Ca levels than the random values. This was reflected also in the mean Ca levels for the 19 fruits. The mean Ca levels of the smallest and randomly selected embryos were not significantly different by a t-test. The mean Ca level of the largest embryos was significantly different at $P=0.05$ from both of the other means.

DISCUSSION

While there was about a 3-fold difference in Ca level between the two cucurbit species there was also considerable variation in Ca level within each species. The larger *C. andreana* embryos had more Ca both on a per embryo and per gram embryo basis than did the smaller-sized embryos of this species. The larger the *C. maxima* embryo the more Ca it contained but not proportionately to its size increase. *C. maxima* embryos that were heavy, long and wide contained less Ca per gram than smaller embryos of this species. In Chapter 7 it was shown that larger fruits produced larger seeds. The largest fruits were grown in the field but the correlation with seed size was less significant for these fruits than for the smaller fruits grown indoors. On the other hand, some of these field fruits had embryos with a reciprocal relationship between embryo size and Ca level while the smaller indoor fruits had no such relationship. Whether these differences were related to the difference in growth rate and phloem flow for the larger field grown fruits is not known. The embryo Ca level varied

Figure 8 : 3

The Effect of Embryo Size on Concentration of Ca per Gram *C. maxima* Embryos.



µg Ca^{2+}/g Embryo (Mean ± SD)

Average - Sized Embryos	o	194.9 ± 42.8	a
Small Embryos	*	213.5 ± 76.0	a
Large Embryos	Δ	167.1 ± 45.4	b

a,b - means significantly different at P = 0.05

more plant-to-plant than fruit-to-fruit on the same plant indicating some genetic control of the Ca accumulation. Ca level varied within embryos of single fruits but to a variable degree.

A number of unsolved problems relating to fruit and seed growth also prevent the full understanding of how Ca accumulates in developing embryos. One of these problems involves the relative contributions by xylem and phloem to fruits and seeds. Although phloem transport is believed to be predominant in fleshy fruits, Ca is present in higher amounts in cucurbit xylem than phloem (Richardson et al , 1982). Phloem transport alone cannot supply enough Ca to the embryos and it has been found that 68% of the vascular input of Ca into lupine seeds was delivered via the xylem (Pate and Hocking, 1978). However, the combined delivery of xylem and phloem only accounted for 34% of the Ca accumulated in the lupine seeds (Hocking et al ,1978). The authors suggested that Ca was acquired via some non-vascular route which has not yet been defined. An additional complication is the observed backflow of water out of some fruits (Pate and Hocking, 1978). In Cucurbita maxima such transport of water via the xylem away from the fruits also included the removal of Ca from the fruit (Ziegler, 1963, 1975). The function of the reverse flow and its predominance during development is not clear.

The extent of Ca redistribution from fruit walls and seed coats is poorly understood and appears to vary in amount and timing in the different species examined so far (Hocking and Pate, 1977; Hocking, 1982). The pericarp of the cucurbit fruit is thick and contains Ca which could be redistributed to the embryos. Tyler and Lorenz (1964) found that the Ca level in cantaloupe fruits decreased as the fruits reached maturity. In

the early stages of fruit growth the seeds were enclosed in parenchyma tissue which could contribute Ca to the seed coats and subsequently to the embryos. Underground peanut fruits accumulated Ca in their hulls and testas by direct uptake from the soil solution (Keisling et al ,1982). The amount of Ca taken up by the hulls and the amount that was redistributed from the hulls to the embryos varied from one cultivar to another (Beringer and Tekete, 1979). Peanut seeds growing in Ca-rich medium reached a larger seed size but had no relationship between seed size and Ca concentration within the seeds (Keisling et al , 1982). If cucurbit seeds were capable of direct uptake of Ca from the fruit tissue the pericarps of fruits with embryos containing a high level of Ca should also be rich in Ca. Hocking and Pate (1977) suggested that the uptake of Ca must be selective in order to achieve the levels seen in the embryos despite the low availability of Ca. Beringer and Tekete (1979) ascribe a regulatory role in Ca uptake to the seed coats in peanuts. Variability in Ca uptake and in the release of the Ca from cucurbit seed coats could account for some of the variability in Ca seen within these seeds. Studies on apple fruits have shown that Ca may be more mobile within fruit tissues than suspected (Perring, 1981), but there was no mention of Ca uptake into apple seeds. Fruits of cucurbit size have the potential to have areas of low and high Ca, perhaps as a result of proximity to vascular strands. Such local differences in available Ca could produce seed-to-seed differences in Ca level found within individual fruits.

A third type of problem is related to growth rate and how that affects the accumulation of Ca. Cucurbit fruits have extremely rapid fruit growth and their phloem flow rates are also very high (Crafts and Lorenz,

1944a,b; Richardson et al , 1984). Whether rapid fruit growth is paralleled by rapid seed growth is uncertain. Seed growth rates are higher in larger seeds largely because of their higher number of cells (Davies, 1977; Egli et al , 1981). High growth rate implies a rapid accumulation of embryo dry matter. Hocking and Pate (1977), studying developing legumes, classified Ca as an element which habitually accumulated in the embryos slower than the dry matter. The lower Ca level per gram tissue in the larger cucurbit embryos suggests that at the higher growth rate experienced by these large embryos, the lag in Ca accumulation may be exaggerated. The effect of this lag in Ca is most pronounced in embryos that are both extra heavy and extra long.

The mentioned selectivity in Ca accumulation is particularly marked in the large cucurbit embryos where there appears to be a specific pattern in sequestering of the element (Lott et al , 1978). As the embryo develops its cells would initially contain cytoplasm and vacuoles and would tend to actively extrude any Ca to maintain low cytoplasmic concentrations (Hanson, 1984). This occurs in most mesophyll cells which lack Ca in their storage reserves (Lott et al , 1978). However, in many of the cells predestined to be vascular cells on germination Ca was incorporated into phytin and accumulated in the vacuoles. The control mechanisms for this distribution are not known but appear to be related to embryo size. C. andreana embryos which are small have a more even Ca distribution than do C. maxima embryos. Other cucurbit species with small seeds have Ca distribution similar to that of C. andreana while the large-seeded species have embryos with a distribution pattern similar to the C. maxima embryos (Lott and Vollmer, 1979). Some of the Ca within

embryos must be in the cell walls but there is at present no information on the distribution of Ca between the walls and storage organelles in cucurbit or other embryos.

It has also been suggested that resource allocation may not be random so that some seeds would be better nourished than others (Temme, 1986). Whether such deliberate allocation also applies to individual minerals and could contribute to the overall variation in Ca level in embryos is not known. Large seeds represent a larger investment in resources. Seed size plays a role in the accumulation of Ca but seed size is believed to be under some genetic control (Harper et al ,1970; Egli, 1981). Thus factors that control both seed size and Ca level in embryos are both complex and varied and as yet not fully understood.

This chapter has established that Ca level in C. maxima embryos varies more than embryo size and can be negatively correlated with embryo size. The variation in Ca level could be of the same degree in embryos from different fruits or from within single fruits. Some genetic control of the Ca level was indicated by the higher variation in Ca level in embryos produced on different plants than in fruits from the same plant. In C. andreana embryos the degree of variation in embryo size and Ca level was more similar and no significant relationship was detected between embryo size and Ca level.

Chapter 9.

VARIATION IN SEED SIZE AND CALCIUM LEVEL IN RECIPROCAL HYBRIDS OF C. MAXIMA AND C. ANDREANA .

INTRODUCTION

Reciprocal hybridization of the cultivated C. maxima with the wild C. andreana was reported by Whitaker (1951). Both hybrids were fertile. This hybridization was more successful than usually found between species of cultivated Cucurbita (Whitaker and Davis, 1962). Numerical taxonomy showed that C. maxima and C. andreana clustered closely together when 25 Cucurbita species were compared (Bemis et al , 1970). Members of the genus Cucurbita were found to have a high degree of intraspecific uniformity and a large amount of interspecific variation. The clustering of C. maxima and C. andreana was due to their genetic compatibility and not their phenotypic similarities. Fruits and seeds of the two species are different in both appearance and size.

Within the family Cucurbitaceae the genetics of the genus Cucumis (cucumber) has been studied most extensively (Robinson et al , 1976). This genus has the diploid number of 7 pairs of chromosomes making it easier to study than the genus Cucurbita where most diploid species have 20 pairs of chromosomes (Robinson et al , 1976; Singh, 1979; Whitaker and Bemis, 1975). Singh (1979) found no evidence of natural polyploidy in the Cucurbita genus. This genus is very variable morphologically with considerable variation in fruit and seed characteristics but only a few of

its genes have been studied (Robinson et al , 1976). Most of the defined Cucurbita genes deal with fruit characteristics such as rind texture, rind colour, markings, thickness and flesh colour. Warty fruit and yellow flesh of C. maxima is dominant to the smooth rind and whitish flesh of C. andreana . Hard rind of C. andreana is dominant to the soft rind of C. maxima . Whitaker (1951) found this to be true for the inheritance of the gourd-like rind of C. andreana . C. andreana mottling and bitterness are mainly controlled by a single dominant gene (Robinson et al , 1976; Whitaker, 1951). A single gene is responsible for the tan seed coat of C. andreana as opposed to the white seed coat of C. maxima . No genes have been defined for cucurbit seed size let alone for the control of Ca concentration.

Since C. maxima and C. andreana had a marked difference in seed size and Ca distribution (Lott and Vollmer, 1979), reciprocal hybrids offered a useful system for studying factors affecting Ca accumulation in seeds. This chapter presents characteristics of hybrid seeds and their Ca level. Comparison of Ca levels to that of the other storage minerals is shown in Chapter 10 and the distribution of Ca in axes and cotyledons of the parent and hybrid embryos is discussed in Chapter 11.

MATERIALS AND METHODS

Production of Hybrids :

The parent species C. maxima cv. Warted Hubbard and C. andreana were grown as described in Chapter 7. Cross-pollination between these two species produced reciprocal hybrids named C. maxima x C. andreana and C. andreana x C. maxima according to taxonomic rules of the Botanical Code

TABLE 9:1

Size and Seed Content of Fruits of *C. maxima* x *C. andreana* and *C. andreana* x *C. maxima* and a Comparison of These Parameters With Those of the Female Parent.

Fruit Parameters	Species	Mean ± S.D.	c.v.	Range	Largest / Smallest	Differences from Female Parent
Weight (kg)	A	2.1 ± 1.9	90.4%	0.5 - 6.0	12.0	Smaller, narrower range
	B	0.14 ± 0.08	57.1%	0.04 - 0.45	11.3	Larger, broader range
Circumf. (cm)	A	51.6 ± 11.4	22.1%	34.0 - 75.0	2.2	Fewer field fruit, smaller
	B	20.2 ± 3.4	16.8%	13.5 - 30.0	2.2	Similar
Length (cm)	A	22.2 ± 6.8	30.6%	15.0 - 36.0	2.4	Smaller
	B	8.0 ± 1.7	21.3%	4.5 - 13.0	2.9	Similar
Width (cm)	A	16.4 ± 3.7	22.6%	10.0 - 24.0	2.4	Smaller
	B	6.6 ± 2.2	33.3%	4.0 - 9.0	2.3	Slightly larger
No. of Seeds	A	228 ± 129	56.6%	99 - 470	4.7	Similar, narrower range
	B	255 ± 149	58.4%	43 - 684	15.9	Similar, higher maximum
Total Seed Wt. (g)	A	50.7 ± 34.3	67.6%	14.8 - 122.2	8.3	Lower
	B	5.0 ± 3.0	60.0%	0.9 - 13.2	14.5	Slightly higher
Av. Wt. per Seed (g)	A	0.21 ± 0.04	19.0%	0.14 - 0.27	1.9	Same as parent indoor fruit
	B	0.020 ± 0.004	20.0%	0.008 - 0.029	3.6	Similar

A - *C. maxima* x *C. andreana* N = 12

B - *C. andreana* x *C. maxima* N = 46

(Pringle, 1973). Since only the Warted Hubbard cultivar of C. maxima was used in this study the cultivar name has been omitted from the hybrid names.

Seed Preparation and Ca Analysis :

Seeds were removed from fruits as described in Chapter 7 and were prepared for analysis as described in Chapters 2 and 8. The number of seeds sampled per fruit was based upon the number selected for the parent species as outlined in Chapter 8. All Ca analyses reported in this chapter were carried out by AAS and statistical comparisons were made using MTB.

RESULTS

The problems mentioned in Chapter 7 related to fruit production also apply to the hybrid fruits. The fruit set was lowest with C. maxima as the female parent. Table 9:1 shows for the hybrids the same fruit and seed characteristics as shown for the parent fruit in Tables 7:1 and 7:2. In all cases fruits with hybrid seeds were very similar to their female parent. Most of the 12 C. maxima x C. andreana fruits were grown indoors and the fruit size was thus more similar to that of the C. maxima fruits produced indoors (Table 7:2). Coefficients of variation were highest for fruit weight and also very high for the number of seeds per fruit and total seed weight. Average seed weight had lower coefficients indicating a lower variability in this parameter. These results parallel the parental fruit and seed characteristics. C. andreana x C. maxima fruits, which were produced in larger numbers, though similar to the female parent showed two differences. In this study the fruits produced by hybridization tended to be somewhat larger and the maximum seed number of 684 was higher

TABLE 9:2

Correlation Among Fruit Size and Seed Characteristics in *C. maxima* x *C. andreana* and *C. andeana* x *C. maxima* Hybrids.

A. *C. maxima* x *C. andreana* B. *C. andreana* x *C. maxima*	Fruit Weight	Fruit Circumf.	Fruit Length	Fruit Width	No. of Seeds	Total Seed Wt.
A. Circumference	0.95**	–	–	–	–	–
Length	0.95**	0.92**	–	–	–	–
Width	0.88**	0.94**	0.89**	–	–	–
No. of Seeds	0.94**	0.94**	0.96**	0.90**	–	–
Total Seed Wt.	0.95**	0.95**	0.93**	0.89**	0.98**	–
Av. Wt. per Seed.	0.40	0.46	0.29	0.43	0.46	0.54
B. Circumference	0.95**	–	–	–	–	–
Length	0.89**	0.90**	–	–	–	–
Width	0.85**	0.80**	0.77**	–	–	–
No. of Seeds	0.78**	0.76**	0.73**	0.66**	–	–
Total Seed Wt.	0.71**	0.73**	0.75**	0.70**	0.93**	–
Av. Wt. per Seed	-0:01	0.08	0.16	0.13	-0.14	0.20

** Significant at $P < 0.01$

TABLE 9:3

Variation in Weights of Seeds and Embryos and in Lengths and Widths of the Embryos of *C. maxima* x *C. andreana* and *C. andreana* x *C. maxima* Hybrids.

Hybrid	Means ± S.D. and Ranges (in Parentheses)				
	Seed Weight (g)	Embryo Weight (g)	Embryo Length (mm)	Embryo Width (mm)	% Seed Coat
C. maxima x *C. andreana* N = 12	0.21 ± 0.04 c.v. 19.0% (0.14 - 0.27)	0.16 ± 0.03 c.v. 18.8% (0.10 - 0.20)	15.8 ± 1.0 c.v. 6.3% (14.5 - 17.3)	8.8 ± 0.6 c.v. 6.8% (7.8 - 9.5)	25.1 ± 7.8%
C. andreana x *C. maxima* N = 46	0.020 ± 0.004 c.v. 20.0% (0.008 - 0.029)	0.013 ± 0.003 c.v. 23.1% (0.007 - 0.024)	5.8 ± 0.3 c.v. 5.2% (5.0 - 6.5)	3.4 ± 0.3 c.v. 8.8% (2.9 - 3.9)	34.0 ± 8.6%

N = represents number of fruits measured. (60 seeds measured/fruit for *C. maxima* x *C. andreana*, and > 60 seeds measured for *C. andreana* x *C. maxima*).

than in the parent species. Under the growth conditions used, C. andreana x C. maxima hybrid fruit were easier to produce than a selfed C. andreana fruits. Hybrid seed size was essentially the same as that of its female parent.

Table 9:2 gives correlation coefficients for fruit and seed characteristics which can be compared to those of the parent species as reported in Table 7:4 and 7:5. Correlations were similar to the parental and were very highly significant for all the fruit measurements. Heavy fruits were large in all measured dimensions and tended to have a heavier total mass of seeds and a higher seed number. But, unlike seeds of the parent fruits, there was no correlation between individual seed size and any of the other parameters. Table 9:3 gives the variation in seed and embryo size for the two hybrid seeds. This table can be compared to the parental measurements in Table 7:8. Hybrid seed and embryo weights were more variable than their lengths and widths just as they were in the respective parental species. All measurements were similar to those of the parent species.

The Ca levels of the hybrid embryos as shown in Table 9:4, can be compared to Table 8:3 for Ca levels in embryos of the parents. Ca levels were similar to those of the female parents but with a tendency toward intermediate levels. For C. maxima x C. andreana the Ca levels in embryo tissues were higher than in C. maxima embryos. The increase was particularly evident when parents and hybrids were compared on a per gram embryo basis. The C. andreana x C. maxima hybrid had lower Ca levels than its female parent. Comparison of parent and hybrid Ca levels on both

TABLE 9:4

AAS of Ca in Embryos of *C. maxima* x *C. andreana* and *C. andreana* x *C. maxima*.

	Hybrid		Calcium Concentrations (Mean ± S.D.) and Range	% Difference of Mean from Female Parent*
A.	*C. maxima* x *C. andreana*	µg Ca^{2+}	55.2 ± 12.5	
		c.v.	22.6%	+ 27.5%
	N = 12	Range	29.3 - 74.5	
		Highest	2.5	
		Lowest		
	C. andreana x *C. maxima*	µg Ca^{2+}	7.8 ± 3.4	
		c.v.	43.6%	- 3.7%
		Range (ug)	2.4 - 17.0	
	N = 46	Highest	7.1	
		Lowest		
B.	*C. maxima* x *C. andreana*	µg Ca^{2+}	356.0 ± 104.0	
		c.v.	29.2%	+ 53.1%
		Range (ug)	201.0 - 579.0	
	N = 12	Highest	2.9	
		Lowest		
	C. andreana x *C. maxima*	µg Ca^{2+}	589.0 ± 153.0	
		c.v.	26.0%	- 10.1%
		Range (ug)	341.0 - 1025.0	
	N = 46	Highest	3.0	
		Lowest		

A - Ca^{2+} on a per embryo basis

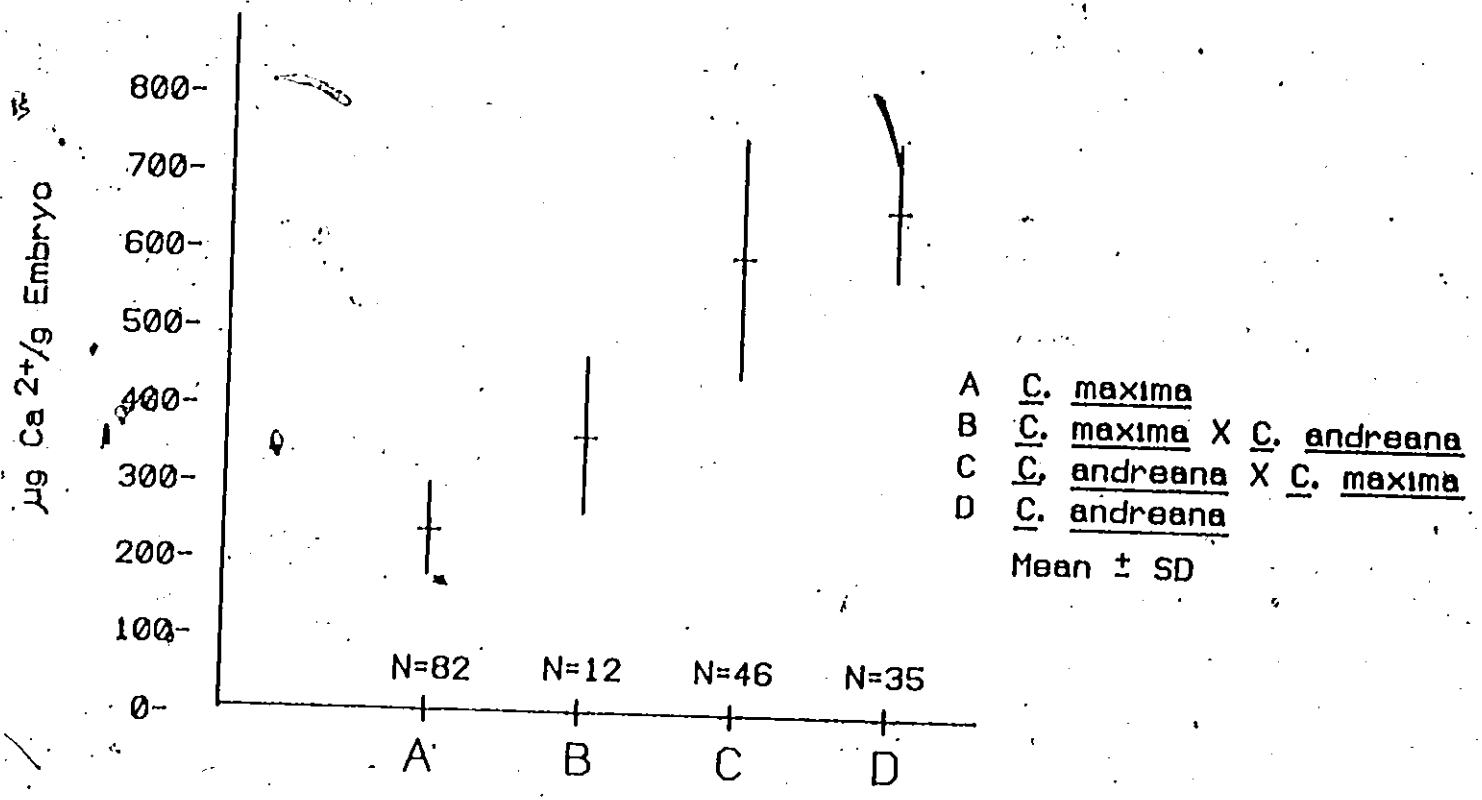
B - Ca^{2+} on a per gram embryo basis

N - represents number of fruits sampled

* compared to means for total crops as shown in Table 8:3

Figure 9 : 1

Variation in Ca Level in Embryos of C. maxima, C. andreana and Their Reciprocal Hybrids.



a per embryo and a per gram embryo basis showed that the means were significantly different at P=0.002 for the C. maxima and its hybrid and at P=0.01 for the C. andreana and its hybrid. The mean Ca levels of the two hybrids were also significantly different from each other at P values of less than 0.001.

Figure 9:1 shows the mean Ca levels with standard deviations for the parent and hybrid embryos. The figure shows that the spread of Ca levels was larger for the hybrid than for the parent embryos. However, the comparison between C. maxima and its hybrid was based on means from very different number of samples. The coefficients of variation of 24.7% for C. maxima and 29.2% for C. maxima x C. andreana were not found to be significantly different when converted to log variances and compared as F-ratios (Falconer, 1960). There was a significant difference between the 13.5% coefficient of variation for C. andreana and 26.0% for C. andreana x C. maxima . The two hybrids did not differ in their coefficients of variation but the coefficients for C. maxima and C. andreana were different. Figure 9:2 shows the frequency of various Ca levels within the parent and hybrid embryos. There was no overlap in the Ca levels of the two parent species and the hybrid embryo Ca levels peaked at concentrations different from each other and from either of the parents.

Table 9:5 shows correlations between Ca levels in hybrid embryos and fruit and seed parameters. C. maxima x C. andreana hybrids had no significant correlation between any of the parameters and the per embryo Ca level thus making it different from its female parent which had strong correlations between most of the measured parameters and the per embryo Ca levels (Table 8:6). When the comparison was done with embryo size

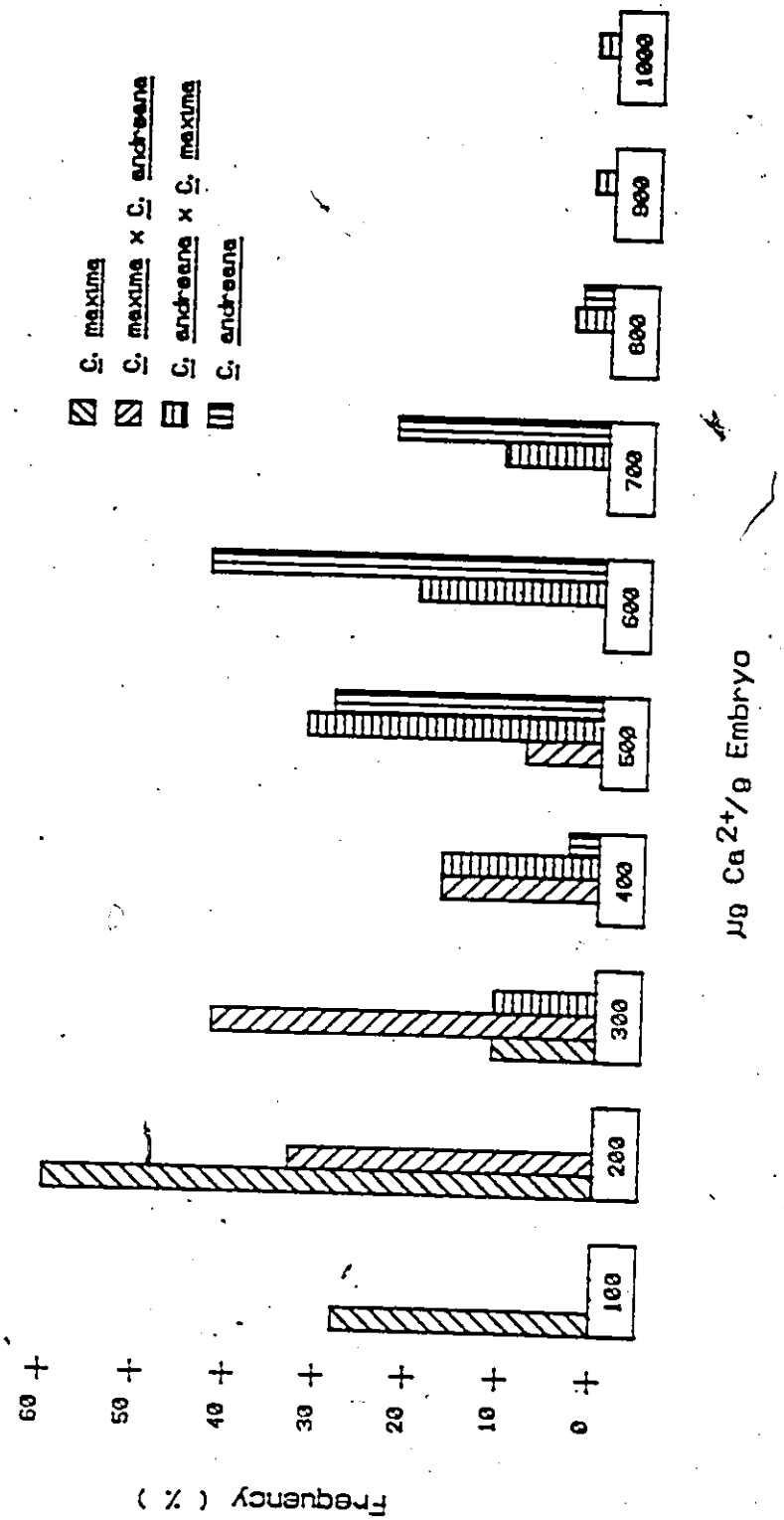


Figure 9 : 2

Distribution of Ca Levels Within Parent and Hybrid Embryos.

TABLE 9:5

Correlation Between Ca Level in Hybrid Embryos and Fruit, Seed and Embryo Size.

Fruit, Seed and Embryo Parameters	Correlation Coefficients and r^2 (in Parentheses)			
	C. maxima x *C. andreana* N = 12		*C. andreana* x *C. maxima* N = 46	
	µg Ca^{2+} per Embryo	µg Ca^{2+}/ g Embryo	µg Ca^{2+} per Embryo	µg Ca^{2+}/ g Embryo
Fruit Weight	0.01	-0.35	0.09	0.03
Fruit Length	-0.08	-0.43	0.22	0.11
Fruit Width	-0.13	-0.49	0.16	0.12
F. Circumference	-0.14	-0.53	0.18	0.12
Seed Number	-0.13	-0.49	-0.14	-0.14
Total Seed Wt.	-0.06	-0.51	0.04	-0.03
Av. Wt. per Seed	0.20	-0.53	0.65** (49.1)	0.43** (17.4)
Embryo Length	-0.39	-0.71** (49.8)	0.19	-0.02
Embryo Width	0.38	0.41	0.26	0.05
Embryo Weight	0.25	-0.56* (28.0)	0.75** (54.8)	0.40** (13.9)

r^2 - coefficient of determination - only given if significant correlation is present

* - significant at P = 0.05

** -significant at P = 0.01

measurements there was strong negative correlation between per gram embryo Ca level and both embryo length and weight. This correlation was stronger than in most of the comparisons of the parent C. maxima embryos from individual fruits and more similar to the correlations found for stock embryos (Figure 8:2). C. andreana x C. maxima also differed from C. andreana in having no significant correlation between per embryo Ca level and fruit size. Both seed and embryo weights were significantly and poritively correlated with the per embryo and per gram embryo Ca level in C. andreana x C. maxima hybrid embryos. In C. andreana (Table 8:7) only the per embryo Ca levels were positively correlated with the size parameter.

DISCUSSION

Production of fruits with hybrid seeds was even less predictable than that of self-pollinated fruits. Stephenson (1981) calculated from Bushnell's (1920) data that 19.8% of the female flowers of Cucurbita maxima matured fruits. This naturally low fruit set coupled with asynchronous growth of male and female flowers limited the ease of hybrid production. The fact that hybrids were produced and had similar seed number and seed size to their female parent attests to the fact that successful pollen tube growth and fertilization must have occurred. Nevertheless it is possible that differences between the success in producing the two kinds of hybrids may be related to pollen tube growth within styles of inappropriate lengths. Kwack and Fujieda (1985) found differences in the success of pollen tube growth in various Cucurbita crosses.

The lack of correlation between fruit parameters and seed size found with the hybrids was also reported by Whitaker (1951) for his reciprocal hybrids of C. maxima and C. andreana . In this study some hybrid embryos were closer in Ca level to that of their maternal parents while others possessed an intermediate Ca level. Ca ranges in hybrids showed an overlap with each other's values and with those of the female parents. Significant differences in measured Ca levels of C. maxima , C. andreana and their reciprocal hybrids clearly indicated that the genetic makeup of the embryo played a role in Ca accumulation within the embryo. The Ca levels obtained suggested that the Ca concentration was a metric trait controlled, at least in part, by polygenes (Mather and Jinks, 1982; Suzuki et al , 1981). Since the Ca levels tended toward an intermediate level, there appeared to be no polygenic dominance. However, since the Ca levels of the two hybrids were significantly different from each other and each approached the levels of its female parent, the female parent also must have been exerting an influence on Ca accumulation. The most obvious way of exerting such an influence would be via differences in vascular supply. That the embryo also exerted an effect on the maternal tissue was shown in the increase in the size of fruits which carried hybrid seeds. C. andreana fruits had a mean weight of 0.11 kg (Chapter 7) while C. andreana x C. maxima fruits had a mean weight of 0.14 kg. This was a 27% increase but hybrid seeds were only 5% heavier. The ability of the maternal tissue to respond to growth of abnormally large seeds was shown by a bee pollinated 2 kg C. andreana fruit. Its seeds were 0.13 g in weight making them 10 times larger than the normal C. andreana seeds. When these large seeds were grown they produced hybrid plants which bore

fruits resembling the C. maxima cv. Hungarian Mammoth. This cultivar, which had been growing in the vicinity, has seeds at least two times larger than Warted Hubbard.

There are more studies of inheritance of seed size than of inheritance of Ca level. Harper et al (1970) stated that mean seed weight within a species was inherited additively with little to no dominance but they cautioned that any interpretation must account for both maternal and paternal effects. Such effects would be even more prominent in hybrid crosses and would apply to both seed size and Ca. Mazer et al (1986) studying inheritance of seed weight in wild radish seeds (Raphanus) concluded that both maternal and paternal effects contributed significantly to the variation in seed size, but that maternal contribution was greater. Maternal effects could be exerted via the ovule number and the availability of resources. Paternal effects resulted from the amount of pollen, pollen germination rate and the success of pollen tube growth and fertilization. Chackravarty and Basak (1983) studying the inheritance of seed weight in Hibiscus found that both seed weight and seed number were controlled mainly by additive gene action and were modified by epistasis and heterosis. Inheritance of Ca level has been studied in peanut (Crompton et al , 1979) and the conclusion was that both additive and non-additive genetic effects were important in all six varieties studied. Whether maternal effects were prominent and whether cytoplasmic and nuclear gene interactions were present was variable within the studied varieties. They also found no significant correlation between peanut embryo size and Ca content.

How much of the significant difference between parental and hybrid

Ca level is due to genetic factors is not possible to determine without further biometric analysis involving diallel studies of parents, crosses and backcrosses (Hayman, 1954; Jinks, 1954; Mather and Jinks, 1982). Environmental effects have been shown to mask the phenotypes produced by polygenes. The problem with cucurbits for such studies is the long fruit maturation period, the unpredictable success in fruit set and the large space required by each plant limiting the number that can be grown at one time. For these reasons diallel analysis would be very time-consuming. However, information on the genetics of Ca inheritance is not vital to gaining understanding of the distribution of Ca within the embryos. Calcium distribution has been studied within both axes and cotyledons and is reported in Chapter 11.

This Chapter has shown that Ca accumulation in embryos is under some genetic control. However, within the species with the large embryos (C. maxima and C. maxima x C. andreana) seed size also influences Ca accumulation. Calcium levels in these long and heavy embryos may represent a balance between the low Ca mobility and the higher growth rates of the larger embryos.

Chapter 10.

VARIATION IN CALCIUM LEVEL IN THE TWO PARENT SPECIES AND HYBRIDS AS RELATED TO THE LEVELS OF THE OTHER ELEMENTS STORED IN THE EMBRYOS.

INTRODUCTION

The embryos of Cucurbita maxima contain stores of Mg, K, Ca and P (Lott, 1975). The uptake and storage of Ca within Cucurbita maxima and C. andreana embryos has been discussed in Chapter 8 and the Ca content of the embryos of their reciprocal hybrids has been described in Chapter 9. This chapter deals with the storage of Mg, K and P and the relationship of these three elements to Ca storage. Since Mg, K and P must be taken up from the soil and then transported to the seeds, I will begin by considering the uptake characteristics and mobilities of these elements.

Magnesium content of the soils is in the range of 0.05–0.15% and the element is present as both ferromagnesium minerals and clay minerals (Mengel and Kirkby, 1982). Magnesium is similar to Ca in that its uptake is believed to be passive, but it differs from Ca in that Mg is mainly concentrated in the symplast. Magnesium is mobile in the phloem and can be mobilized from older leaves as needed (Clarkson and Hanson, 1980; Marme, 1983; Mengel and Kirkby, 1982). However, the overall distribution of Mg is similar to that of Ca with lower concentrations in roots than shoots and higher levels in older rather than younger leaves (Bengtsson and Jensen, 1983). Despite the greater mobility of Mg in the phloem, Pate and Hocking

(1978) found discrepancies between the amount of Mg delivered to embryos by the phloem and the actual amount accumulated. They concluded that at least some Mg must be delivered by the xylem.

Organic soils tend to have lower K concentrations (0.03%) than soils rich in clay (4%) but such clay soils, if they are of alkaline pH, can bind K very strongly decreasing its availability to plants (Mengel and Kirkby, 1982). The uptake of K^+ by the roots is an active process with the ions entering the symplast for phloem transport (Chino, 1981; Mengel and Kirkby, 1982). In Cucurbita pepo , all parts of the root were capable of absorbing and translocating K (Harrison-Murray and Clarkson, 1973). In plant cells K is the most abundant cellular cation with concentrations of 100 mM or greater (Clarkson and Hanson, 1980). It is by far the most abundant cation in the phloem (Hocking, 1982; Ziegler, 1975). Phloem-fed tissues such as fruits tend to be high in K (Ziegler, 1975). Potassium tends to be fairly evenly distributed between roots and shoots in Cucumis and has a higher concentration in these tissues than either Ca or Mg (Bengtsson and Jensen, 1983). From work on mineral uptake by legume seeds it was found that K was mobilized from the leaves, pods and testa to the embryo with 60-90% efficiency as opposed to less than 20% efficiency for Ca and that the accumulation of K within the embryo was acceptably close to that of the estimated delivery via phloem (Hocking and Pate, 1977; Pate and Hocking, 1978).

The total soil P content is in the range of 0.02-0.15%, most of it present as orthophosphate. As soils age their organic P content increases (Mengel and Kirkby, 1982). In soil, P is the most immobile of the major nutrients (Mengel and Kirkby, 1982) in contrast to being one of the most

mobile nutrients within plants (Bieleski, 1973). Soils are more commonly deficient in P than in Ca, K or Mg and at best the P concentration of the soil solution is seldom as high as 10 uM. Soil solutions with modal concentrations of P as low as 1.5 μM had modal concentrations of 90 μM, 700 μM and 1000 μM for K, Ca and Mg respectively (Bieleski, 1973). Most P is taken up by the roots as the monovalent ion $H_2PO_4^-$ which exists in equilibrium with the divalent form, HPO_4^{2-} (Bieleski, 1973; Mengel and Kirkby, 1982). While some P can enter the roots by passive diffusion, most P is taken up by energy-dependent carriers and rapid uptake can leave depletion zones around the roots (Bieleski, 1973). Associations with mycorrhizal fungi result in higher phosphorus levels in plants and such associations have been reported for cucurbits (Saif, 1977). Harrison-Murray and Clarkson (1973) found that P was absorbed better by the younger regions of the Cucubita roots and that movement within the older portions of the root was inhibited by the suberized endodermis. In general, P can be transported both in the xylem and phloem and in both organic or inorganic form (Bieleski, 1973; Mengel and Kirkby, 1982; Ziegler, 1975). Concentrations in the xylem can be 20-100 times higher than the soil levels and P has been found to pass easily from xylem to phloem though rarely in the opposite direction (Bieleski, 1973). Pate and Hocking (1978) found that most of the P delivered to legume embryos was carried in the phloem and Hocking and Pate (1977) reported a 60-90% mobilization of P to the embryos from the leaves, pods and testa.

Mature embryos contain more P than any organ system within the plant (Hocking and Pate, 1977). Most of the P within embryo tissues is present as phytin and most of the phytin in squash embryos is concentrated

in the globoid crystals (Lott, 1984). The exact conformation of the phytic acid molecule and how many cations can be bound to it has been the subject of much debate (Cosgrove, 1966; Johnson and Tate, 1969; Maga, 1982; Posternak, 1965). It is currently accepted that in sodium phytate the ratio of bound sodium to phosphorus is 2:1 thus indicating that 12 of the potentially hydrolyzable hydrogens have been replaced by sodium. Within embryo tissues the phytic acid binding sites are occupied by K, Mg and Ca but the relative proportions of each and the relative strengths of the *in vivo* binding have not been defined. The physical arrangement of phytin molecules within the spherical globoid crystals is as yet unknown but there are indications that globoid crystal size may be related to the solubility of the phytate which varies with the ratio of divalent to monovalent cations (Lott *et al*, 1985). The mineral analyses presented in this chapter were all carried out on mature and dry cucurbit embryos and represent the total mineral content accumulated under the growth conditions described in Chapter 7.

MATERIALS AND METHODS

Stock embryos and embryos of some fruits selected from the fruits described in Chapter 7 were analyzed by NAA using the procedure given in Chapter 3. Analyses were performed on unashed, pooled, ground embryo mixtures. These were the same ones used for AAS analysis. Seed coats were also ground and analyzed in the unashed state. Statistical analyses were carried out using MTB.

RESULTS

The concentrations of Mg, K, Ca and P in the embryos of C. maxima and C. andreana stocks are shown in Table 10:1. On a per embryo basis the 0.21 g embryos of C. maxima contained a higher amount of all four elements. On a percent basis the C. andreana embryos had more Mg and Ca while K and P were in similar concentrations to those of C. maxima. Calcium showed more difference between the species than the other three minerals. The embryos of C. maxima were 17.5 times heavier than those of C. andreana and when the mineral levels in C. andreana were multiplied by 17.5 the mineral levels appeared higher in C. andreana than in C. maxima.

Analysis of pooled embryos from individual fruits is shown in Table 10:2. The high coefficients of variation seen here were due to the fruit-to-fruit mineral level differences. Of the four minerals analyzed, Ca was again unusual in having the greatest fruit-to-fruit variation in C. maxima and the two hybrids but not in C. andreana. Although the ranges appeared quite broad, for all four minerals the ratio of the highest to lowest concentration of Mg, K and P was remarkably constant at 1.5-1.6. The mineral levels from Table 10:2 are shown graphically in Figure 10:1. Phosphorus represented as much as 50% of the total mineral content in both parent and hybrid embryos. Calcium made up about 1% of the mineral complement in C. maxima and about 3% in C. andreana. Potassium and Mg each made up between 20 and 30% of the total mineral content. The parent species differed from each other in their Mg to K ratios. Both hybrids followed the C. maxima pattern in having higher K than Mg levels. As was shown in Chapter 9, Ca levels of the hybrid embryos tended to be more

TABLE 10:1

Neutron Activation Analysis of Mg, K, Ca and P in Embryos of the Parental Seed Stocks of *C. maxima* and *C. andreana*.

Element	Concentration	*C. maxima* (A)	*C. andreana* (B)	*C. andreana* x 17.5 (C)	B/A	C/A
Mg	µg/Embryo	677.2 ± 38.1*	65.2 ± 3.8	1141.0	0.10	1.68
	%	0.38 ± 0.02	0.54 ± 0.03	--	1.42	--
K	µg/Embryo	853.0 ± 39.4	61.5 ± 3.1	1076.3	0.07	1.26
	%	0.48 ± 0.02	0.51 ± 0.03	--	1.06	--
Ca	µg/Embryo	35.1 ± 1.3	7.94 ± 0.35	139.0	0.22	3.96
	%	0.020 ± 0.001	0.066 ± 0.002	--	3.30	--
P	µg/Embryo	1728.7 ± 148.4	120.3 ± 17.3	2105.3	0.07	1.21
	%	0.97 ± 0.08	0.99 ± 0.14	--	1.02	--

A mean embryo wt. = 0.21 g, 6 subsamples analyzed from 300 pooled embryos
B mean embryo wt. = 0.012 g, 6 subsamples analyzed from 300 pooled embryos
C wt. ratio A/B = 17.5
* mean ± SE

TABLE 10:2

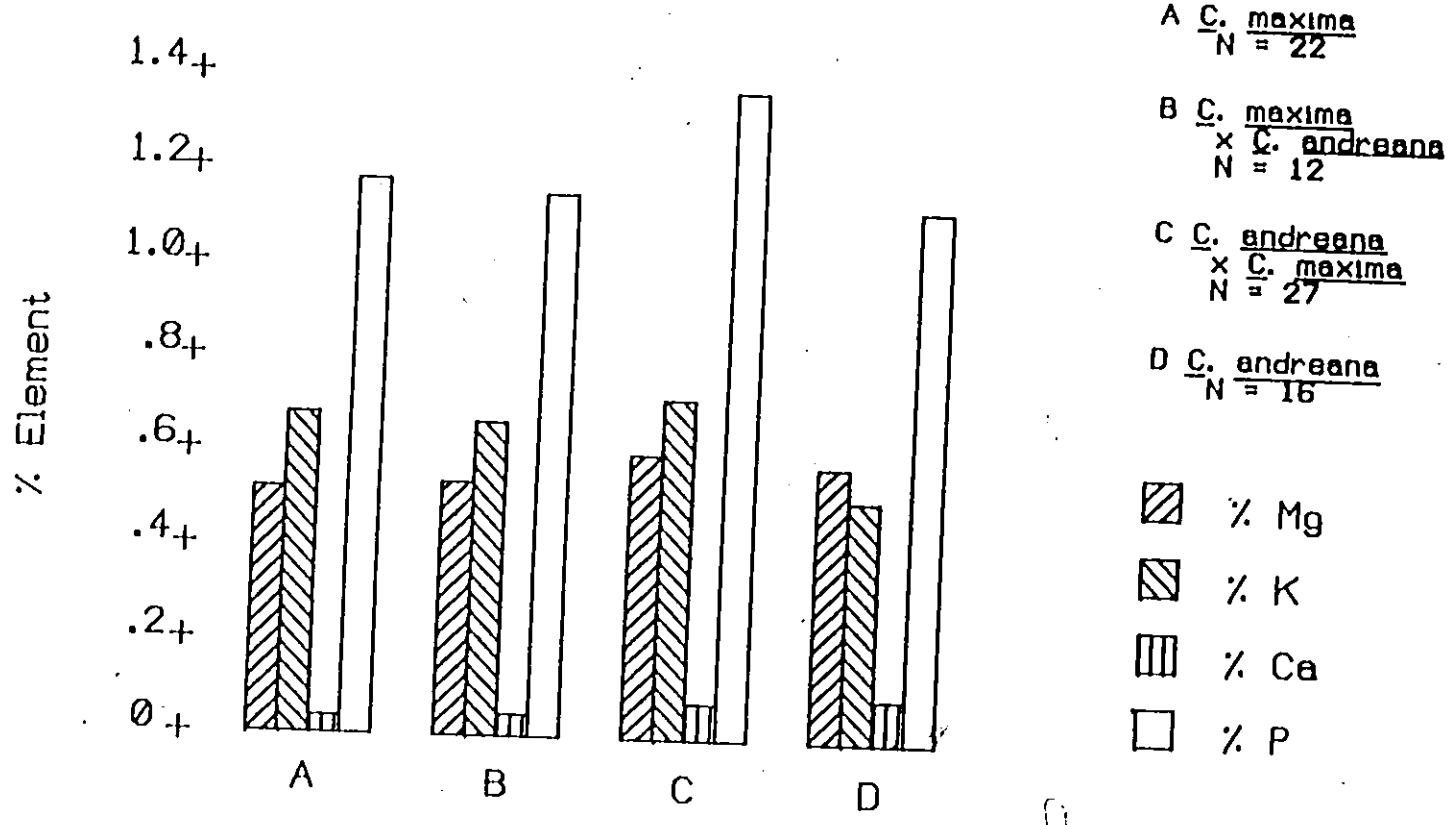
Percent Mineral in Embryos of Parent Species and Hybrids Grown for this Study.

Parent Species and Hybrids	N	% Mineral ± SE, c.v., Range and Ratio Highest % / Lowest %			
		Mg	K	Ca	P
C. maxima	22	0.50 ± 0.01 11.4% 0.40 – 0.60 1.5	0.66 ± 0.02 11.7% 0.52 – 0.79 1.5	0.024 ± 0.002 33.3% 0.012 – 0.045 3.8	1.18 ± 0.03 9.8% 1.03 – 1.55 1.5
C. maxima x C. andreana	12	0.52 ± 0.02 11.9% 0.43 – 0.64 1.5	0.65 ± 0.03 15.7% 0.53 – 0.86 1.6	0.035 ± 0.003 31.4% 0.018 – 0.061 3.4	1.16 ± 0.05 15.1% 0.94 – 1.51 1.6
C. andreana x C. maxima	27	0.60 ± 0.02 12.1% 0.50 – 0.75 1.5	0.71 ± 0.03 15.0% 0.55 – 0.88 1.6	0.056 ± 0.004 28.6% 0.033 – 0.088 2.7	1.36 ± 0.05 15.3% 1.14 – 1.72 1.5
C. andreana	16	0.58 ± 0.02 13.0% 0.47 – 0.71 1.5	0.50 ± 0.03 16.6% 0.41 – 0.64 1.6	0.069 ± 0.003 13.0% 0.049 – 0.078 1.6	1.15 ± 0.05 14.1% 0.94 – 1.49 1.6

N represents the number of fruits sampled

Figure 10 : 1

Relative Concentrations of Mg, K, Ca and P in Embryos of *C. maxima*, *C. andreana* and Their Reciprocal Hybrids.



similar to those of their respective female parents. Table 10:3 shows the mineral levels from Table 10:2 expressed on a per embryo basis and again Ca levels show a greater variability than the levels of the other minerals. Table 10:4 shows that the mean Ca levels were significantly different for the parent and hybrid embryos on a percent basis but not on a per embryo basis. Based on the mineral ratios the main difference between C. maxima and its hybrid was in Ca level while C. andreana and its hybrid differed most in K level.

Table 10:5 shows that, when considered on a percent mineral basis, levels of all four minerals were not significantly correlated with embryo weights but the overall trend was toward negative correlation with weight. Correlations among K, Ca and P were generally not significant. Magnesium was significantly correlated with K levels in all four types of embryos. Correlation between Mg and Ca was significant only in C. maxima and its hybrid. Correlation between Mg and P was significant in both parent but not the hybrid embryos. Multiple regression and stepwise multiple regression of the mineral levels showed no consistent pattern in the variation of the mineral levels in the parent and hybrid embryos. In the C. maxima embryos variation in P level could explain about 30% of the variation in Mg. In C. maxima x C. andreana , K was the more important indicator (R^2=32%). All three elements interacted in the variation in Mg level in C. andreana x C. maxima with an overall R^2 of 90%. For C. andreana the R^2 was 70% with only K and Ca being involved. When batches of embryos with a 50% difference in Ca level were compared those with the higher Ca levels had about 10% more Mg and P and about 10% less

TABLE 10:3

Mineral Content of Embryos of the Two Parent Species and Hybrids.

Parent Species and Hybrids	N	Concentration in µg/Embryo ± SE, c.v., Range				
		Mg	K	Ca	P	Embryo Weights (g)
C. maxima	22	969 ± 50 23.9% 535 - 1521	1278 ± 59 21.7% 817 - 1870	46.6 ± 3.7 36.9% 24.1 - 85.3	2317 ± 115 23.3% 1404 - 3378	0.195 ± 0.008 19.5% 0.114 - 0.270
C. maxima x C. andreana	12	834 ± 47 19.7% 564 - 1112	1021 ± 52 17.5% 771 - 1364	54.0 ± 4.2 26.9% 25.6 - 81.3	1792 ± 93 17.9% 1274 - 2194	0.160 ± 0.008 18.1% 0.098 - 0.201
C. andreana x C. maxima	27	78.6 ± 3.8 18.1% 58.5 - 108.8	92.3 ± 3.1 12.5% 73.8 - 108.3	7.57 ± 0.80 39.6% 3.47 - 14.75	176.3 ± 8.0 17.1% 121.3 - 251.0	0.013 ± 0.002 15.4% 0.010 - 0.016
C. andreana	16	80.3 ± 4.4 17.2% 64.8 - 105.8	69.2 ± 3.9 17.8% 51.0 - 89.0	9.46 ± 0.46 15.4% 7.40 - 11.34	157.1 ± 6.4 12.3% 132.1 ± 185.3	0.014 ± 0.001 7.1% 0.012 - 0.016

TABLE 10:4

Mineral Levels in Parent and Hybrid Embryos.

Species	%Mg	H/P	%K	H/P	%Ca	H/P	%P	H/P
A. *C. maxima* (P)	0.50a	1.04	0.66a	0.98	0.024a	1.46	1.18a	0.98
C. maxima x *C. andreana* (H)	0.52a,c		0.65a		0.035b		1.16a	
C. andreana x *C. maxima* (H)	0.60b	1.03	0.71a	1.42	0.056c	0.81	1.36b	1.18
C. andreana (P)	0.58b,c		0.50b		0.069d		1.15a	

B. Species	Mg µg/Embryo	H/P	K µg/Embryo	H/P	Ca µg/Embryo	H/P	P µg/Embryo	H/P
C. maxima (P)	969a	0.86	1278a	0.80	46.6a	1.16	2317a	0.77
C. maxima x *C. andreana* (H)	843a		1021b		54.0a		1792b	

C.								
C. andreana x *C. maxima* (H)	78.6a	0.98	92.3a	1.33	7.57a	0.80	176.3a	1.12
C. andreana (P)	80.3a		69.2b		9.46a		157.1a	

A The 4 means in each column were compared by analysis of variance followed by Tukey's test for differences between means (Zar, 1984) - means with different letter names are significantly different at P = 0.05 or less.

B and C The two means in each column were compared by a t-test.

TABLE 10:5

Correlation Among the % Mineral Concentrations and Embryo Weights Within Parent and Hybrid Embryos.

Parent Species and Hybrids	Mineral Being Measured	Correlation Coefficients (r) Embryo Wt.	% Mg	% K	% Ca
C. maxima N = 22	% Mg	0.06	—	—	—
	% K	-0.15	0.45*	—	—
	% Ca	-0.03	0.47*	-0.17	—
	% P	-0.01	0.55**	-0.24	0.28
C. maxima x *C. andreana* N = 12	% Mg	-0.30	—	—	—
	% K	-0.54	0.57*	—	—
	% Ca	-0.52	0.55*	0.46	—
	% P	-0.43	0.28	0.44	0.07
C. andreana x *C. maxima* N = 27	% Mg	-0.10	—	—	—
	% K	-0.30	0.59*	—	—
	% Ca	0.13	0.15	-0.49	—
	% P	0.20	0.38	-0.02	-0.27
C. andreana N = 16	% Mg	-0.13	—	—	—
	% K	-0.26	0.82**	—	—
	% Ca	-0.29	0.48	0.13	—
	% P	-0.51	0.74**	0.76**	0.57

Correlations based on mineral levels in Table 10:2
* significant at P = 0.05
** significant at P = 0.01

K. Thus, variation in the other three minerals was much less than variation in Ca level and the differences were generally not significant.

The correlations between individual minerals and embryo weights were quite different on a per embryo basis (Table 10:6). C. maxima and its hybrid had a strong positive correlation with embryo weight for all minerals except Ca in the hybrid embryos. C. andreana and its hybrid embryos had no correlation with embryo weight. Magnesium had the strongest correlation with the other elements and this was true for all four kinds of embryos. Potassium level was correlated with P but not with Ca. In C. andreana x C. maxima hybrids K was not correlated with P. Calcium was correlated with P in all but the C. maxima x C. andreana hybrid. Regression analysis on the per embryo values produced essentially the same results as on the percent basis. Phosphorus was most significantly related to variation in Mg in C. maxima while K was more important in C. maxima x C. andreana . All three elements interacted with Mg in C. andreana x C. maxima . Potassium and Ca but not P interacted in variation in Mg level in C. andreana . Thus, if the concentration of Mg was high, the concentration of K, Ca and P was also likely to be high. Calcium level was not dependent on the variation in K but was linked to the levels of Mg and P. Comparing Tables 10:5 and 10:6 it can be seen that some of the correlations remained significant in both methods of comparing mineral levels but on the whole there was less significant correlation when the comparison was done on a percent basis.

When the divalent cation concentration was compared to that of K, a fairly strong positive correlation was obtained for per embryo mineral levels in both parent and hybrid embryos (Table 10:7 A). Correlations were

TABLE 10:6

Correlation Among Mineral Concentrations
On a Per Embryo Basis Within Parent Species and Hybrids.

Parent Species and Hybrids	Per Embryo Concn. (µg)	Correlation Coefficients (r) Embryo Wt.	µg Mg/ Embryo	µg K/ Embryo	µg Ca/ Embryo
C. maxima	Mg	0.86**	—	—	—
	K	0.83**	0.86**	—	—
	Ca	0.53**	0.65**	0.36	—
	P	0.89**	0.92**	0.85**	0.57**
C. maxima x *C. andreana*	Mg	0.80**	—	—	—
	K	0.68*	0.81**	—	—
	Ca	0.21	0.49	0.29	—
	P	0.67*	0.67*	0.58*	-0.02
C. andreana x *C. maxima*	Mg	0.46	—	—	—
	K	0.46	0.70**	—	—
	Ca	0.22	0.72**	0.21	—
	P	0.43	0.77**	0.25	0.55*
C. andreana	Mg	0.51	—	—	—
	K	0.37	0.86**	—	—
	Ca	0.43	0.67*	0.33	—
	P	0.34	0.83**	0.79**	0.66*

Correlations based on mineral levels in Table 10:3
* significant at P = 0.05 or less
** significant at P = 0.01 or less

TABLE 10:7

Correlations Among Minerals Within Parent and Hybrid Embryos.

A. Correlation Coefficients (Ca + Mg) vs. K

	for % Mineral r	for % Mineral r^2	for µg mineral/embryo r	for µg mineral/embryo r^2
C. maxima	0.40	14.9%	0.85**	70.5%
C. maxima x C. andreana	0.59*	28.0%	0.80**	60.2%
C. andreana x C. maxima	0.46	14.5%	0.64**	36.1%
C. andreana	0.79**	56.9%	0.83**	65.7%

B. Correlation Coefficients (Mg + K + Ca) vs. P

	for % Mineral r	for % Mineral r^2	for µg mineral/embryo r	for µg mineral/embryo r^2
C. maxima	0.45*	16.0%	0.92**	83.2%
C. maxima x C. andreana	0.42	8.9%	0.64*	34.9%
C. andreana x C. maxima	0.14	0.0%	0.61*	31.5%
C. andreana	0.80**	93.6%	0.85**	68.1%

Correlations based on mineral levels in Tables 10:2 and 10:3
* significant at P = 0.05
** significant at P = 0.01

also determined between total cation concentration and P level (Table 10:7 B). On a percent basis there was significant correlation only for the two parent species but on a per embryo basis all four types of embryos had a significant correlation. This indicated that if the embryos contained high levels of the three cations their P concentration was also higher.

Ratios of the minerals to each other within the parent and hybrid embryos were calculated (Tables 10:8 and 10:9). Since Ca levels were different in C. maxima and C. andreana any ratios involving Ca were also different for the two parent species and their hybrids (Table 10:8). Ratios not involving Ca were relatively similar for all four groups of embryos with the exception of ratios involving Mg and K in C. andreana . Table 10:9 shows that the contribution by Ca was very small compared to that of K and Mg, which together were almost equivalent to the amount of P present in the embryos. The ratio of total cations to P was virtually the same for both parent and hybrid embryos and showed a 1:1 balance between cations and P.

The ratio of one for cations to P, shown in Table 10:9, did not indicate that there were exactly the right number of cations to bind with the phosphorus on the phytic acid. When mineral concentrations were converted to the number of atoms present using the gram atomic weight and Avogadro's number (Appendix C) the number of binding sites needed for the cations were determined (Table 10:10). Phytic acid has six phosphorus atoms in phosphate groups and has 12 binding sites for the cations (Cosgrove, 1966). It has been determined for Cucurbita moschata that 85% of the cotyledon P was present as phytate (Splittsoesser, 1982). The results in Table 10:10 are shown using all the measured P and assuming

TABLE 10:8

Ratios of Mineral Percentages Within Parent and Hybrid Embryos.

Species	Mg/K	Mg/Ca	K/Mg	K/Ca	Ca/Mg	Ca/K	P/Mg	P/K	P/Ca
C. maxima	0.78	20.83	1.32	27.50	0.05	0.04	2.36	1.79	49.17
C. maxima X C. andreana	0.80	14.85	1.25	18.57	0.07	0.05	2.23	1.78	33.14
C. andreana x C. maxima	0.85	10.71	1.18	12.68	0.09	0.08	2.26	1.92	24.29
C. andreana	1.16	8.40	0.86	7.25	0.12	0.14	1.98	2.30	16.67

TABLE 10:9

Percent Cation Concentration of the Embryos Relative to the % P Concentration.

Species	Mg / P	K / P	Ca / P	Mg + K / P	Mg + Ca / P	K + Ca / P	Mg + K + Ca / P
C. maxima	0.42	0.56	0.02	0.98	0.44	0.58	1.00
C. maxima X C. andreana	0.45	0.56	0.03	1.01	0.48	0.59	1.04
C. andreana x C. maxima	0.44	0.52	0.04	0.96	0.48	0.56	1.00
C. andreana	0.50	0.43	0.06	0.94	0.56	0.49	1.00

that only 85% of the measured P was in the phytate form. When the total phosphorus value was used there was a cation deficit. When 85% of the P level was used in the calculation the ratio of cation sites needed to those available on the P was close to unity. Assuming that 85% of the measured P was in phytic acid and using the formula for anhydrous phytic acid (Cosgrove, 1966), the phytic acid contents of the embryos were calculated. C. maxima , C. maxima x C. andreana , C. andreana x maxima and C. andreana embryos contained 3.6, 3.5, 4.1 and 3.5% of their weight as phytic acid.

In Chapter 8 it was pointed out that Ca levels were higher by about 13% in the embryos of C. maxima fruits grown indoors. Embryos of C. maxima fruits used for NAA had a difference between the indoor and outdoor Ca levels of only 6%. Differences for the other three minerals were even less being 4% for P and less than 1% for Mg and K. Hence growth location was not contributing to variation in mineral levels.

Seed coats were analyzed for some batches of embryos (Table 10:11). Standard errors were within similar ranges for seed coats and embryos and have been omitted for easier comparison. In all cases Mg was lowest in outer seed coats and highest in the embryos. Magnesium was particularly low in C. maxima outer seed coats. Potassium was the opposite in being highest in the outer seed coat and lowest in the embryos of all but C. maxima where it was the lowest in inner seed coats. Calcium was highest in the inner seed coats. Phosphorus was much higher in the embryos than in the two seed coats. If the total mineral levels in both seed coats were considered the Ca and K exceeded the levels of the embryos but the amount of Mg and P was less than that present in the embryos

TABLE 10:10

The Number of Binding Sites Needed by the Measured Cations and the Number of Anionic Sites Available on Phytic Acid.

Species	Mineral Concn.	No. of Binding Sites	Cation Binding Sites / Anionic Sites on PA
C. maxima	0.50% Mg 0.66% K 0.024% Ca	2.46×10^{20} 1.00×10^{20} 0.072×10^{20}	$\frac{3.53 \times 10^{20}}{4.58 \times 10^{20}} = 0.77$
	% Total P 1.18% % P as PA 0.97%*	4.58×10^{20} 3.89×10^{20}	$\frac{3.53 \times 10^{20}}{3.89 \times 10^{20}} = 0.91$
C. andreana	0.58% Mg 0.50% K 0.069% Ca	2.86×10^{20} 0.77×10^{20} 0.20×10^{20}	$\frac{3.83 \times 10^{20}}{4.47 \times 10^{20}} = 0.86$
	% Total P 1.15% % P as PA 0.94%	4.47×10^{20} 3.79×10^{20}	$\frac{3.83 \times 10^{20}}{3.79 \times 10^{20}} = 1.01$

* based on 85% P as Phytate (PA) (see Appendix C)

TABLE 10:11

Analysis of Minerals in Seed Coats and Embryos by NAA.

Species	Seed Part	% Mg	% K	% Ca	%P
*C. maxima**	Outer Seed Coat	0.046	0.825	0.058	0.136
	Inner Seed Coat	0.247	0.515	0.201	0.591
	Embryo	0.498	0.659	0.024	1.178
C. maxima x *C. andreana*	Outer Seed Coat	0.143	1.960	0.069	0.256
	Inner Seed Coat	0.224	0.710	0.144	0.472
	Embryo	0.523	0.649	0.035	1.157
C. andreana x *C. maxima*	Outer Seed Coat	0.172	1.790	0.064	0.380
	Inner Seed Coat	0.238	0.810	0.099	0.570
	Embryo	0.595	0.707	0.056	1.364
C. andreana	Outer Seed Coat	0.169	1.635	0.060	0.402
	Inner Seed Coat	0.200	0.730	0.166	0.446
	Embryo	0.578	0.500	0.069	1.150

* N = 2 seed coat samples analyzed from a pooled mixture of 50 or more seed coats from seeds of 10-25 fruits
Embryo values from Table 10:2

Table 10:12 presents the mineral concentrations from Table 10:11 as ratios to each other obtained by arbitrarily multiplying the percent mineral levels by 20. The phloem and xylem exudate mineral contents are rough estimates to indicate which minerals predominate in the two vascular exudates. The mean mineral levels used were derived from ranges given by Richardson et al (1982) for bulked phloem sap of C. maxima and root pressure xylem exudate. Their millimolar concentrations were converted to percent levels and also arbitrarily multiplied by 20 (Table 10:12). In both exudates K was the predominant cation. In phloem the ratio of K to Mg was 400:1 while in xylem it was about 20:1. The amount of all four minerals in the vascular solutions was lower than that accumulated in the seed coats and embryos. Potassium was the most abundant mineral in the seed coats but within the embryos it was surpassed by P. Although Ca was more abundant than P and Mg in the exudates and was relatively abundant in the seed coats it became least abundant in the embryos.

DISCUSSION

Cucurbit embryos had more P than Mg, K or Ca. The Ca concentrations were very low compared to that of the other three elements. The levels obtained in this study agreed well with those for C. maxima embryos from Lott et al (1978). In many legume embryos there is more P than Mg or Ca but K concentration often surpasses that of P (Branch and Gaines, 1983; Guardiola and Sutcliffe, 1972; Hocking, 1980). The castor bean embryo and endosperm also have P present in the highest amount and Ca in the lowest (Lott et al , 1982). Seeds of some species contain Ca deposits. For example, the seeds of Hibiscus esculentus L. have druse

TABLE 10:12

Ratios of Mineral Levels in Seed Coats and Embryos as Compared to the Ratios of Minerals in *C. maxima* Xylem and Phloem Exudates.

	Mg	:	K	:	Ca	:	P
C. maxima							
Outer Seed Coat	0.9	:	16.5	:	1.2	:	2.7
Inner Seed Coat	4.9	:	10.3	:	4.0	:	11.8
Embryo	10.0	:	13.2	:	0.5	:	23.6
C. andreana							
Outer Seed Coat	3.4	:	32.7	:	1.2	:	8.0
Inner Seed Coat	4.0	:	14.6	:	3.3	:	9.3
Embryo	11.6	:	10.0	:	1.4	:	23.0
Phloem Exudate*	0.02	:	7.0	:	0.06	:	0.04
Xylem Exudate**	0.05	:	1.1	:	0.33	:	0.17

* based on average levels of Richardson *et al* (1982) obtained for various organs.

** based on average levels of Richardson *et al* (1982) obtained for root pressure sap.

crystals of calcium oxalate in some cells (Webb and Arnott, 1981). Seeds with oxalate crystals may contain twice as much Ca as P (Spitzer and Lott, 1982).

The cucurbit species studied here differed more in their Ca levels than in levels of Mg, K and P. In addition, there was a greater variation in Ca within each species than there was in the variation of the other three minerals. No consistent pattern of correlation among the minerals was found within the parent and hybrid embryos. Such inconsistency was also reported for peanuts (Branch and Gaines, 1983) and soybeans (Raboy et al , 1984). In peanut seeds Mg was found to be correlated with P but not with K and Ca and there was a strong negative correlation between Ca and K. In soybean seeds (Glycine max) Ca level was significantly correlated with P but in G. soja there was no correlation between the two minerals. Factors that govern mineral interrelationships within embryos have not been adequately defined for most species.

Posternak (1965) stated that phytic acid in seeds represented 65-90% of the organically bound P. In a review by Lott (1984) it was reported that within dicot species 40-86% of the total seed P was found in phytic acid. In various legumes 40-60% of the total P may be present as phytic acid (Griffiths and Thomas, 1981; Lolas and Markakis, 1975) but the percent was as high as 85% for cucurbits (Splittstoesser, 1982). In various monocot and dicot species a strong positive correlation between total P and phytic acid P has been reported (Griffiths and Thomas, 1981; Lolas and Markakis, 1975; Lolas et al , 1976; Raboy and Dickinson, 1984). The high proportion of P as phytic acid in cucurbit embryos and the overall low cation concentrations resulted in a 1:1 ratio of cations to P.

The ratio of cations to P for soybean, peanut and sunflower was 2.7, 2.1 and 1.5 respectively (Chapter 6, Table 6:6). The physiological reason for the need for higher P in cucurbit embryos is not known but may be related to the function of the large cotyledons as photosynthetic leaves during seedling growth. When the needed cationic binding sites for Mg, K and Ca were compared to the available sites on phytic acid there was frequently a small cation deficit. Sharma and Dieckert (1975) found a cation deficit in their isolated globoid crystal fraction from peanuts and an excess of cations in the non-globoid fraction. They suggested that an organic cation substituted for the missing inorganic cations. Raboy and Dickinson (1984) multiplied P levels by a factor of 3.55 to obtain the amount of phytic acid. Their factor was derived from the molecular weight of an anhydrous phytic acid molecule and yielded phytic acid levels of 3.5-4.0% for cucurbit embryos (50% oil). These levels were in good agreement with the 7.7-9.1% phytic acid obtained for defatted seed flours of three Cucurbita species (Bolley and McCormack, 1952).

In most of the results it has been assumed that the bulk of the measured cations were bound to phytic acid. The ratios of cations to P and the comparison of the needed and potential binding sites on phytic acid indicated that all cations could be bound. However, at least some of the Ca and possibly some Mg could be in the cell walls (Hanson, 1984). Even if as much as 50% of the Ca were in the cell walls the change in the total cation balance would be very small since Ca is so much lower in concentration than K and Mg. The solubility of phytates varies with the type of cation bound to it with alkali metal phytates being more soluble than those of alkaline earth metals (Posternak, 1965). Thus potassium

phytates are more soluble and more likely to be present in the proteinaceous matrix outside of the globoid crystals as is quite commonly the case with legume embryos (Lott and Buttrose, 1978 a). Lott (1975) detected no K in the proteinaceous matrix of Cucurbita maxima although a small amount of P was detected. Studies on seed tissues of other species indicate a variation in the presence and absence of phytin outside the globoid crystals (Lott and Buttrose, 1978a,b,c). In some of the cases K without P can also be detected outside of the globoid crystal. Hence the proportion of K that is bound to phytin is not known for cucurbits but it is likely to be high. At present it is difficult to relate the ratios of mineral present in whole embryos to the actual distribution of the minerals within the embryos cells or to conclude what percent of the measured mineral is actually bound to phytic acid.

The testas of peas and lupines did not have the very high K levels found in cucurbits (Ferguson and Bollard, 1976; Guardiola and Sutcliffe, 1972; Hocking, 1980). Guardiola and Sutcliffe (1972) found pea testas relatively rich in Ca compared to the other minerals but their embryos contained more Ca than the testas. Ferguson and Bollard (1976) and Hocking (1980) studying peas and lupines found higher Ca in the testas than in the embryos. In these legumes the Ca levels of the embryos were higher than found in the cucurbits.

In Cucurbita maxima xylem the most abundant anion was nitrate, while the most abundant cation was ammonium followed by K (Richardson et al , 1982). Magnesium and Ca were much lower in concentration and, unlike the situation in most phloems studied, Mg was in lower concentration than Ca. In C. maxima phloem, K was the predominant cation while chloride was

the main anion. Magnesium, Ca and P were all lower in the phloem than in the xylem. Cucurbit seed coats had a high concentration of K but the overall mineral ratios in seed coats and embryos did not indicate the predominance of one vascular system over the other. Ziegler (1975) stated that the transport of Ca, K, Mg and P varied with their relative mobilities in the phloem and xylem. However, Pate and Hocking (1978) found for legumes that the vascular transport could not account for all the Mg and Ca accumulated in embryos. This problem remains even more obscure in the less studied cucurbit embryos. Since all four minerals were present in both testas, the likelihood of redistribution of minerals to the embryos from the seed coats is high.

Thus, in cucurbit embryos Ca was found to be a more variable element compared to Mg, K and P both between and within the species. The two parent species were different in their relative proportions of K and Mg and the reciprocal hybrids both resembled the C. maxima parent in this respect. Phosphorus concentration was essentially the same in the two parent species and was equal to or higher than the combined concentration of Mg, K and Ca. This low cation-to-P content resulted in the binding of Ca in the cucurbit ash but the significance of the ratio of the minerals to P for germination and seedling growth are not understood.

Chapter 11

DISTRIBUTION OF MAGNESIUM, POTASSIUM, CALCIUM AND PHOSPHORUS BETWEEN AXES AND COTYLEDONS OF PARENT AND HYBRID EMBRYOS WITH SPECIAL EMPHASIS ON CALCIUM.

INTRODUCTION

Cucurbit embryos are similar to the embryos of legume seeds in having two large cotyledons and a small root-shoot axis. The embryos are protected by two rather than the usual one seed coat. The structure of cucurbit seed coats has been described by Barber (1909), Singh (1953) and Singh and Dathan (1972) and was studied under the scanning electron microscope by Lott (1973). The seeds of C. maxima are dull white, flat, elongated or broadly oval with a strong marginal rim which is interrupted at the micropylar end (Whiting, 1938). This description applies as well to the smaller C. andreana seeds though the seed coats are tan rather than white. The shape of the embryo matches that of the seed. The cotyledons are flat and unfolded and the root-shoot axis brings the embryo to a narrow point at the micropylar end. The inner seed coat is thin and membranous and is believed to consist in part of remnants of both the nucellus and the endosperm (Singh and Dathan, 1972). Cucurbit seeds contain no endosperm. On the surface of the cotyledons the outline of provascular strands can be seen (Whitaker and Davis, 1962). In cross-section the cotyledons consist of an upper epidermis, two to three layers of palisade mesophyll, up to twenty layers of spongy mesophyll cells and a lower epidermis (Nelson, 1932). The provascular strands run

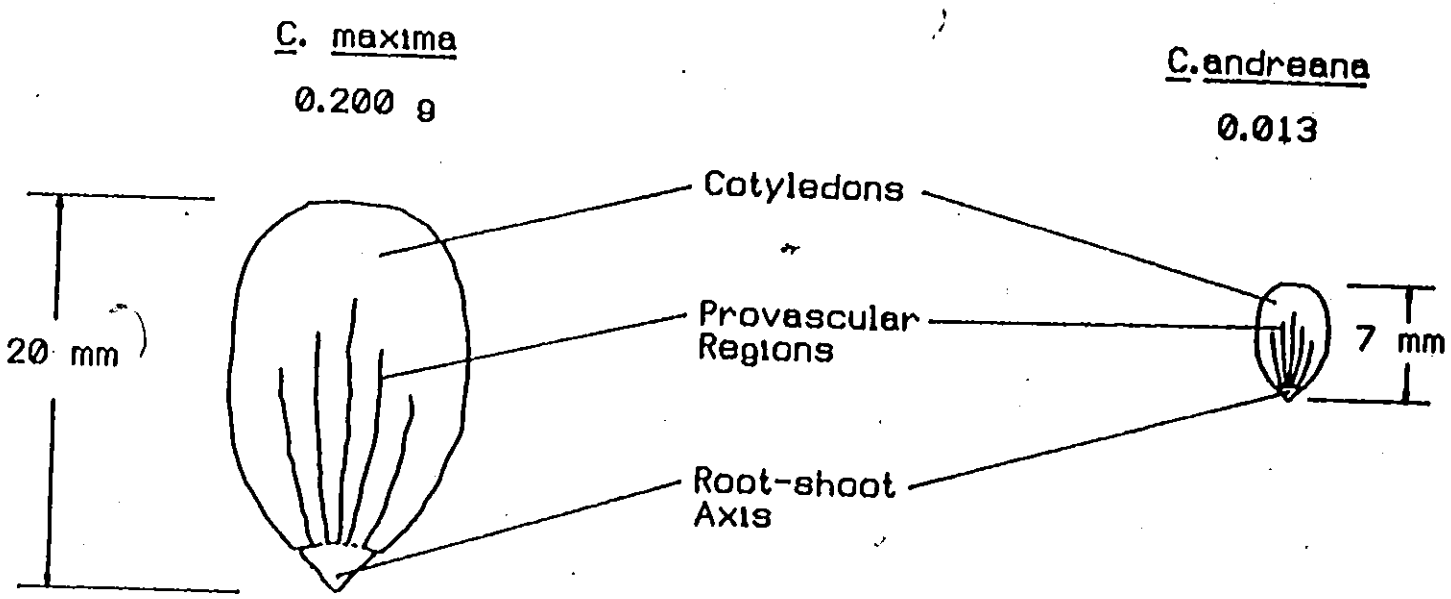
mainly through the spongy mesophyll (Whitaker and Davis, 1962).

The ultrastructure of the cotyledon cells in C. maxima has been studied by Lott et al (1971) and Lott and Vollmer (1973b). Cotyledon cells contain most of the storage reserves as lipid and protein bodies. Minerals are stored within the protein bodies as electron dense globoid crystals. Squash protein bodies consist of a proteinaceous matrix bound by a single membrane and within this matrix are one or more protein crystalloids and one or more globoid crystals. Globoid crystals can be surrounded by a less dense area called the soft globoid. Wiley (1971) found the diameters of C. maxima protein bodies, protein crystalloids and globoid crystals to be 5-12 μm, 4-10 μm and 1-5 μm respectively.

Whiting (1938) described C. maxima shoot axis as having a small growing point over-arched by the primordium of the first leaf. Whiting stated that the whole structure remained inconspicuous up to 6 days after germination even though primordia of 5 more leaves may have differentiated during this time. It was found in this study that a small, thin, pyramidal-shaped structure was just visible above the flat surface of the shoot area of the root-shoot axis. The structure was examined under the scanning electron microscope in both embryos from the dry seeds and in specimens that showed radicle protrusion (4 days after imbibition). There was no difference in the appearance of the pyramidal-shaped structure in the two kinds of specimens so that it was assumed that the structure represented the primordium of the first leaf. The area at the base of the primordium was flat in the dry embryo axis but showed a slight protrusion in the germinated specimen. This area thus corresponded to the meristematic region. The simplicity of the root-shoot axis aided in its

Figure 11 : 1

Cucurbita Embryos



removal from the cotyledons. Figure 11:1 shows the embryo structure and indicates the relative sizes of the two parent cucurbit species.

The initial studies of globoid crystals in C. maxima cotyledon cells with energy dispersive x-ray analysis showed that globoid crystals contained P, K and Mg with occasional traces of Ca (Lott, 1975). Subsequent work showed that C. maxima embryos did not have a random Ca distribution (Table 11:1). Calcium was more commonly found in globoid crystals from the root-shoot axis than in those from cotyledon cells (Lott et al , 1978). Within the cotyledons, future epidermal cells were more likely to have Ca-containing globoid crystals. Provascular cells and often also the adjacent layer of mesophyll cells frequently contained Ca in their globoid crystals but the bulk of the mesophyll cells were devoid of Ca in their globoid crystals. When a number of cucurbit species were compared, the small-seeded species such as C. andreana were were found to have a more homogeneous distribution of Ca within the cotyledon globoid crystals. However, Mg, K and P were more evenly distributed in globoid crystals of species with both the large and small embryos.

The results of analyses of Ca in axes and cotyledons by AAS are presented in this chapter. NAA was also applied to the analysis of isolated axes and cotyledons to compare the levels of Mg, K and P to Ca.

MATERIALS AND METHODS

The growth of fruits and preparation of seeds for analysis has been described in Chapters 2 and 7. Axes were removed from embryos by cutting with a razor blade at the point of connection between the axis and

TABLE 11:1

Distribution of Ca in Globoid Crystals of Cucurbita Embryos.

Embryo Size	Location of Globoid Crystals Containing Ca			
	Root-Shoot Axis	Cotyledons		
		Epidermis	Provascular	Mesophyll
> 50 mg	+ + +	+	+ +	0
< 50 mg	+ + +	+ + +	+ + +	+ + +

+ + + Ca^{2+} usually present in globoid crystals
+ + Ca^{2+} often present in globoid crystals
+ Ca^{2+} occasionally present in globoid crystals
0 Ca^{2+} rarely present except in globoid crystals in cells around provascular regions

Embryos of > 50 mg - C. maxima, C. mixta, C. moschata
Embryos of < 50 mg - C. andreana, C. pepo, C. foetidissima

Lott, et al (1978) Plant Physiol. 61: 984-988.
Lott, et al (1979) Plant Physiol. 63: 847-851.
Lott and Vollmer (1979) Plant Physiol. 63: 307-311.

the cotyledon. Weights of axes were obtained by weighing a batch of axes and dividing by the number pooled. Axes of C. andreana were particularly small and as a result fruits with small numbers of seeds did not contain enough tissue for separate analyses on axes and cotyledons. This accounts for the smaller sample numbers used in this chapter. Samples used for mineral analysis of the axis tissues were also bordering on being too small due to the problems described in Chapter 4. However, repeat analyses and comparison between the two analytical techniques showed that the accuracy was acceptable. It is believed that the much higher Ca levels in the axes resulted in minimization of the effects seen with small samples of C. maxima embryo tissues. The representative Ca levels per fruit for parent and hybrid embryos (Tables A:3, A:5, A:6 and A:9) were segregated into high and low Ca groups. Those with Ca levels above the median Ca level for the crop were called high Ca embryos and those with Ca levels below the median were called low Ca embryos. The AAS and NAA procedures were shown in Chapters 2 and 3. Statistical analyses were made using MTB.

RESULTS

Weights and Ca levels of axes and cotyledons of parent and hybrid embryos are shown in Table 11:2. Axes were small compared to cotyledons and contained less Ca on a per structure basis. However, on per gram basis the axes contained more Ca than the cotyledons in both parents and hybrids. Table 11:3 A shows the ratios and percent levels for the data from Table 11:2. Axes were larger relative to cotyledon size in C. andreana embryos as seen in the cotyledon to axis weight ratio of about 45 for C. maxima and about 19 for C. andreana . The axis made up about

TABLE 11:2

Sizes and Ca Concentrations of Whole Embryos, Axes and Cotyledon Pairs of Parent and Hybrid Embryos as Determined by AAS.

Species and N.	Wt. (g) (Mean ± SD)			µg Ca^{2+}/Tissue (Mean ± SE)			µg Ca^{2+}/g Tissue (Mean ± SE)		
	Axis	Cotyl. Pr.	Embryo	Axis	Cotyl. Pr.	Embryo	Axis	Cotyl. Pr.	Embryo
C. maxima N = 79	0.0041 ± 0.0006	0.184 ± 0.040	0.187 ± 0.040	6.34 ± 0.16	37.3 ± 1.5	43.3 ± 1.5	1546.3 ± 31.0	202.7 ± 6.2	231.6 ± 6.3
C. maxima x *C. andreana* N = 11	0.0037 ± 0.0005	0.153 ± 0.028	0.159 ± 0.029	5.99 ± 0.31	47.6 ± 3.4	55.2 ± 3.6	1618.9 ± 89.0	311.1 ± 31.0	347.2 ± 30.0
C. andreana x *C. maxima* N = 33	0.00058 ± 0.00009	0.0121 ± 0.0023	0.0130 ± 0.0027	0.86 ± 0.06	6.57 ± 0.53	7.40 ± 0.46	1482.8 ± 71.0	543.0 ± 27.0	569.2 ± 22.0
C. andreana N = 21	0.00066 ± 0.00013	0.0122 ± 0.0030	0.0125 ± 0.0031	1.08 ± 0.74	7.50 ± 0.45	8.13 ± 0.38	1636.4 ± 62.0	600.0 ± 17.7	650.4 ± 14.9

TABLE 11:3

Embryo Sizes and Ca Distribution Between Axes and Cotyledon Pairs of Parent and Hybrid Embryos.

A.

Species and N	Weight (g)			$\mu g\ Ca^{2+}$/Tissue			$\mu g\ Ca^{2+}$/g Tissue
	Axis / Cotyl.*	Cotyl* / Axis	% in Axis	Axis / Cotyl.*	Cotyl* / Axis	% in Axis	Axis / Cotyl.*
C. maxima N = 79	0.02	44.9	2.2	0.17	5.9	14.6	7.6
C. maxima x C. andreana N = 11	0.02	41.4	2.3	0.13	7.9	10.9	5.2
C. andreana x C. maxima N = 33	0.05	20.9	4.5	0.13	7.6	11.6	2.7
C. andreana N = 21	0.05	18.5	5.3	0.14	6.9	13.3	2.7

B.

Parameter Compared	Ratios Calculated	
	C. andreana / C. maxima	C. maxima / C. andreana
Wt. Axis (g)	0.16	6.21
Wt. Cotyl. (g)	0.07	15.08
Wt. Embryo (g)	0.07	14.96
$\mu g\ Ca^{2+}$/Axis	0.17	5.87
$\mu g\ Ca^{2+}$/Cotyl.	0.20	4.97
$\mu g\ Ca^{2+}$/Embryo	0.19	5.33
$\mu g\ Ca^{2+}$/g Axis	1.06	0.94
$\mu g\ Ca^{2+}$/g Cotyl.	2.96	0.34
$\mu g\ Ca^{2+}$/g Embryo	2.81	0.36

* Cotyl. = Cotyledon Pair

2% of the embryo weight in C. maxima and 5% in C. andreana . Hybrid embryos were similar to their female parents. On a per tissue basis, the Ca distribution between cotyledons and axes was about 6:1 in C. maxima and 7:1 in C. andreana . The percent of the Ca in the axis was in the 13-15% range for both species thus the axes contained a disproportionately higher amount of Ca compared to the cotyledons. The hybrid embryos had more Ca in the cotyledons relative to the axes than the parents. On a per gram basis the axes contained nearly 8 times more Ca than the cotyledons in C. maxima and about 2.5 times the amount in C. andreana . The C. andreana x C. maxima embryos were more similar to their female parent than the C. maxima x C. andreana embryos were to their female parent. In Table 11:3B shows the ratios of the Ca concentrations in the two parent species. At per tissue levels C. andreana embryos contained much less Ca in both axes and cotyledons due to their small size. On a per gram basis Ca in the axes was about the same in both species but the concentration in C. andreana cotyledons was three times higher than in C. maxima cotyledons.

Chapter 8 showed that there was considerable variation in Ca level in embryos of the parents and hybrids. The variation in Ca level was of a different magnitude in the axes than in the cotyledons (Table 11:4). Embryos with higher Ca level contained more Ca in both axis and cotyledons but the increase was greater in the cotyledons for C. maxima and C. maxima x C. andreana . This increase was accompanied by a small decrease in weight of both axes and cotyledons. Thus embryos which had higher Ca levels tended to be smaller in both their axes and their cotyledons. C. andreana x C. maxima and C. andreana embryos with the high Ca level had

TABLE 11:4

Calcium Levels in Axes and Cotyledon Pairs of Embryos Containing Low and High Ca Levels as Determined by AAS.

Species N	Wt. (g) and Ca^{2+} (µg)	Low Ca			High Ca			% Difference High to Low Ca	
		Axis	Cotyl. Pair	% in Axis	Axis	Cotyl. Pair	% in Axis	in Axis	in Cotyl. Pair
C. maxima N = 42 (Low) N = 38 (High)	Wt.	0.0042	0.187	2.2	0.0040	0.180	2.2	- 4.8	- 3.7
	Ca^{2+}	5.83	30.8	15.9	6.91	44.6	13.4	+ 18.5	+ 44.8
C. maxima x C. andreana N = 6 (Low) N = 5 (High)	Wt.	0.0038	0.160	2.3	0.0036	0.144	2.4	- 5.3	- 10.0
	Ca^{2+}	5.91	40.0	12.9	6.10	56.6	9.7	+ 3.2	+ 41.5
C. andreana x C. maxima N = 15 (Low) N = 18 (High)	Wt.	0.00054	0.0109	4.7	0.00061	0.0131	4.8	+ 13.0	+ 20.2
	Ca^{2+}	0.62	4.26	12.7	1.05	8.49	11.0	+ 69.4	+ 99.3
C. andreana N = 7 (Low) N = 14 (High)	Wt.	0.00063	0.0121	4.9	0.00067	0.0123	5.2	+ 6.3	+ 1.7
	Ca^{2+}	0.86	6.44	11.8	1.19	8.03	11.8	+ 38.3	+ 24.7

Ca^{2+} - called "low" if levels below the median value
- called "high" if levels above the median value

TABLE 11:5

Correlation Between Weights of Axes and Cotyledon Pairs and Ca Levels in Parent and Hybrid Embryos.

Parameters Correlated	C. maxima N = 79	C. maxima x C. andreana N = 11	C. andreana x C. maxima N = 33	C. andreana N = 21
Axis Wt. vs. Cotyledon Wt.	0.59**	- 0.09	0.88**	0.87**
µg Ca^{2+}/Axis vs. µg Ca^{2+}/Cotyl. Pr.	0.73**	0.42	0.83**	0.81**
Ca^{2+}/g Axis vs. Ca^{2+}/g Cotyledon	0.67**	0.38	0.75**	0.72**
Axis Wt. vs. µg Ca^{2+}/Axis	0.61**	0.27	0.79**	0.86**
Axis Wt. vs. µg Ca^{2+}/g Axis	- 0.05	- 0.53*	0.55**	0.39*
Cotyl. Wt. vs. µg Ca^{2+}/Cotyl. Pr.	0.62**	0.17	0.88**	0.88**
Cotyl. Wt. vs. µg Ca^{2+}/g Cotyl.	- 0.01	- 0.61*	0.72**	- 0.11

* significant at P = 0.05 or less
** significant at P = 0.01 or less

heavier axes and cotyledons. C. andreana embryos were different from the other three kinds of embryos in having a higher increase in Ca level in the axis rather than in the cotyledons.

Table 11:5 presents correlation coefficents for size and Ca levels of axes and cotyledons of parent and hybrid embryos. Weights and Ca levels were positively correlated in C. maxima embryos but not for Ca levels per gram embryo parts. The C. maxima x C. andreana hybrid was unusual in having few significant correlations and those few were negative hence opposite to that of the C. maxima parent. C. andreana and C. andreana x C. maxima embryos were more similar to each other and had positive correlations for most of the compared parameters.

Tables 11:6 and 11:7 show the distribution of Mg, K, Ca and P in axes and cotyledons of parent and hybrid embryos as determined by NAA. Because Mg, K and P levels were much higher than Ca levels it was convenient to express the mineral levels in percent rather than per gram. In all cases (Table 11:6) axes contained a higher percent of the four minerals than the cotyledons but the highest difference was found with Ca levels. Higher concentration of P in the axis represents about 8% phytic acid as opposed to about 4% found in cotyledons and whole embryos (Chapter 10). Table 11:7 shows that the percent of the total Ca that was in the axis was higher in both parent and hybrid embryos than the percent of each of the other three minerals. The variations in mineral levels, as indicated by coefficients of variation, were similar for most axes and cotyledons. Variation in Ca level in C. maxima cotyledons was exceptionally high.

Table 11:8 indicates considerable variation between species,

TABLE 11:6

% Hg, K, Ca and P in Axes and Cotyledons of Parent Species and Hybrids as Determined by NAA.

Species	% Hg		% K		% Ca		% P	
	Axis	Cotyl.**	Axis	Cotyl.	Axis	Cotyl.	Axis	Cotyl.
C. maxima N = 17	0.943 ±0.032* 14.0%	0.472 ±0.011 10.0%	0.920 ±0.023 10.4%	0.641 ±0.017 11.1%	0.185 ±0.012 24.3%	0.0197 ±0.002 36.5%	2.328 ±0.120 21.3%	1.132 ±0.017 6.2%
Ratio Axis / Cotyl. Pr.	2.0		1.4		9.4		2.1	
C. maxima x *C. andreana* N = 8	1.004 ±0.003 9.2%	0.501 ±0.019 10.6%	0.945 ±0.062 18.5%	0.639 ±0.043 18.9%	0.198 ±0.011 15.2%	0.0337 ±0.0036 30.6%	1.864 ±0.097 14.7%	1.078 ±0.052 13.7%
Ratio Axis / Cotyl. Pr.	2.0		1.5		5.9		1.7	
C. andreana x *C. maxima* N = 15	0.808 ±0.028 7.5%	0.544 ±0.025 10.5%	0.878 ±0.055 14.1%	0.702 ±0.043 13.7%	0.148 ±0.018 27.0%	0.0507 ±0.0062 27.4%	1.955 ±0.146 16.7%	1.211 ±0.039 7.3%
Ratio Axis / Cotyl. Pr.	1.5		1.3		2.9		1.6	
C. andreana N = 9	0.863 ±0.019 3.7%	0.550 ±0.036 11.3%	0.810 ±0.046 9.8%	0.489 ±0.049 17.4%	0.187 ±0.012 11.2%	0.0613 ±0.0023 6.4%	2.246 ±0.213 13.4%	1.071 ±0.081 13.2%
Ratio Axis / Cotyl. Pr.	1.6		1.7		3.1		2.1	

* Mean ± SE, % values represent c.v. ** Cotyl. = Cotyledon pair

TABLE 11:7

Distribution of Minerals Between Axes and Cotyledons of Parent and Hybrid Embryos as Determined by NAA.

Species		µg Mg per Region		µg K per Region		µg Ca per Region		µg P per Region	
C. maxima		Axis	Cotyl.*	Axis	Cotyl.	Axis	Cotyl.	Axis	Cotyl.
N = 17	Mean	40.4	916	39.2	1228	7.87	38.7	98.8	2201
	S.E.	± 1.6	± 57	± 1.2	± 59	± 0.55	± 4.2	± 5.2	± 119
	c.v.	16.6%	25.7%	12.2%	20.0%	28.7%	43.9%	21.8%	22.4%
	% in Axis	4.2%		3.1%		16.9%		4.3%	
C. maxima x *C. andreana*									
N = 8	Mean	37.0	739	35.2	935	7.27	48.2	69.4	1579
	S.E.	± 1.9	± 48	± 3.1	± 66	± 0.43	± 3.2	± 5.8	± 86
	c.v.	14.6%	18.3%	25.0%	20.0%	16.8%	18.9%	23.6%	15.5%
	% in Axis	4.8%		3.6%		13.1%		4.2%	
C. andreana x *C. maxima*									
N = 15	Mean	4.86	68.8	5.30	88.2	0.90	6.50	11.75	154.6
	S.E.	± 0.24	± 4.2	± 0.45	± 4.8	± 0.14	± 1.04	± 0.98	± 4.6
	c.v.	11.0%	15.7%	18.7%	12.1%	34.4%	36.0%	18.6%	6.6%
	% in Axis	6.6%		5.7%		12.2%		7.1%	
C. andreana									
N = 9	Mean	6.11	71.0	5.71	63.0	1.32	7.94	16.5	145.5
	S.E.	± 0.39	± 4.78	± 0.25	± 6.0	± 0.08	± 0.54	± 3.7	± 13.1
	c.v.	11.1%	11.7%	7.5%	16.7%	10.6%	11.7	31.7%	15.6%
	% in Axis	7.9%		8.3%		14.3%		10.2%	

* Cotyl. – Cotyledon pair

between embryo parts and between the two methods of presenting mineral levels. For example, Ca levels were not significantly different in axes of the parent and the hybrid embryo types but the cotyledon Ca levels were different. Phosphorus levels were similar in the four kinds of embryos in both axes and cotyledons if compared on the percent mineral levels but were different for C. maxima and its hybrid embryos when the actual level per embryo parts were compared. Variability was also seen in correlations between the minerals and between the minerals and weights of the embryo parts. Most correlation coeffients for the weights and mineral levels were not significant. The strongest correlations were between Mg and the other three minerals in both the axes and cotyledons. Overall the correlations, even when significant, were weak and there was a frequent lack of correspondence between correlations on the per tissue and the per gram tissue level. No consistent pattern of mineral interaction between the embryo parts emerged from either correlation or regression analysis. Tables showing the correlations mentioned are found in Appendix D.

Table 11:9 shows the relative proportions of the mineral within axes and cotyledons based on arbitrarily equating the Mg concentrations to one. The concentrations of Mg and K are close to 1:1 in the axes of both parent and hybrid embryos. Potassium is somewhat higher in proportion to Mg in all but the C. andreana cotyledons. Calcium was very low in relation to Mg, K and P but was higher in the axes of all embryos. There was about twice as much P as Mg or K in both axes and cotyledons. Comparing total cation content to P, as was done for whole embryos in Chapter 10, it was found that axes tended to have a cation deficit while cotyledons had a slight excess. C. maxima x C. andreana hybrid was an

TABLE 11:8

Comparison of Mean Levels of Mg, K, Ca and P in Parent and Hybrid Embryo Parts.

A. % Mineral (Mean Value)

Species	%Mg Axis	%Mg Cotyl.	%K Axis	%K Cotyl.	%Ca Axis	%Ca Cotyl.	%P Axis	%P Cotyl.
C. maxima	0.94a	0.47a	0.92a	0.64a	0.19a	0.020a	2.33a	1.13a
C. maxima x C. andreana	1.00a	0.50ab	0.95a	0.64a	0.20a	0.034b	1.86a	1.08a
C. andreana x C. maxima	0.81b	0.54b	0.88a	0.70a	0.15a	0.051c	1.96a	1.21a
C. andreana	0.86b	0.55b	0.81a	0.49b	0.19a	0.061c	2.25a	1.07a

B. µg Mg, K, Ca and P per Embryo Part

	Mg (µg) Axis	Mg (µg) Cotyl.	K (µg) Axis	K (µg) Cotyl.	Ca (µg) Axis	Ca (µg) Cotyl.	P (µg) Axis	P (µg) Cotyl.
C. maxima	40.4a	915.9a	39.2a	1227.7a	7.87a	38.7a	98.9a	2201.0a
C. maxima x C. andreana	37.0a	738.8b	35.2a	934.6b	7.28a	48.2a	69.5b	1579.1b
C. andreana x C. maxima	4.86a	68.8a	5.30a	88.2a	0.90a	6.50a	11.8a	154.6a
C. andreana	6.11a	71.0a	5.71a	63.0b	1.32b	7.94a	16.5a	145.5a

A means in each column were separated by Tukey's test (Zar, 1985) following analysis of variance on MTB.

B means of each hybrid and parent pair were analyzed by a t-test (MTB).

A and B same letter name indicates no significant difference in the means at P = 0.05.

Cotyl. = cotyledon pair

TABLE 11:9

Mineral Ratios in Axes and Cotyledons of Parent and Hybrid Embryos.

Species N	Embryo Part	Ratio Mg	: K	: Ca	: P	$\frac{Mg + K + Ca}{P}$
C. maxima N = 17	Axis	1	: 1	: 0.2	: 2.5	0.88
	Cotyl.	1	: 1.4	: 0.04	: 2.4	1.00
C. maxima x *C. andreana* N = 8	Axis	1	: 0.9	: 0.2	: 1.9	1.15
	Cotyl.	1	: 1.3	: 0.07	: 2.2	1.09
C. andreana x *C. maxima* N = 15	Axis	1	: 1.1	: 0.2	: 2.4	0.94
	Cotyl.	1	: 1.3	: 0.1	: 2.2	1.07
C. andreana N = 9	Axis	1	: 0.9	: 0.2	: 2.6	0.83
	Cotyl.	1	: 0.9	: 0.1	: 1.9	1.03

Cotyl. = cotyledon pair

exception in having an excess of cations even in the axis, a result due to the lower P concentration.

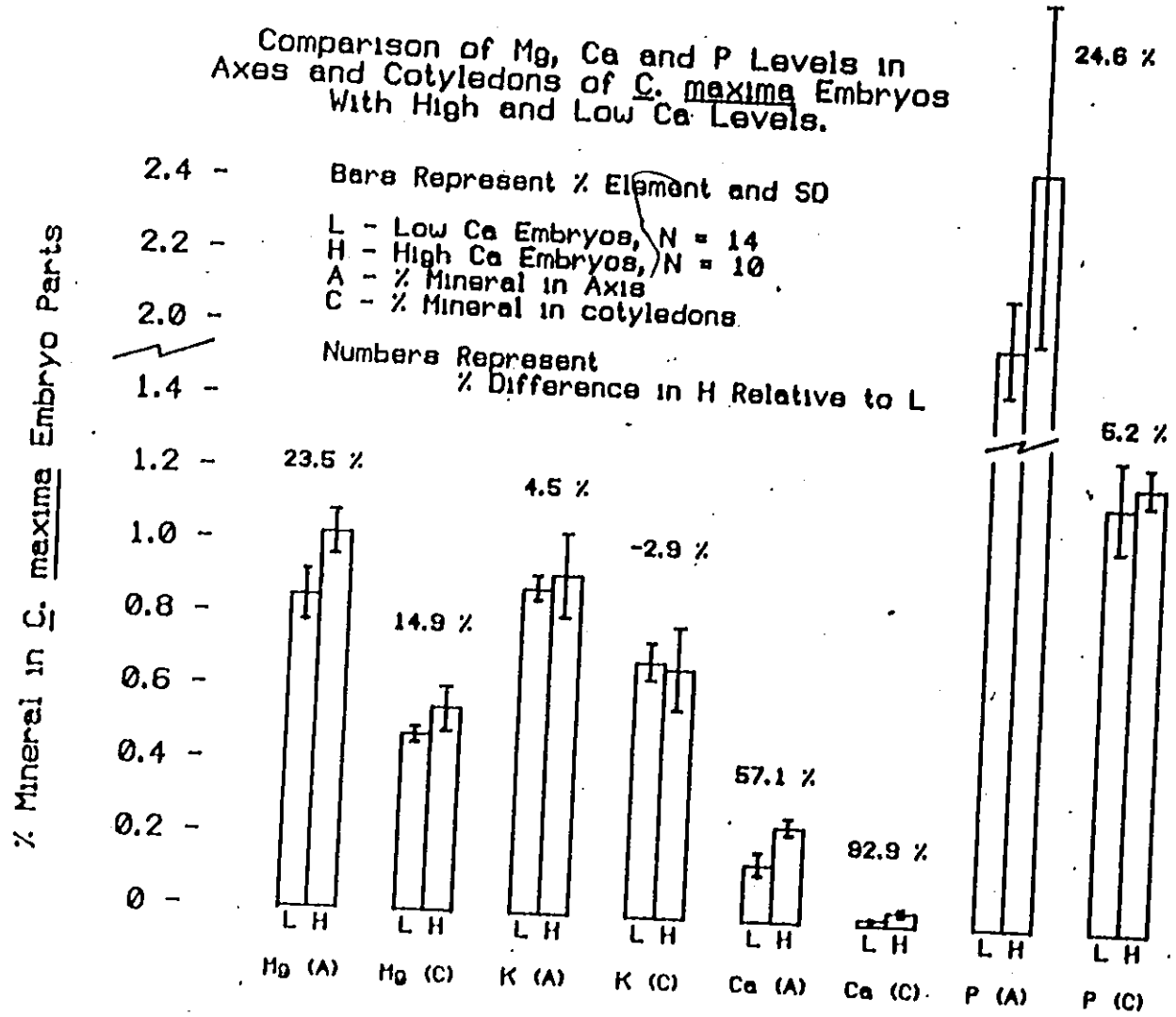
The main difference in the C. maxima embryos with high and low Ca was in their cotyledons (Table 11:4). In Figure 11:2 it can be seen that Mg and P are higher in embryos with higher Ca levels but their increase is greater in the axes than in the cotyledons. There is only a small change in the K level and in the cotyledons of high Ca embryos the K tends to be lower although the difference was not significant. The percent difference in the Ca levels is very much higher than that of the other minerals but the overall Ca concentration is very small compared to the levels of Mg, K and P. Concentrations of oil were determined for embryos to confirm that differences in oil content were not responsible for mineral level differences. High Ca embryos contained 46.2 ± 2.3% oil while low Ca embryos had 47.5 ± 3.2%; the amount of oil in axes was not markedly different from oil contents of the cotyledons.

DISCUSSION

The distribution of elements in axes and cotyledons of the parent cucurbit species explained why Ca-containing globoid crystals were found more commonly in the root-shoot axes of both C. maxima and C. andreana (Lott and Vollmer, 1979). The per gram Ca concentrations in the axis of the two species were almost identical. C. maxima cotyledons, which were 15 times heavier than those of C. andreana ,contained only a third of the Ca found in the small C. andreana cotyledons. Lott et al (1978, 1979) established that the amount of Ca found in C. maxima cotyledons was very specifically sequestered as described earlier. It is still not certain

Figure 11 : 2

Comparison of Mg, Ca and P Levels in Axes and Cotyledons of C. maxima Embryos With High and Low Ca Levels.



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whether there is an actual restriction against Ca storage in the mesophyll cells or whether the distribution could be affected by available Ca concentrations.

When compared to the other three main storage minerals, Ca remained exceptional in being present in the lowest amount and in being more variable within and between the species. Calcium was also different in that a higher percent of the total content was stored in the axis than was the case for the other three minerals. The variability in Ca levels was less in the axes than in the cotyledons. Whether the higher and less variable Ca levels in the axes were related to its low mobility and hence the need to have it in the tissues which would begin growth on germination is not certain. It is interesting that analysis of mineral concentrations in pea axes and cotyledons (Ferguson and Bollard, 1976; Guardiola and Sutcliffe, 1972) did not find a larger proportion of Ca in the axes of this legume. The percent of total Ca present in the axes of pea seeds ranged from 1.6 to 2.6%. In peas total cation concentration was much higher relative to P and the K level was very similar in the axes and the cotyledons. Pea cotyledons, though contributing nutrients during germination, do not become photosynthetic leaves.

Studies on mineral accumulation, particularly as related to distribution of the elements between embryo parts, remain few. Greenwood et al (1984) reported some differences between cotyledons and axes of castor beans. However, it must be cautioned that castor beans have a large endosperm at maturity and thus have a complex mineral storage pattern. Castor bean root-shoot axes contained 45% lipid by weight compared to 70% in the cotyledons. This difference in lipid content was not found in

cucurbit embryos. Castor bean embryos also differed from cucurbits in having less phytic acid (1%) in the root-shoot axis than in the cotyledons (1.4%). Greenwood et al (1984) also found that the mineral accumulation was completed earlier in the axes than in the cotyledons.

The fact that correlations between minerals in cucurbit axes and cotyledons showed no consistent patterns between tissues and between species indicated that there was no strong link between individual minerals. Each of the four minerals could vary independently within the axes and cotyledons but the causes of the variation remain undefined. The small cation deficit in the cotyledons may be due to the higher K and lower P concentration.

Thus cucurbit embryos store a high proportion of their total mineral content in the small root-shoot axes. The proportion of Ca stored in the axes can be 2 to 4 times that of Mg, K and P and the proportion of the total Ca stored in the axes of C. maxima is higher than that stored in C. andreana . Concentrations of Ca per gram axis are similar in the two species but the cotyledons show the 3:1 difference seen in whole embryos. In embryos known to have higher Ca levels, the main increase in Ca is also in the cotyledons. The requirements for high axis Ca for germination and seedling growth will be demonstrated in the next chapter.

Chapter 12

COMPARISON OF CALCIUM AND OTHER STORAGE MINERAL DISTRIBUTION IN DRY EMBRYOS AND SEEDLINGS OF *C. MAXIMA* AND *C. ANDREANA*.

INTRODUCTION

Germination of cucurbit seeds can, in practice, be achieved under a wide range of conditions although the seeds germinate best in the dark and may be under phytochrome control (Fritts and Loy, 1981). Ba-Amer and Bemis (1968) reported that cucurbit seeds removed directly from a mature fruit will not germinate and require a period of after-ripening outside the fruit for about 25 days. While many of the non-endospermic legumes, such as *Phaseolus vulgaris*, have embryos similar to those of the cucurbits with large cotyledons acting as the main storage reserves, their cotyledons undergo autolysis after the reserves are depleted (McKersie and Senaratna, 1983). Cucurbit cotyledons become fully photosynthetic (Lott, 1970a) and may persist for several weeks (Lasley and Garber, 1978).

The degradation of storage reserves in cucurbit embryos is markedly influenced by the testa. Slack *et al* (1977) and Davies and Chapman (1979a) found that little degradation of protein and lipid occurred as long as the seed coats remained on the cotyledons. It has been shown that the cucurbit seed coats restrict oxygen uptake by the embryos (Brown, 1940; Pesis and Ng, 1986). To ensure testa removal at the appropriate stage of germination cucurbits have a protuberance called a peg in the root-shoot transition area (Witztum and Gersani, 1975). This

peg presses on the lower half of the seed coat and enables the hooked, elongating hypocotyl to pull the cotyledons out of the seed coat.

Mobilization of mineral reserves involves the breakdown of phytin. Scott and Loewus (1986) discussed the current biochemical information on the degradation of phytin but pointed out that much still remains unclear. The enzymes involved are acid phosphatases called phytases. These enzymes increase markedly after germination begins (Mandal and Biswas, 1970) and may be present or absent in the dry seeds (Maga, 1982; Mandal and Biswas, 1970). Splittstoesser (1982) found no phytase activity in dry pumpkin seeds. Goel and Sharma (1979) have found multiple forms of phytase in germinating C. maxima cotyledons and suggested that this was compatible with the accepted stepwise removal of phosphate groups from the inositol moiety. The released inositol is believed to be converted to uronic acid and arabinose and incorporated into seedling cell walls (Loewus, 1971; Sasaki and Taylor, 1984). In addition inositol may be incorporated into phospholipids in the plasma membranes (Loewus and Loewus, 1983). The release and transport of Ca, Mg and K out of the cucurbit cotyledons is not documented.

The ultrastructural evidence on Cucurbita indicates that globoid crystal digestion proceeds along with protein degradation (Lott and Vollmer, 1973a). In peas Guardiola and Sutcliffe (1971) found that a higher percent of phytin than protein was hydrolyzed in the same time period during germination. Studies on legumes have shown a linear relationship between the export of minerals and the decrease in cotyledon dry matter (Collins and Sutcliffe, 1977; Hocking, 1980). These authors found, however, that individual minerals were not mobilized from the

cotyledons at the same rate nor to the same extent. Guardiola and Sutcliffe (1972) suggested that the degree of mobilization of individual minerals may be related to their mobilities in the phloem. Cotyledons may also act as sinks especially for Ca (Guardiola and Sutcliffe, 1972; Hocking, 1980; Marshall and Kozlowski, 1975).

Mobilization of minerals out of cucurbit cotyledons was studied mainly in Cucurbita maxima with emphasis on Ca. Mobilization of minerals from Cucurbita andreana seeds and differences in mobilization during light and dark growth were also determined. Mineral composition of fully expanded photosynthetic cotyledons was studied for comparison.

MATERIALS AND METHODS

Ca Levels in Dry Seed Cotyledons, Cotyledons of Etiolated Seedlings and Photosynthetic Cotyledons :

One hundred seeds of Stokes C. maxima cv. Warted Hubbard were imbibed for an hour in deionized distilled water. They were germinated in the dark at room temperature on ashless filter paper in Petri dishes moistened with deionized distilled water. Fifty seeds with the most prominent radicle protrusion on the third day after imbibition were placed on perforated plastic tops over aerated deionized distilled water tanks (1.5 litre capacity). The seedlings were grown in the dark in a growth chamber at 30°C and were harvested on the 10th day. The lengths of the hypocotyls and cotyledons of each seedling were measured. The cotyledons were removed and weighed before and after drying for 24 hr. at 105°C and were analyzed for Ca by AAS (Chapter 2).

Twenty-five randomly selected seeds were also imbibed and then

both seed coats were removed and the embryos were separated into axes and cotyledons for AAS analysis as ungerminated controls. Older cotyledons and several recently expanded green foliage leaves from 2 week old C. maxima plants grown in soil in lighted growth chambers were also weighed, measured, dried and analyzed for Ca by AAS.

Ca in Seedlings of Seeds Germinated With and Without Seed Coats :

Four batches of 30 seeds of field grown C. maxima seed stock were individually weighed, measured and imbibed as described above. Throughout the experiment the seeds were kept in numbered positions so that seed and seedling measurements could be related. After imbibition one batch of seeds was placed directly into Petri dishes for germination. Another batch had the outer seed coats removed while the third batch had both seed coats removed before being placed in the Petri dishes. A fourth batch was used as an ungerminated control and was analyzed as separated cotyledon pairs and pooled axes samples.

On the third day following imbibition the seeds were placed in the growth chambers as described above. In this experiment larger tanks (3.7 litre) were used and nylon mesh replaced the perforated top. After 8-10 days the seedlings were measured, separated into cotyledon pairs and the rest of the seedling and dried for AAS analysis. The water in the tanks was analyzed at the beginning, middle and end of the growth period.

Levels of Mg, K, Ca and P in Dark-grown Seedlings :

Thirty seeds of C. maxima and 140 seeds of C. andreana , derived in each case from one field grown fruit, were imbibed, germinated and grown in deionized distilled water in the dark as described above. Only pooled seed and seedling weights were determined but lengths of the seeds,

cotyledons, hypocotyls and roots were obtained for each specimen. Between the third and fourth day after imbibition any seed coats of both species still remaining on the cotyledons were removed manually. Seedlings of C. maxima were harvested on the ninth day and those of C. andreana on the tenth day. The dried and pooled cotyledon pairs and the rest of the seedling portions were ground and analyzed for Mg, K, Ca and P by NAA (Chapter 3).

Levels of Mg, K, Ca and P in Light-grown Seedlings :

Thirty seeds of the same field-grown C. maxima fruit were germinated and grown as above except that a 12 hour light/dark cycle was used. After 7 days the seedlings were harvested, separated into the two portions, dried, ground and analyzed by NAA. In addition 12 seeds were planted in soil, watered with 1/4 strength Hoagland's solution and grown under a 12 hour light/dark cycle. Only the cotyledons were harvested for analysis at 7 days.

Leakage of Mg, K and Ca from Seed Coats and Embryos :

Two batches of thirty embryos were selected from the same seed stock used for the germination experiments. The outer seed coats were removed from both batches. The embryos with the inner testa and the outer seed coats of each batch were covered with 50 ml of deionized distilled water and placed on a shaker. One batch of embryos and seed coats were shaken for one hour while the other batch was left to soak on the shaker for 24 hours. The supernatant was removed by filtration through ashless filter paper and made up to the initial volume of 50 ml. Four subsamples of each solution were analyzed for Mg, K and Ca by NAA.

TABLE 12:1

Some Measurements of Dry Seeds, Etiolated Seedlings and Photosynthetic Cotyledons of *C. maxima*.

Parameters Measured	Cotyledons of Dry Embryos (Mean ± SD)	Etiolated Seedling Cotyledons* (Mean ± SD)	Photosynthetic Cotyledons** (Mean ± SD)
No. Cotyl. Pairs	25	45	2
Wt. Hydrated Cotyl. Pr. (g)	0.20 ± 0.04	0.57 ± 0.16	2.30 ± 0.22
% Moisture	4.7 ± 0.3	78.4 ± 4.7	92.7 ± 0.3
Dry Weight Cotyl. Pr. (g)	0.19 ± 0.03	0.13 ± 0.04	0.17 ± 0.01
Range Dry Wts. (g)	0.13 - 0.26	0.07 - 0.25	0.16 - 0.18
Length Hydrated Cotyl. (cm)	1.6 ± 0.1	2.5 ± 0.4	6.8 ± 0.4
Width Hydrated Cotyl. (cm)	0.9 ± 0.1	1.5 ± 0.2	3.8 ± 0.4
µg Ca^{2+} per Cotyl. Pr.	32.2 ± 14.3	38.8 ± 16.1	4,826 ± 929
Range Ca^{2+} per Cotyl. Pr. (µg)	15.4 - 83.6	11.2 - 91.0	4,019 - 5,769
µg Ca^{2+}/g Cotyl.	171.5 ± 55.5	306.0 ± 136.0	28,540 ± 4,151

* from 10 day old seedlings

** green cotyledons of 2 week old plants grown in soil, watered with 1/4 strength Hoagland's solution

RESULTS

Ca Levels in Dry Seed Cotyledons, Cotyledons of Etiolated Seedlings and Photosynthetic Cotyledons :

Growth in the dark produced etiolated seedlings with long hypocotyls and yellow, partially expanded cotyledons. Plumule growth was minimal. By the tenth day after imbition the seedlings had stopped growing and necrotic areas appeared along the hypocotyl and in some of the plumules. The experimental conditions did not permit the full functioning of the peg so that some seed coats remained at least partly on the cotyledons. Table 12:1 shows that the oily dry seed tissue with about 5% moisture changed during seedling growth to become a more typical plant tissue with close to 80% moisture. During charring traces of oil were evident in the etiolated cotyledons indicating that not all the lipid had been mobilized. Cotyledon expansion in both length and width was less than two-fold. The difference in dry weights of the cotyledons indicated a loss of dry matter of 31.5% during the growth period. The 45 harvested seedlings showed a wide range of hypocotyl lengths of 3.3-14.5 cm with a mean length of 6.2 $\pm$ 2.4 cm. Statistical correlation of the measurements showed that length of the hypocotyl was correlated with longer and wider cotyledons as well as a higher wet weight. Hypocotyl length was not significantly correlated with the dry weight of the etiolated cotyledons. The photosynthetic cotyledons had a more than 4-fold expansion over dry embryo cotyledon length.

The mean Ca levels (Table 12:1) were higher for the etiolated cotyledon pairs but were not significantly different at P=0.05 from the Ca levels of dry embryo cotyledons. The ranges for the two groups were.

TABLE 12:2

Caloium Levels in Embryos and Seedlings of *C. maxima* Grown in the Dark in Nutrient-free Medium for 8 - 10 Days and Analyzed by AAS.

$\mu g\ Ca^{2+}$ per Tissue (Mean $\pm$ SD)

Embryo Region Measured	Control Ungerminated Embryo	Germination and Growth Conditions		
		With Both Seed Coats	With Inner Seed Coats	Without Seed Coats
Cotyledons	37.4 ± 12.5 $N = 30$	36.0 ± 15.5 $N = 26$	31.1 ± 12.1 $N = 28$	31.7 ± 11.7 $N = 33$
Axis or Rest of Seedling	6.3 ± 1.7 $N = 30$	10.0 ± 2.9 $N = 26$	12.9 ± 4.0 $N = 28$	13.3 ± 5.3 $N = 33$
Whole Embryo or Seedling	43.7 ± 14.0 $N = 30$	46.0 ± 17.2 $N = 26$	44.0 ± 15.6 $N = 28$	44.9 ± 13.5 $N = 33$
ug Ca Gained by Axis		3.7	6.6	7.0
Gain as % of Ca over Control Cotyl.		9.9%	17.6%	18.7%

TABLE 12:3

Distribution of Weight and Ca Between *C. maxima* Embryo and Seedling Parts.

All Percentages Based on Dry Weights (% Moisture in Brackets)	% Weight in Cotyledons and Axis or ROS			
	Dry Seed N=30	Etiolated Seedlings		
		With Both Seed Coats N=26	With Inner Seed Coats N=28	Without Seed Coats N=30
Cotyledons	98.2 ± 0.5% (6.2 ± 0.3%)	88.7 ± 3.6 (57.2 ± 8.6%)	90.0 ± 2.2 (73.0 ± 7.1)	90.4 ± 3.2 (70.0 ± 8.1)
Axis or ROS **	1.8 ± 0.5% (6.2 ± 0.3%)	11.3 ± 3.6 (89.8 ± 3.5%)	10.0 ± 2.3 (94.0 ± 1.6)	9.6 ± 3.2 (91.0 ± 3.7)

All Percentages Based on μg Ca^{2+} per Dry Weight of Given Tissue	% Ca (by AAS) in Cotyledons and Axis or ROS			
	Dry Seed N=30	Etiolated Seedlings		
		With Both Seed Coats N=26	With Inner Seed Coats N=28	Without Seed Coats N=30
Cotyledons	83.3 ± 5.5%	77.2 ± 5.9	70.8 ± 4.7	69.7 ± 9.6
Axis or ROS	16.7 ± 5.5%	22.8 ± 5.9	29.3 ± 4.5	30.3 ± 9.6

* seedlings grown for 8 - 10 days in the dark in the nutrient-free medium.
* ROS - rest of seedling after cotyledon removal.

similar suggesting that there was little change in Ca during the growth period. The dry seeds showed a strong positive correlation ($r=0.90$, $P=0.001$) between weight and Ca level per cotyledon pair but no significant correlation between cotyledon weight and the Ca level per gram. Although the dry and wet weights of the etiolated cotyledons were significantly correlated ($P=0.001$, $r=0.60$), the Ca level was correlated only with the dry weight ($r=-0.36$, $P=0.01$). The heavier etiolated cotyledons had less Ca per gram tissue. When compared per cotyledon pair rather than per gram the larger cotyledons had more Ca and were attached to seedlings with longer hypocotyls. The Ca levels of the two week old photosynthetic cotyledons were about 150 times higher than the levels of dry seed cotyledons and resembled the levels of 27,000 µg Ca^{2+} per gram obtained for the foliage leaves. The Ca levels of 20,900 µg, 30,000 µg and 13,500 µg for NBS leafy standard materials (Gladney et al ,1984) of Orchard Leaves, Tomato Leaves and Spinach Leaves, respectively, show that the cucurbit leaf Ca levels were similar those of other leaves.

Ca in Seedlings of Seeds Germinated With and Without Seed Coats :

The Ca levels in seedlings produced by the three germination conditions are shown in Table 12:2. Analysis of variance showed that there was no significant difference in the means for the total Ca levels of embryos and seedlings. Thus the seed coats did not appear to contribute any Ca to the seedling. The small decrease in Ca in the cotyledons and the small gain by the axes showed that there was only a limited amount of Ca being mobilized. The amount was highest for the embryos which had the outer seed coats removed. There was no Ca detectable in the water tanks throughout the growth period.

TABLE 12:4

The Distribution of the Weight and Ca Level Between *C. maxima* Embryo and Seedling Parts.

A.

Measured Parameter	Dry Wt. Embryo Parts N=30	Dry Wt. of 8–10 Day Old Seedlings With Both Seed Coats (N=26)	With One Seed Coats (N=28)	Without Seed Coats (N=33)
Wt. Axis or ROS * (g)	0.004	0.020	0.019	0.017
Wt. Cotyl. Pr. (g)	0.202	0.159	0.164	0.160
Wt. Embryo or SDL (g)	0.206	0.179	0.183	0.177
% Wt. Difference $\frac{\text{SDL}}{\text{Embryo}}$		- 13.1%	- 11.2%	- 14.1%
% Wt. Difference $\frac{\text{SDL cotyl.}}{\text{Embryo cotyl.}}$		- 21.3%	- 18.8%	- 20.8%
Degree of Wt. Gain By Axis		5.0-fold	4.8-fold	4.3-fold

B.

$\frac{\text{Wet Wt. Cotyl.}}{\text{Wet Wt. Axis or ROS}}$	—	2.1	2.0	3.4
$\frac{\text{Dry Wt. Cotyl.}}{\text{Dry Wt. Axis or ROS}}$	56.2	8.8	9.6	10.6

* ROS - rest of seedling minus cotyledons
SDL - whole seedling

Table 12:3 shows the relative distribution of dry matter and Ca in the seeds and seedlings. The small axis showed a weight gain while the cotyledons had a corresponding loss of dry matter. The axis had a proportionately higher amount of Ca and acquired more from the cotyledons as growth occurred. However, the bulk of the Ca in the cotyledons was not moved to the growing axis. While the three germination procedures produced seedlings with very similar Ca levels, their growth varied. Seedlings with both seed coats grew well until the tenth day. Seedlings with one seed coat grew well until the eighth day when necrotic areas and incipient hypocotyl collapse of several seedlings necessitated the termination of the growth period. The testaless embryos germinated faster than those with seed coats and continued to show vigour for several days before beginning to lag behind. Two batches of 30 testaless embryos were pooled to give the 33 (Table 12:2) results due to the high attrition of seedlings after the fifth day. A characteristic of this group was uneven expansion of the cotyledons with one well expanded and one partially expanded one present on many seedlings. Table 12:4A shows that there was an 11-14% loss in dry weight during the transition from embryo to seedling. The cotyledons lost about 20% of their dry matter but only half of this was incorporated into the dry matter of the root and shoot tissue. The axis had about a 5-fold gain in dry weight in the transition to seedling state. The root and shoot areas were more highly hydrated than the compact cotyledons (Table 12:3) and thus appeared heavier as seen in Table 12:4B. The cotyledons were only twice as heavy as the hydrated shoot and root portions. This was a change from the 56-fold difference in weight between the cotyledons and axis of the dry seed. When compared in the dry state the cotyledons still retained

TABLE 12:5

Mean Measurements and AAS Ca Levels of 8-10 Day Old *C. maxima* Seedlings Grown With and Without Seed Coats.

Parameter Measured	Seedling Type and Mean ± SD		
	Grown With Both Seed Coats (N=26)	Grown With One Seed Coat (N=28)	Grown Without Seed Coats (N=33)
Seed Wt. (g)	0.281 ± 0.047	0.286 ± 0.039	0.282 ± 0.031
Seed L. (mm)	20.9 ± 1.4	20.8 ± 1.6	20.6 ± 1.3
Wet Wt. COT* (g)	0.383 ± 0.093a	0.640 ± 0.174b	0.523 ± 0.166c
Wet Wt. ROS (g)	0.224 ± 0.119a	0.374 ± 0.240b	0.206 ± 0.115a
Dry Wt. COT (g)	0.159 ± 0.032	0.164 ± 0.024	0.160 ± 0.025
Dry Wt. ROS (g)	0.020 ± 0.007	0.019 ± 0.005	0.017 ± 0.006
Dry Wt. SDL (g)	0.179 ± 0.033	0.185 ± 0.023	0.176 ± 0.025
L. Hypocotyl. (cm)	3.3 ± 1.4a	3.3 ± 1.0a	2.3 ± 1.0b
µg Ca^{2+}/Dry COT	36.0 ± 15.5	31.1 ± 12.1	31.7 ± 11.7
µg Ca^{2+}/g Dry COT	234.0 ± 116.0	191.6 ± 73.7	202.3 ± 78.4
µg Ca^{2+}/Dry ROS	10.0 ± 2.9a	12.9 ± 4.0b	13.3 ± 5.3b
µg Ca^{2+}/g Dry ROS	518.0 ± 140.0a	692.0 ± 133.0b	806.0 ± 230.0c
µg Ca^{2+}/Dry SDL	46.0 ± 17.2	44.0 ± 15.6	44.9 ± 13.5

* COT - cotyledon pair
ROS - rest of seedling minus cotyledons
SDL - whole seedling

a, b, c - means in one row with different letter names are different at P = 0.05 or less as separated by analysis of variance and Tukey's test (Zar, 1984)

9-10 times the weight found in the rest of the seedling. While the Ca levels showed a higher mobilization from the testaless cotyledons, the higher amount of dry weight retained by these cotyledons (12:4B) suggested that mobilization of C and N may have been less efficient in the testaless embryos.

Table 12:5 presents the mean values for various measurements on seeds and seedlings germinated with and without seed coats and indicates which were significantly different in each category. The wet weight of the seedling parts was very variable while the dry weights were not significantly different. Hypocotyls were significantly shorter for the testaless batch. While Ca remaining in the cotyledons was not significantly different for the three germination procedures, the differences in Ca between the whole seeds and testaless specimens were significantly different for the "rest of seedling" portion.

The results were also used to determine the relationship between seed and seedling sizes and Ca levels. Correlation coefficients were calculated comparing all the parameters and regression analyses were carried out on all and on selected combinations of measurements. The strongest correlation was between seed weight and seedling weight ($r=0.92$, $P=0.001$) and regression analysis showed that 85% of the variation in one of the parameters could be explained by the variation in the other. The next strongest correlation was between hypocotyl length and seedling Ca ($r=0.72$, $P=0.001$). Regression analysis showed that 52% of the variation in the hypocotyl length was explained by the variation in Ca level and seedlings with long hypocotyls tended to have high Ca levels. Heavy seeds tended to produce heavy seedlings but such seedlings did not always have

TABLE 12:6

Measurements of Dark-grown 9 Day Old Seedlings of *C. maxima* and 10 Day Old Seedlings of *C. andreana*.

A. Actual Measurements:

Parameter Measured	*C. maxima*		*C. andreana*	
	Dry Seed	Seedling	Dry Seed	Seedling
Number Measured	30	30	140	122
Seed Wt. (g)	0.380	—	0.023	—
Wt. Embryo ¦—Wet	—	1.39	—	0.118
or SDL (g) ¦—Dry	0.270	0.241	0.016	0.014
Wt. COT ¦—Wet	—	0.831	—	0.030
(g) ¦—Dry	0.266	0.214	0.015	0.010
Wt. Axis ¦—Wet	—	0.556	—	0.088
or ROS (g) ¦—Dry	0.004	0.027	0.001	0.004
COT Length (cm)	2.3	3.3	0.7	0.8
Hypocotyl L. (cm)	—	5.2	—	4.2
Root L. (cm)	—	6.2	—	2.8

B. Comparisons of Measurements Within Each Species:

Parameters Compared	*C. maxima*	*C. andreana*
Hypocotyl L. to Seed L.	4.3	8.4
Wt. COT to Wt. ROS	7.9	3.5
Seedling COT L. to Embryo COT L.	1.8-fold	1.3-fold
% Loss Dry Wt. (SDL)	10.7%	12.5%
% Loss Dry Wt. COT	19.5%	33.3%
Gain in Wt. by Axes	6.8-fold	4-fold

C. Comparisons Between the Two Species:

Parameters Compared	*C. maxima*/*C. andreana*
Embryo Wt.	16.8
Seedling Wt.	17.2
Embryo Cotyl. L.	3.3
Seedling Cotyl. L.	4.1
Hypocotyl L.	1.2
Root L.	2.2

* SDL - Whole seedling
ROS - rest of seedling minus cotyledons
COT - cotyledon pair

long hypocotyls. High Ca in seedling cotyledons was correlated with high Ca in the rest of the seedling. Seed length was negatively correlated with each of Ca per gram cotyledons, hypocotyl length and total seedling weight but these correlations just missed being significant at P=0.05.

Levels of Mg, K, Ca and P in Dark-grown Seedlings :

While the procedure for seedling growth was adaptable to the smaller seeds and seedlings of C. andreana , many more seeds had to be germinated to obtain large enough sample for NAA analysis. One hundred percent germination was obtained with both species but some of the C. andreana specimens showed very poor subsequent growth so that only 122 out of the 140 seedlings were processed for analysis. The seed and seedling measurements are shown in Table 12:6. The C. andreana seedlings had considerably smaller measurements for all size parameters except hypocotyl and root lengths (Table 12:6A). Comparing hypocotyl length to the initial seed length showed that the small seeds grew longer hypocotyls than the larger seeds (Table 12:6B). This was also reflected in the relative distribution of dry matter between the cotyledons and the rest of seedling. C. andreana had proportionately more weight in the rest of the seedling than in the cotyledons but the cotyledon expansion was lower. In Table 12:6C the ratios of seed and seedling weights were very similar for the two species showing that the total dry matter remained unchanged for the two seeds relative to each other. As in the previous experiments, there was a net loss in dry matter of about 10-12% but the cotyledons lost an even larger percent of their total weight. The cotyledonary loss represented, at least in part, mobilization of C and N reserves which occurred to a greater extent in the C. andreana cotyledons.

TABLE 12:7

Mineral Levels in *C. maxima* and *C. andreana* Embryos and Seedlings as Determined by NAA.

Dry Embryo*		*C. maxima*			*C. andreana*		
		Cotyledon Pair	Axis	Whole Embryo	Cotyledon Pair	Axis	Whole Embryo
µg/ Tissue	Mg	1471	49.7	1521	89.4	7.8	97.2
	K	1828	41.8	1869	71.7	6.6	78.3
	Ca	54.1	9.2	63.2	9.5	1.6	11.1
	P	3157	95.5	3253	155.7	17.8	173.5

Etiolated Seedling*		Cotyledon Pair	Rest of Seedling	Whole Seedling	Cotyledon Pair	Rest of Seedling	Whole Seedling
µg/ Tissue	Mg	1420	113	1533	78.6	11.5	90.1
	K	1357	781.7	2139	25.7	82.1	107.8
	Ca	56.6	16.7	73.3	6.7	4.7	11.4
	P	2795	314.8	3109	131.2	45.1	176.3

* N = 3 subsamples analyzed from pooled embryo or seedling parts of each species *C. maxima* seedlings 9 days old; *C. andreana* seedlings 10 days old.

TABLE 12:8

Distribution of Minerals Within Embryos and Seedlings of Dark-grown *C. maxima* (9 Days Old) and *C. andreana* (10 Days Old).

Element Measured	*C. maxima*				*C. andreana*			
	Ratio C : A* C : RS	% Mobilized I**	II	III	Ratio C : A C : RS	% Mobilized I	II	III
Mg	96.7 : 3.3 92.6 : 7.4	4.1%	3.4%	4.3%	92.0 : 8.0 87.2 : 12.8	4.8%	12.1%	4.1%
K	97.8 : 2.2 63.4 : 36.5	34.3%	25.7%	40.5%	91.6 : 8.4 23.8 : 76.2	67.8%	64.2%	105.3%
Ca	85.4 : 14.6 77.2 : 22.8	8.2%	-4.6%	13.9%	85.6 : 14.4 58.8 : 41.2	26.8%	29.5%	32.6%
P	97.1 : 2.9 89.9 : 10.1	7.2%	11.5%	6.9%	89.7 : 10.3 74.4 : 25.6	15.3%	15.7%	17.5%

* C : A - % total mineral in dry cotyledon pair : % in dry axis
C : RS - % total mineral in seedling cotyledon pair : % in rest of seedling

** I - based on difference derived from ratios
II - based on loss in cotyledon pair
III - based on gain in axis

The mineral levels in dry embryo and seedling parts are shown in Table 12:7. Since the seedlings were grown in demineralized water no mineral uptake should have occurred. For both species the Mg and P levels for the whole embryos and seedlings were within 10% of each other. The Ca levels for C. andreana were also similar but the Ca levels for C. maxima were 16% higher for the whole seedling than for the dry embryos. Although the "rest of seedling" portion of C. maxima shows a gain in Ca level over that of the dry axis there is no corresponding loss of Ca in the cotyledon pair. In both species the seedling cotyledons have lost K while the 'rest of seedling' portions have gained K. However, the total seedling K was higher than that of the dry embryos. The increase in K was 14% for C. maxima and 37.7% for C. andreana . Experiments with soaking seed coats (Table 12:14) showed that K was being lost from the seed coats on soaking while the loss of Ca was minimal. Thus only the gain in K could have resulted from the ability of the seedling to scavenge some K derived from the seed coats which were in contact with the medium.

The extent to which the four minerals were mobilized from the cotyledons of the two cucurbit species is shown in Table 12:8. Because of the above-mentioned discrepancies in total mineral levels, the percent mobilized was calculated in three ways. Generally at least two of the methods gave very similar percentages. Overall agreement between the methods was better for C. andreana than C. maxima . Despite some disagreement, the trends were not reversed. Magnesium was the least mobilized element in both species. Phosphorus was mobilized to a lesser extent than Ca. Calcium was mobilized to a higher degree in C. andreana and these embryos also had more extensive hypocotyl growth and a greater

TABLE 12:9

Mineral Ratios for Dark-grown *C. maxima* and *C. andreana* Embryos as Determined by NAA.

Ratio*	Tissue Measured	*C. maxima*		*C. andreana*	
		Embryo	Seedling	Embryo	Seedling
$\frac{Mg}{K}$	COT	0.80	1.05	1.24	3.06
	A, ROS	1.19	0.14	1.19	0.14
$\frac{Mg + Ca}{K}$	COT	0.83	1.08	1.38	3.32
	A, ROS	1.41	0.17	1.41	0.20
$\frac{Ca}{K}$	COT	0.03	0.04	0.13	0.26
	A, ROS	0.19	0.02	0.20	0.06
$\frac{Mg}{P}$	COT	0.47	0.51	0.57	0.60
	A, ROS	0.52	0.36	0.43	0.25
$\frac{K}{P}$	COT	0.58	0.48	0.46	0.20
	A, ROS	0.44	2.48	0.37	1.82
$\frac{Ca}{P}$	COT	0.02	0.02	0.06	0.05
	A, ROS	0.10	0.05	0.09	0.10
$\frac{Mg + Ca}{P}$	COT	1.04	0.99	1.03	0.80
	A, ROS	0.96	2.84	0.84	2.08
$\frac{Mg + K + Ca}{P}$	COT	1.06	1.01	1.09	0.85
	A, ROS	1.05	2.89	0.90	2.18

* based on % mineral in dry embryo or seedling part

COT - embryo or seedling cotyledon pairs
A - embryo axis
ROS - rest of seedling (minus cotyledons)

TABLE 12:10

Seedling Growth of Dark and Light-grown C.maxima.

Parameter Measured	Grown in Distilled Water in the Dark (Mean ± SD)	Grown in Distilled Water in the Light (Mean ± SD)	Grown in Full Nutrient Medium in the Light* (Mean ± SD)
Cotyledon L. (cm)	3.3 ± 0.4	3.2 ± 0.4	6.4 ± 0.4
Cotyl. Expansion*	1.4 - fold	1.4 - fold	2.8 - fold
Hypocotyl L. (cm)	5.2 ± 1.2	1.7 ± 0.2	- 5
Root Length (cm)	6.2 ± 1.2	4.5 ± 0.8	—
Age (Days)	9	7	7
N	30	30	10

* grown in soil and watered with 1/4 strength Hoagland's solution
** based on length of the embryo of 2.3 cm

TABLE 12:11

Mineral Distribution in C. maxima Seedlings Grown in Nutrient-free Medium in Dark or Light and Analyzed by NAA.

Mineral (µg)	Dark Grown COT	ROS	SDL	Ratio COT/ROS	Light Grown COT	ROS	SDL	Ratio COT/ROS
Mg	1420.5	113.0	1533.5	12.6	1698.6	93.7	1792.3	18.1
K	1357.9	781.7	2139.6	1.7	1522.1	501.8	2023.8	3.0
Ca	56.6	16.7	73.3	3.4	58.7	13.1	71.8	4.5
P	2795.0	314.8	3109.8	8.9	3948.3	284.7	4233.0	13.9

COT - cotyledon pairs
ROS - rest of seedling
SDL - whole seedling

loss of dry matter from the cotyledons (Table 12:6B). Potassium was mobilized to the highest extent of all four minerals and twice as much K was mobilized from the small C. andreana cotyledons as from C. maxima. The 105% mobilization of K by method III reflected the higher total amount of K in the seedling than found in the dry embryo.

Mineral ratios, such as were shown in Chapter 10, were also calculated for the seedlings (Table 12:9). In C. maxima and C. andreana the ratios for Mg/K reflected the low mobilization of Mg as compared to K. The Ca/K ratio also indicated the low mobilization of Ca relative to K. Seedling ratios for Mg/P were not markedly different from those of the dry embryos. The K/P ratios showed accumulation of K in "the rest of seedling" portion. The ratios of cations to P showed little change in the cotyledons of C. maxima but a small drop in cations in C. andreana. The "rest of seedling" portion of both species had a cation excess largely due to the increase in K. This type of cation excess relative to P is compatible with the tissue having changed from storage to leafy-type tissue. In the cotyledons of seedlings grown in the soil (Table 12:12) the cation to P ratio was 3.9.

Levels of Mg, K, Ca and P in Light-grown Seedlings :

Light-grown cotyledons of C. maxima seedlings grown in deionized distilled water, showed no greater expansion than their dark-grown counterparts (Table 12:10). Cotyledons from plants grown in soil which was watered with Hoagland's solution were large, green and leafy while those of the seedlings grown in the light in distilled water were smaller, still fleshy and not as uniformly green. Hypocotyl growth, while longer in the dark than in the light, was probably limited by the lack of nutrients such

TABLE 12:12

Mineral Levels in Cotyledons of Dry Embryos of C. maxima and Those of C. maxima Seedlings Grown Under Various Conditions.

Type of Cotyledons	µg Mineral per Cotyledon Pair (Mean ± SE)			
	Mg	K	Ca	P
Dry Seed	1472 ± 101	1828 ± 106	54.0 ± 2.9	3158 ± 59
Dark, Deficient Growth (Etiolated) (9 Days Old)	1421 ± 125	1358 ± 105	56.6 ± 3.6	2795 ± 102
Light, Deficient Growth (Low Expansion) (7 Days Old)	1699 ± 110	1522 ± 88	58.7 ± 4.0	3948 ± 227
Light, Growth in Soil (Fully Expanded) (7 Days Old)	1800 ± 134	9484 ± 613	2778 ± 192	3576 ± 687

TABLE 12:13

Relative Amounts of Mg, K, Ca and P in Dry Embryo Cotyledons of *C. maxima* and Cotyledons of *C. maxima* Seedlings Grown Under Various Conditions.

Growth Conditions	Relative Concentrations of Minerals in Cotyledons*			
	Mg	K	Ca	P
Dry Embryo Cotyledons	2.9	3.7	0.1	6.3
Etiolated Seedling Cotyledons Dark-grown in Distilled H_2O	2.8	2.7	0.1	5.6
Green Cotyledons Light-grown in Distilled H_2O	3.4	3.0	0.1	7.9
Green Cotyledons Light-grown in Soil	3.6	19.0	5.6	7.2

* derived from values in Table 12:12 by arbitrarily dividing the concentrations by 500.

as Ca. Total seedling K and Ca levels were very similar for dark or light growth (Table 12:11). Total concentration of Mg was 16.8% and that of P was 36.6% higher in the light-grown seedlings than in the dark-grown. This could have been due to some analytical problems but such differences were also found in the separately measured cotyledons from soil grown seedlings (Table 12:12) suggesting that perhaps a genuine pick-up of Mg and P may have occurred during growth in the light. Mobilization of minerals from the light-grown seedling cotyledons was less than from dark grown ones as shown by comparison of the ratios (Table 12:11).

Table 12:12 shows the mineral levels in C. maxima cotyledons produced under different growth conditions. Cucurbit cotyledons acquired more minerals as they became photosynthetic. The greatest increases were in K and Ca while the increases in Mg and P were much lower. Table 12:13 shows the same results as ratios of the minerals to each other. It can be seen that while there was about a 50-fold increase in Ca level, its initially low concentration made it still only the third most abundant of the four minerals. Potassium exceeded the P in the photosynthetic cotyledons.

Leakage of Mg, K and Ca from Seed Coats and Embryos :

To determine whether sufficient K may have been released locally from seed coats that fell from the cotyledons and were in contact with the growth medium, the outer seed coats were soaked separately while the inner seed coats and the embryos were soaked together. The content of K was high in both the inner and outer testas (Table 10:11). The supernatants, from soaking both the outer seed coats and embryos plus the inner seed coats, contained more K than Mg and Ca. This was reflected in the higher amounts

TABLE 12:14

Loss of Mg, K and Ca from Embryos and Seed Coats of *C. maxima* Soaked in Deionized Distilled Water.

		Embryo + Inner Seed Coat N = 30		Outer Seed Coat N = 30	
		1 hr.	24 hrs.	1 hr.	24 hrs.
Loss in µg per Embryo or Seed Coat After Soaking*	Mg	0.0	2.4	4.8	3.0
	K	14.6	88.0	338.3	322.7
	Ca	0.4	2.0	5.7	4.7
Loss as % of Total in Embryo or Seed Coat	Mg	0%	0.2%	14.9%	9.3%
	K	0.9%	5.6%	58.6%	55.9%
	Ca	0.6%	3.0%	14.0%	11.6%

*compared to mineral levels in unsoaked tissues:

		Embryo + Inner Seed Coat	Outer Seed Coat
µg Mineral per Tissue Part	Mg	1,172.7	32.2
	K	1,561.3	577.5
	Ca	65.9	40.6

of K than Mg and Ca lost from both types of samples (Table 12:14). The difference between the one hour and the 24 hour soaking period for the seed coats was not marked. Soaking the embryos and the inner testas for the longer period did produce an increase in the amount leached but the overall amount leached was much smaller than from the outer testas.

DISCUSSION

The characteristics of the etiolated seedlings agree well with the descriptions of Wiley and Ashton (1967) and Lott (1970a) except that the overall hypocotyl length in the present study was lower, probably due to the growth in nutrient-free medium. In the etiolated state the cotyledon expansion was much reduced. Lovell and Moore (1970) found a 22-fold expansion in light-grown Cucurbita pepo cotyledons and a 50-fold expansion in Cucumis sativus . Nelson (1932) and Fofanova and Khokhlova (1983) studied the expansion and found that the enlargement was due to cell division followed by expansion in the epidermis and palisade mesophyll while cell enlargement and development of intercellular spaces accounted for the enlargement of the spongy mesophyll. The decrease in dry matter from dry seed to etiolated cotyledon was only 30%. This low level may be related to the smaller hypocotyl growth and to the incomplete mobilization of the lipid and protein reserves. Wiley and Ashton (1967) found equivalent amount of proteolytic activity in light and dark grown squash seedlings but Davies et al (1981) found a higher amount of lipid degraded in the light. The traces of oil on charring attest to the presence of some undegraded lipid. The complete loss of 10% of the dry matter in the transition from dry seed to seedling may be from losses due

to respiration or perhaps due to leaching from roots (Hocking, 1980).

Hypocotyl collapse in seedlings grown in distilled water has been reported as evidence of Ca deficiency (Helms, 1971). Necrotic areas seen by the tenth day were also likely due to a lack of Ca (Hocking, 1980). Okamoto (1962) found that Vigna sesquipedalis seedlings could grow without any Mg or K but failed to grow if Ca was absent from the medium. The higher Ca level in etiolated cotyledons in the first experiment could have been caused by sampling bias. Since the seeds with the earliest germination were selected, their greater vigour may have been accompanied by higher Ca levels. Contamination of the water and derivation of Ca from the seed coats during germination and early seedling growth were other possibilities. These were tested and no contribution of Ca was detected from either of these sources. No acquistion of Ca by pea embryos from the testas was detected by Hocking (1980). While testas of many species, including peas, have more Ca than K this Ca seems to be unavailable to the embryos during germination and seedling growth (Hocking, 1980). The heavier cucurbit embryos produced the larger seedlings and this trend was also found for pea embryos (Ferguson and Bollard, 1976). In Chapter 8 it was shown that some batches of embryos had a negative correlation between embryo length and Ca level. The germination experiments showed that embryo length was negatively correlated with seedling weight perhaps due to the lower Ca levels of the long embryos.

While there was no difference in the overall mineral content in seedlings grown with and without seed coats, there was about a two-fold increase in Ca mobilization in those without the outer testa. The requirement of oxygen for the resumption of metabolic activity

(Rasi-Caldogno and De Michelis 1978) would be more easily satisfied without the seed coat since Brown (1940) showed that both inner and outer testas of Cucurbita pepo had a low permeability to oxygen. Hara et al (1976b) stated that improved gas exchange and freedom from mechanical resistance promoted germination of testaless cucurbit seeds. The testaless embryos did indeed have more rapid germination and early growth, but this was followed by a lag and eventually poorer growth. Larson (1968) germinated pea seeds without seed coats and found that the resulting seedlings had reduced growth while Powell and Matthews (1978) found a reduced respiration in pea seeds germinated without testas. The authors blamed damage to the outermost cell layers as a result of too rapid water intake for the poorer seedling growth. The lower removal of dry matter from the testaless cotyledons of cucurbit embryos did not prevent a higher removal of Ca from these embryos.

The linearly related mobilization of dry matter and minerals from legume cotyledons has been reported by a number of authors (Collins and Sutcliffe, 1977; Guardiola and Sutcliffe, 1972; Hocking, 1980). In this study there was a higher percent of dry matter lost from the C. andreana cotyledons and a higher percent of each of the minerals mobilized. Mobilization of Ca has been found to be lower than that of Mg, K and P in legumes (Ferguson and Bollard, 1976). The mobilization of Mg was lower than that of Ca in both C. maxima and C. andrena . Guardiola and Sutcliffe (1972) reported that Ca appeared to be quite mobile and was probably removed in the phloem in the initial growth after germination. They suggested that the high concentration of Ca present as a result of its release from phytin may have enhanced its transport. The C. andreana

embryos contained three times more Ca in their cotyledons than those of C. maxima and about three times as much Ca was mobilized from the C. andreana cotyledons. While mobilization of dry matter from cotyledons of seedlings grown in distilled water was reported as slower and less complete, the mobilization of Ca has been found to be greater under the nutrient-free conditions (Guardiola and Sutcliffe, 1972; Hocking, 1980). Guardiola and Sutcliffe (1972) and Hocking (1980) found that if Ca was present in the growth medium the seedling cotyledons showed a net gain in Ca in both peas and lupines. The reported mobilization of Ca from these species has been in the range of 20-31% (Ferguson and Bollard, 1976; Guardiola and Sutcliffe, 1972; Hocking, 1980). Guardiola and Sutcliffe (1972) suggested that the mobilizable Ca represented the amount bound to phytin while the rest may not be available for removal. Exhausted pea cotyledons were low in K and P but rich in Ca. Calcium movement was enhanced by light but movement of K and P has been variously reported as inhibited or promoted by light (Guardiola and Sutcliffe, 1972; Marshall and Kozlowski, 1975).

Studies on legumes have shown considerable species-to-species differences in the amount of minerals mobilized and in how these amounts are affected by the environmental conditions. The amount of transport and the duration of the transport period depended on how soon the cotyledons senesced (Guardiola and Sutcliffe, 1972). The legume cotyledons studied did not become photosynthetic leaves. The overall percent of Mg, K and P mobilized from legume cotyledons were much higher than in cucurbits, reaching 90% for K and P in some species. The fact that even less was mobilized out of the light-grown cucurbit cotyledons, which had become

leafy, indicated that perhaps a different requirement existed for mobilization of minerals in cucurbits. Studies of mobilization of mineral reserves from cotyledons that do become photosynthetic are few compared to the legume studies and the main results are on seedlings of woody plants. Marshall and Kozlowski (1974, 1975) found that the photosynthetic cotyledons of tree seedling could transport mineral reserves out of the cotyledons, accumulate more minerals in the cotyledons or do both. The cucurbits under the conditions used in this study seemed to transport some minerals out of the cotyledons in deficient medium but in more natural conditions appeared to accumulate minerals.

The particularly low export of Mg from cucurbit cotyledons may be related to the requirement of this element for chlorophyll. Greening of the cucurbit cotyledons takes place very early in seedling growth with the base of the cotyledons turning green even before the seed coats are removed by the peg (Lott, 1970b). The experiments of Marshall and Kozlowski (1975) were carried out only in distilled water to which Ca had been added and are not comparable to the cucurbit results obtained for growth in water without Ca. In their study more minerals were moved into cotyledons in the light and were exported out of the cotyledons only when they began senescing. In the green cotyledons of the soil-grown C. maxima plant there was a many-fold increase in Ca and K compared to the dry seed. Marshall and Kozlowski (1975) found considerable variation between the seedlings of the various woody species and such variation was also seen between the two closely related Cucurbita species.

The high loss of K from the outer testas has been shown for other species. Simon and Mathavan (1986) studying seeds of various species found

that K leakage was more rapid in the early soaking period and much slower during the subsequent hours of imbibition. Cucurbit embryos when enclosed in the inner testa, which itself had more K than the embryo, did not lose as much K as the outer testas. The source of the large amounts of K and Ca in soil-grown cotyledons must have been the transport of minerals from the soil. The origin of K, Mg and P in the deficient seedlings is still uncertain but possible mobilization from the testas cannot be ruled out. Dmitreva and Sobolev (1985) reported new synthesis of phytin in the photosynthetic cotyledons of castor bean during seedling growth but this species has a large endosperm reserve lacking in cucurbits so that the species may function differently.

Thus C. maxima seedlings, when grown under normal conditions, retained most of their minerals in the cotyledons and began uptake from the medium early in seedling growth. Growth in deficient medium did result in some transfer of minerals to the growing axis. While Ca mobilization was lower in C. maxima than in pea and lupine seedlings, C. andreana cotyledons lost Ca in amounts comparable to some legume species. Unlike legumes, cucurbit cotyledons lost less of their Mg and P. Cucurbit embryos could germinate well without seed coats and could mobilize more Ca from their cotyledons but the seedling vigour rapidly declined and many seedlings succumbed to symptoms of Ca defiency. C. andreana embryos grown in the dark in deficient medium had a lower cotyledon expansion but longer hypocotyls and a higher percent of mineral mobilization. Despite differences in the amounts mobilized, the overall pattern of mineral movement was similar in the two cucurbit species and followed the pattern shown by other seedlings with photosynthetic cotyledons.

Chapter 13

GENERAL CONCLUSIONS.

The goals of this study have been fulfilled and it has been shown that both genetic factors and seed size exert an effect on Ca accumulation in cucurbit embryos. The role of these two factors and the unusual features of Ca storage compared to those of Mg, K and P are summarized in Tables 13:1 and 13:2. Unique characteristics of cucurbit ash, discovered during the standardization of the atomic absorption procedure, are outlined in the next section.

ANALYTICAL PROCEDURE

The different procedures for mineral analysis used in this study served their designated purposes. Atomic absorption analysis was used to analyze Ca in embryos from all Cucurbita fruits produced for the study. Neutron activation analysis was used to obtain the concentrations of Mg, K, Ca and P in selected batches of embryos of both parent species and hybrids. The measured Ca levels from the two techniques were acceptably close indicating no major problems with either procedure. However, this was only true after the resolution of two problems which arose in the atomic absorption analysis of Ca in cucurbit embryos. Neither problem was mentioned in previous reports of Ca analysis in embryos of other species. One of the problems I called the "sample size effect" and the other was the binding of Ca in the ash produced at temperatures above 550°C. Because of these two problems a disproportionate amount of time and effort was expended on the standardization of the analytical procedure as shown

by the presence of Chapters 4 to 6 in this thesis. However, the recognition and resolution of the problems is of general importance to analysts of plant tissues. I have shown that it is important to consider the sample composition and how that can affect sample-crucible interaction. For example, enchancement of Ca in small tissue samples arose in part from the interaction of potassium with crucible walls. Seed tissues vary in their relative proportions of Mg, K, Ca and P and this was shown to affect how easily Ca could be extracted from the ash. Had more information been available on the effects of tissue composition on ashing characteristics, some of the analytical problems encountered might have been avoided.

STORAGE OF CALCIUM IN CUCURBIT EMBRYOS

As was pointed out in Chapter 1 there are many unanswered questions both with respect to mineral functions and seed physiology. While there are many studies of minerals in plant tissues there is a distinct lack of studies of mineral accumulation in seeds. There are some valid reasons for this lack. Seeds are more difficult to study than vegetative plant tissues, for example, many of the seeds can be very small, the seed coats can be tough to remove and separation of coiled or folded embryos into component parts can be virtually impossible. The fact that mineral reserves usually form less that 5 % of the dry weight necessitates the use of large samples which require lengthy seed preparation. Most studies of mineral reserves in seeds have been limited to the major crop plants. The differences in Ca requirements by monocot and dicot plant tissues make comparisons between cereal seeds and

cucurbits unsuitable. Among the dicot species legumes are studied most frequently but there are also distinct differences between legume and cucurbit seeds. Throughout the thesis some comparisons have been made between published results on legume seeds and the cucurbit results. However, the limited number of species studied within both legumes and non-legumes makes it difficult to assess whether differences in growth characteristics result in differences in seed mineral accumulation. For example, legume seeds are produced in pods containing a small number of seeds. Cucurbit fruits are large and fleshy and contain hundreds of seeds. Although some legume and cucurbit embryos are similar in that their large cotyledons are the main storage organs, the ultimate function of the cotyledons is different. Legume cotyledons generally senesce shortly after germination while cucurbit cotyledons become the first photosynthetic leaves and persist for several weeks.

Tables 13:1 and 13:2 summarize the main results obtained during my studies of cucurbit embryos. Comparable analysis of minerals in legume embryos, embryo parts and seed coats have been carried out by Pate, Hocking and associates in papers referred to earlier in this thesis. Cucurbit results are representative of mineral levels in seed produced in large, multi-seeded fruits and of rather simple embryos with cotyledons that become photosynthetic after germination. There are no comparable studies on other cucurbits or other non-legume, dicot embryos. Previous work by Lott and associates considered the distribution of Ca within embryos tissues but did not consider the concentration of the minerals. This early work on cucurbit mineral reserves showed that the distribution of Ca in C. maxima was different from that found in C. andreana and was

TABLE 13:1

Characteristics of Ca Storage in Cucurbit Embryos.

Parameter Studied	Main Findings	Tables With Results
Total Embryo Calcium	- C. andreana embryos (0.012 g) contained less Ca per embryo than C. maxima embryos (0.2 g)	8:3
	- C. andreana embryos contained three times more Ca per gram embryo tissue than C. maxima embryos	8:3
	- hybrid embryos had Ca levels different from each of their parents; C. maxima x C. andreana also differed in Ca level from C. andreana x C. maxima	9:4
	- Ca levels were more variable within C. maxima and both types of hybrid embryos than within C. andreana	8:3, 9:4, Fig. 9:1
	- Ca levels in C. maxima embryos varied more plant-to-plant than fruit-to-fruit	pp. 141
	- variation in Ca level within seeds of a single fruit could be more or less than the overall variation	8:10
	- longer and heavier C. maxima embryos tended to have a lower Ca level per gram embryo than smaller embryos	Figs. 8:1, 8:2
	- heavier C. andreana embryos tended to to contain more Ca per gram embryo tissue than their smaller counterparts	8:5
Ca in Axis vs. Ca in Cotyledons	- C. maxima axis made up about 2% of embryo weight but contained about 15% of the total embryo Ca	11:3
	- C. maxima axis had about 8 times more Ca per gram axis tissue than per gram cotyledon tissue	11:3
	- C. andreana axis made up about 5% of embryo weight but contained about 13% of the total embryo Ca	11:3
	- C. andreana axis had about 3 times more Ca per gram axis tissue than per gram cotyledon tissue	11:3
	- differences between embryos with high or low Ca reflected differences mainly in cotyledons	11:4, Fig. 11:2
	- axis and cotyledon weight was positively correlated with Ca level per embryo part	11:5
	- changes in Ca level in the axis were	

	positively correlated with changes in the cotyledons	11:5
	- on a per gram embryo part basis the parent embryos had no correlation between weight and Ca level; C. maxima x C. andreana had a negative and C. andreana x C. maxima had a positive correlation between cotyledon weight and cotyledon Ca level	11:5
Storage of Ca vs. Storage of Mg, K, P	- in both parent and hybrid embryos Ca level was lower than that of Mg, K and P	10:1, 10:2
	- in C. maxima and the two kinds of hybrid embryos, the Ca levels varied more than Mg, K and P levels	10:3
	- the parent and hybrid embryos differed more in their Ca level than in the other three minerals	10:4
	- distribution of Mg, K and P was similar and more even between the axes and the cotyledons than found for Ca	11:6
	- C. maxima axes contained about 5% of the total amount of Mg, K and P while C. andreana axes had about 10% of each of the minerals	11:7
Ca vs. Mg, K, P in Germination	- cucurbit seeds germinated and seedlings grew up to 10 days without external Mg, K, Ca and P; then Ca deficiency symptoms developed	12:1, 12:5
	- there was poorer overall growth but more Ca mobilization in seedlings grown without seed coats	12:2
	- mobilization of Mg, Ca and P from the cotyledon was low (less than 20%) and lower in light-grown than in dark-grown cotyledons	12:8, 12:11
	- more Mg, Ca and P mobilized from C. andreana cotyledons than from C. maxima	12:8
	- between 40 and 60 % of the K was mobilized from the cotyledons of both species but total seedling K levels were higher than that of the dry embryos	12:7, 12:8
	- seedlings grown with available nutrients showed marked increases in cotyledon K and Ca but only small increases in Mg and P	12:12

TABLE 13:2

General Characteristics of Mineral Accumulation in Cucurbit Embryos.

Main Findings	Tables With Results
Embryo Ca level represented only 1% of the total mineral content in *C. maxima*, 3% in *C. andreana* and between 1 and 3% in the hybrid embryos.....	10:1, 10:2
Phosphorus was the predominant storage mineral making up about 50% of the mineral content; Mg and K each make up about 25%.....	10:1, 10:2
C. maxima had a lower Mg to K ratio than *C. andreana* and the hybrids both follow the *C. maxima* pattern....	Fig. 10:1
Ratio of the cations of Mg, K and Ca to P is close to 1 and the proportion of P assumed to in phytic acid has sufficient binding sites for the measured cations.....	10:9, 10:10
The trend in the cucurbit embryos was toward a small cation deficit relative to P but in seedlings the hypocotyl and root portion had a cation excess since most of the P is not moved out of the cotyledons.....	12:9
The concentration of minerals in the axes of both parent and hybrid embryos varied less than the concentration in the cotyledons.....	11:8
Magnesium concentration was generally positively correlated with K, Ca and P in both parent and hybrid embryos. Correlation between the other minerals showed less consistency among the different embryos.....	10:5
Comparison of mineral levels in the two seed coats and the embryos showed a selective uptake of the minerals - Mg was lower in both seed coats than in embryos - K was higher in both seed coats than in embryos - Ca was higher in both seed coats but highest in the inner seed coat - P was higher in the embryos than in either of the seed coats.....	10:11
The high K levels relative to levels of Mg, Ca and P in the outer seed coats reflected the high K levels of the phloem but embryo concentrations of these minerals did not suggest the predominance of either xylem or phloem transport.....	10:12

related to embryo size. These findings have been supported by the work in this thesis. My work has additionally shown that differences in distribution of Ca are, in part, the result of differential storage of the minerals in the axes and the cotyledons in the two species. I have also shown that seed size plays a role in Ca accumulation in C. maxima but has little effect in C. andreana . The hybrids produced for this study have never been analyzed before. The study of the hybrid mineral levels showed that mineral accumulation is under genetic control. Hybrid Ca levels of C. maxima x C. andreana showed a negative correlation between embryo size and Ca level per gram embryo tissue which is similar to that seen in the female parent. The C. andreana x C. maxima hybrid lacked this relationship with seed size. The low availability and low mobility of Ca appears to be more limiting for the long and heavy embryos probably because of their higher growth rates. The evidence for the above is the negative correlation between Ca level and embryo size but it is also reflected in the higher variability in the Ca levels in C. maxima than in C. andreana . The storage of higher amounts of Ca in the axes than in the cotyledons and the relatively higher proportion in C. maxima than in the C. andreana axes is also a compensation for the low mobility of Ca. The higher amount of Ca in the C. maxima axis also compensates for the lower mobilization of this mineral from the cotyledons in this large-seeded species. While there is some casual mention in the literature (discussed in Chapter 8) of lower ash contents of larger seeds, the relationship between mineral accumulation and seed size has not been examined carefully until now.

The variation in Ca level with seed size within single fruits may

be due to several factors including local variation in available minerals, differences in growth rate or differences in the genetic makeup of the embryos. While other workers have shown differences in Ca levels between seeds from different areas of a pod, no studies have been made of fruits with a large number of seeds. It is also interesting that in Cucurbita the inner seed coats have more Ca than the embryos. The function of the second seed coats of cucurbits remains obscure and it is not known whether the measured Ca in these seed coats is part of the structure of the seed coat or available for transfer to the embryos. The unanswered question is whether the embryos controlled or limited their Ca uptake or were unable to obtain any more Ca from surrounding tissues. The high levels of P in the embryos relative to that in both vascular exudates and seed coats as well as the lower levels of K despite very large amounts in exudates and the seed coats, supports the existence of specific controls on mineral uptake within the embryos.

Thus, I have found that during development cucurbit embryos take up and store minerals in varied amounts in the axes and in the cotyledons. The storage patterns for Mg, K and P are quite similar but the Ca storage pattern is different. Ca levels are low and more variable especially in the cucurbit species with large embryos, whether parent or hybrid. However, this low amount of Ca was sufficient for considerable seedling growth. The root and stem portions of etiolated seedlings receive only a small percent of the Ca stored in the cotyledons and no Ca was acquired from the seed coats. It became evident that the measured mobilization of the minerals was mainly the result of the growth conditions. Some Ca was mobilized from the cotyledons when seedlings were grown in the dark and in

nutrient-free medium but when grown in the light in full nutrient medium the cotyledons showed marked gains in Ca. Fully expanded photosynthetic cotyledons contained as much as 150 times more Ca than the dry seed cotyledons. It may be that in cucurbits minerals such as Mg, K, Ca and P, stored in the cotyledons are not used by the growing axis and no mobilization of mineral, such as found with legume cotyledons occurs under normal growth conditions. The question is whether the relative proportions of the initially stored minerals are different in these species. One difference that I found during my work with ashing procedures was in the cation-to-P ratio. Legume seeds have a higher ratio of cations to P than do the cucurbits and generally have a lower level of P. Perhaps expanding and greening cotyledons require higher amounts of P. Castor bean embryos also have photosynthetic cotyledons and I found that, like cucurbits, they had a low cation to P ratio. However castor beans also have a large endosperm whereas cucurbits lack endosperm at maturity and so the comparison may not be valid. This study has contributed not only a lot of useful basic knowledge about mineral storage in seeds, but has also raised many new questions. Some suggestions for future research follow.

FUTURE WORK

Information on the chemical composition of ashes of various plant components is not generally available. The evidence that similar oily embryo tissues can produce ashes with such different extraction properties for Ca, points to the need for more investigation into plant ash characteristics. Sample-crucible interactions obviously occur and with cucurbit tissues involve K and perhaps P but how widespread interactions

between crucibles and plant tissues are remains unknown. It would be of interest to test the ashing characteristics of a wider range of seeds, especially those whose cotyledons become photosynthetic. Analysis of ash components after ashing at various temperatures is needed to determine the validity of Wichmann's explanations of the differences in ash solubilities. Microwave ashing and various new lower temperature ashing procedures need to be tested on Ca-binding and non-binding tissues to ensure their general applicability to plant tissues. Whether the low cation-to-P ratio contributes to crucible interactions as well as increasing Ca binding in the ash remains to be determined. In addition, marked differences in mineral ratios between species raise a number of interesting questions as to the varying needs of embryos for different mineral levels.

While the distribution of minerals between axes and cotyledons was studied, the more specific distribution within embryo cells has not been examined in the hybrid embryos. Since the development of cryogenic procedures the use of frozen specimens for mineral analysis by energy dispersive x-ray analysis has been recommended to ensure the *in situ* retention of the minerals. While the procedure is useful for the analysis of cucurbit embryos the freezing and fracturing of oil-rich tissues to obtain the desired cross-section presents challenges not present with non-oily tissues. When the technique is standardized it will be useful not only to establish the pattern of Ca distribution in the hybrid embryos as compared to the parental patterns, but also to compare hybrids with Ca levels closer to an intermediate and to parental Ca levels. It is of interest to determine whether distribution patterns differ for the long

and short C. maxima embryos which I found to have marked differences in total Ca concentration.

Other aspects of mineral accumulation in cucurbit embryos also need to be investigated further. Comparison of mineral levels in the flesh of fruits with embryos with high or low mineral levels would contribute information on the relative importance of redistribution of minerals from surrounding tissues. The timing of the accumulation of the four minerals during cucurbit seed development remains to be defined. Changes in the distribution of elements such as Ca during maturation of the embryos have also not been studied. The analysis of embryos of F_2 hybrid seeds which have seed sizes of intermediate size between the two parent species would contribute information of the role of seed size in Ca accumulation. Because of differences in embryo size and Ca levels, the two cucurbit species and their hybrids remain a useful system for studying mineral storage in dicot embryos.

REFERENCES

ALKEMADE, C.Th.J. (1966). On the interpretation of solute vaporization interference in flame photometry. Anal. Chem. 38: 1252-1253.

ALLEN, S.E.(ed.) (1974). Chemical Analysis of Ecological Materials. New York: John Wiley and Sons. pp. 133-136.

ALVAREZ, R. (1980). NBS plant tissue standard reference materials. J. Assoc. Off. Anal. Chem. 63: 806-808.

AMOS, M.D. and WILLIS, J.B. (1966). Use of high-temperature pre-mixed flames in atomic absorption spectroscopy. Spectrochim. Acta 22: 1325-1343.

ARMSTRONG, M.J. and KIRKBY, E.A. (1979). The influence of humidity on the mineral composition of tomato plants with special reference to calcium distribution. Plant and Soil 52: 427-435.

BA-AMER, M.A. and BEMIS, W.P. (1968). Fruit and seed development in Cucurbita foetidissima. Econ. Bot. 22: 297-299.

BAILEY, L.H. (1949). Manual of Cultivated Plants. New York: Macmillan Publishing Co., Inc. pp. 950-953.

BAIR, C.W. and SNYDER, H.E. (1980). Electron microscopy of soybean lipid bodies. J. Amer. Oil Chemists' Soc. 57: 279-282.

BAKER, G.L. and JOHNSON, L.H. (1954). Effects of anions on calcium flame emission in flame photometry. Anal. Chem. 26: 465-468.

BARBER, K.G. (1909). Comparative histology of fruits and seeds of certain species of Cucurbitaceae. Bot.Gaz. 47: 263-310.

BEMIS, W.P., RHODES, A.M., WHITAKER, T.W. and CARMER, S.G. (1970). Numerical taxonomy applied to Cucurbita relationships. Amer. J. Bot. 57: 404-412.

BEMIS, W.P., SCHEERENS, J.C., BERRY, J.W., DREHER, M.L. and WEBER, C.W. (1977). Accumulation of crude protein and oil contents. J. Amer. Oil Chemists' Soc. 454: 537-538.

BENGTSSON, B. (1982a). Uptake and translocation of calcium in cucumber. Physiol. Plant. 54: 107-111.

BENGTSSON, B. (1982b). The effect of Ca^{2+} stress on fluxes of Ca^{2+} and K^{+} in wheat and cucumber. Physiol. Plant. 56: 415-420.

BENGTSSON, B. and JENSEN, P. (1982). Uptake of calcium in wheat and cucumber roots. Physiol. Plant. 55: 273-278.

BENGTSSON, B. and JENSEN, P. (1983). Uptake and distribution of calcium, magnesium and potassium in cucumber of different age. Physiol. Plant. 57: 428-434.

BERINGER, H. and TEKETE, A. (1979). Influence of mineral nutrition on seed development of two groundnut cultivars. Commun. Soil Sci. Plant Anal. 10: 491-501.

BEWLEY, J.D. and BLACK, M. (1985). Seeds, Physiology of Development and Germination. New York: Plenum Press. pp. 10-25, 264-300.

BIELESKI, R.L. (1973). Phosphate pools, phosphate transport, and phosphate availability. Ann. Rev. Plant Physiol. 24: 225-252.

BLAGROVE, R.J. and LILLEY, G.G. (1980). Characterisation of cucurbitin from various species of the Cucurbitaceae. Eur. J. Biochem. 103: 577-584.

BOLLEY, D.S. and McCORMACK, R.H. (1952). Separation of phytin from oil seed protein flours. J. Amer. Oil Chemists' Soc. 29: 470-472.

BRANCH, W.D. and GAINES, T.P. (1983). Seed mineral composition of diverse peanut germplasm. Peanut Sci. 10: 5-8.

BROWN, R. (1940). An experimental study of the permeability to gases of the seed-coat membranes of Cucurbita Pepo . Ann. Bot. NS. IV: 379-395.

BUSHNELL, J.W. (1920). The fertility and fruiting habit in Cucurbita . J. Amer. Soc. Hort. Sci. 15: 47-51.

CAMERON, J.A., KESZTHELYI, L., MORTON, R., NIR, D. and WENDER, S.A. (1976). Determination of heavy elements in organic substances by x-ray absorption. Rad. and Environm. Biophys. 13: 263-266.

CHAKRAVARTY, K. and BASAK, S.L. (1983). Genetics of seed weight and number in mesta. Seed Res. 11: 212-216.

CHAPMAN, J.M. and DAVIES, H.V. (1983). Control of the breakdown of food reserves in germinating dicotyledonous seeds - a reassessment. Ann. Bot. 52: 593-595.

CHAPMAN, J.M. and GALLESCHI, L. (1985). The control of food mobilization in seeds of Cucumis sativus L. VI. The production of starch. Ann. Bot. 55: 29-34.

CHINO, M. (1981). Species differences in calcium and potassium distributions within plant roots. Soil Sci. Plant Nutr. 27: 487-503.

CHRISTIANSEN, M.N. and FOY, C.D. (1979). Fate and function of calcium in tissues. Commun. Soil Sci. Plant Anal. 10: 427-442.

CLARKSON, D.T. and HANSON, J.B. (1980). The mineral nutrition of higher plants. Ann. Rev. Plant Physiol. 31: 239-298.

CLEGG, M.S., KEEN, C.L., LONNERDAL, B. and HURLEY, L.S. (1981). Influence of ashing techniques on the analysis of trace elements in biological

samples. II. Dry ashing. Biol. Trace Elem. Res. 3: 237-244.

COCCUCI, M. (1984). Increase in calmodulin level in the early phases of radish seed (Raphanus sativus) germination. Plant, Cell and Environ. 7: 215-221.

COLLINS, O.D.G. and SUTCLIFFE, J.F. (1977). The relationship between transport of individual elements and dry matter from the cotyledons of Pisum sativum L. Ann. Bot. 41: 163-171.

COOK, R.E. (1975). The photoinductive control of seed weight in Chenopodium rubrum L. Amer. J. Bot. 62: 427-431.

COOMBE, B.G. (1976). The development of fleshy fruits. Ann. Rev. Plant Physiol. 27: 207-228.

CORLISS, W.R. (1964). Neutron Activation Analysis . United States Atomic Energy Commission, Division of Technical Information. pp. 2-28.

CORNES, J.J.S. (1944). Water-insoluble alkali ash. New Zeal. J. Sci. Tech. 26B: 190.

COSGROVE, D.J. (1966). The chemistry and biochemistry of inositol polyphosphates. Rev. Pure and Appl. Chem. 16: 209-224.

CRAFTS, A.S. and LORENZ, O.A. (1944a). Fruit growth and food transport in cucurbits. Plant Physiol. 19: 131-138.

CRAFTS, A.S. and LORENZ, O.A. (1944b). Composition of fruits and phloem exudate of cucurbits. Plant Physiol. 19: 326-337.

CRANE, J.C. (1964). Growth substances in fruit setting and development. Ann. Rev. Plant Physiol. 15: 303-326.

CROMPTON, C.E., WYNNE, J.C. and ISLEIB, T.G. (1979). Combining ability analysis of seed calcium content and adenosine phosphates in peanuts. Oleagineux 34: 71-74.

CRUDEN, R.W. and MILLER-WARD, S. (1981). Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: an hypothesis. Evol. 35: 964-974.

CUTLER, H.C. and WHITAKER, T.W. (1961). History and distribution of the cultivated cucurbits in the Americas. Antiquity 26: 469-485.

DAVID, D.J. (1959). Determination of calcium in plant material by atomic-absorption spectrophotometry. Analyst 84: 536-545.

DAVIES, D.R. (1977). DNA contents and cell number in relation to seed size in the genus Vicia . Heredity 39: 153-163.

DAVIES, H.V. and CHAPMAN, J.M. (1979a). The control of food mobilisation in seeds of Cucumis sativus L. I. The influence of embryonic axis and testa on protein and lipid degradation. Planta 146: 579-584.

DAVIES, H.V. and CHAPMAN, J.M. (1979b). The control of food mobilisation in seeds of Cucumis sativus L. II. The role of the embryonic axis. Planta 146: 585-590.

DAVIES, H.V. and CHAPMAN, J.M. (1980). The control of food mobilisation in seeds of Cucumis sativus L. III. The control of protein degradation. Planta 149: 288-291.

DAVIES, H.V. and CHAPMAN, J.M. (1981). The control of food mobilisation in seeds of Cucumis sativus L. IV. The pattern of protein degradation. Z. Pflanzenphysiol. Bd. 101S: 347-353.

DAVIES, H.V., GABA, V., BLACK, M. and CHAPMAN J.M. (1981). The control of food mobilisation in seeds of Cucumis sativus L. V. The effect of light on lipid degradation. Planta 152: 70-73.

DAVIES, H.V. and SLACK, P.T. (1981). The control of food mobilization in seeds of dicotyledonous plants. New Phytol. 88: 41-51.

DAVIS, M.E. (1974). In vivo and in vitro breakdown of Cucurbita seed protein bodies. MSc. Thesis, University of California, Davis, California. pp. 16-19.

DeKOCK, P.C., INKSON, R.H.E. and HALL, A. (1982). Blossom-end rot of tomato as influenced by truss size. J. Plant Nutr. 5: 57-62.

DICKSON, R.E. and JOHNSON, C.M. (1966). Interferences associated with the determination of calcium by atomic absorption. Appl. Spec. 20: 214-218.

DINNIN, J.I. (1960). Releasing effects in flame photometry. Anal. Chem. 32: 1475-1480.

DIPPEL, W.A., BRICKER, C.E. and FURMAN, N.H. (1954). Flame photometric determination of phosphate. Anal. Chem. 26: 553-556.

DMITRIEVA, M.I. and SOBOLEV, A.M. (1985). Mobilization of phytin in castor seeds during germination. Soviet Plant Physiol. 31: 799-805.

DURE, L.S. III. (1975). Seed formation. Ann. Rev. Plant Physiol. 26: 259-278.

ECHLIN, P. (1968). Pollen. Sci. Amer. 218: 81-90.

EGLI, D.B. (1981). Species differences in seed growth characteristics. Field Crops Res. 4: 1-12.

EGLI, D.B., FRASER, J., LEGGETT, J.E. and PONELEIT, C.G. (1981). Control of seed growth in soya beans (Glycine max (L.) Merrill). Ann. Bot. 48: 171-176.

EGLI, D.B. and WARDLAW, I.F. (1980). Temperature response of seed growth characteristics of soybeans. Agron. J. 72: 560-564.

ESAU, K. (1977). Anatomy of Seed Plants. New York: John Wiley and Sons. pp. 429-473.

EVANS, C.C. (1970). X-ray fluorescence analysis for light elements in plant and faecal materials. Analyst 95: 919-929.

FALCONER, D.S. (1960). Introduction to Quantitative Genetics. London: Longman Group Ltd. (reprinted 1976) pp.

FASSEL, V.A. and BECKER, D.A. (1969). Chemical or solute vaporization interferences in flame atomic emission and absorption spectrometry. Anal. Chem. 41: 1522-1526.

FENNER, M. (1983). Relationship between seed weight, ash content and seedling growth in twenty-four species of Compositae. New Phytol. 95: 697-706.

FERGUSON, I.B. and BOLLARD, E.G. (1976). The movement of calcium in germinating pea seeds. Ann. Bot. 40: 1047-1055.

FOFANOVA, T.A. and KHOKHLOVA, V.A. (1983). Tissue specificity of the response of cells of isolated pumpkin cotyledons to phytochrome action. Soviet Plant Physiol. 30: 317-324.

FONTES, L.A.N. and OHLROGGE, A.J. (1972). Influence of seed size and population on yield and other characteristics of soybean (Glycine max (L.) Merr.). Agron. J. 64: 833-836.

FRITTS, S.K. and LOY, J.B. (1981). Influence of light quality during seed development and drying on germination in watermelon. J. Amer. Soc. Hort. Sci. 106: 262-266.

GALEN, C. and WEGER, H.G. (1986). Re-evaluating the significance of correlations between seed number and size: evidence from a natural population of the lily, Clintonia borealis . Amer. J. Bot. 73: 346-352.

GATSCHKE, W. and GAWLIK, D. (1980). Simultaneous determination of aluminum and phosphorus by neutron activation analysis. J. Radioanal. Chem. 56: 203-212.

GIFFORD, R.M. and EVANS, L.T. (1981). Photosynthesis, carbon partitioning, and yield. Ann. Rev. Plant Physiol. 32: 485-509.

GIRON, H.C. (1973). Comparison between dry ashing and wet digestion in the preparation of plant material for atomic absorption analysis. At. Abs. Newsl. 12: 28-29.

GLADNEY, E.S., BURNS, C.E., PERRIN, D.R., ROELANDTS, I. and GILLS, T.E. (1984). NBS Special Puablic. 260-88, US Government Printing Office, Washington. pp. 9, 48-49.

GLEIT, C.E. (1963). Electonic apparatus for ashing biologic specimens.

Med. Res. Eng. 2: 112-118.

GLEIT, C.E. and HOLLAND, W.D. (1962). Use of electrically excited oxygen for the low temperature decomposition of organic substances. Anal. Chem. 34: 1454-1457.

GOEL, M. and SHARMA, C.B. (1979). Multiple forms of phytase in germinating cotyledons of Cucurbita maxima . Phytochem. 18: 1939-1942.

GORCHOV, D.L. (1985). Fruit ripening asynchrony is related to variable seed number in Amelanchier and Vaccinium . Amer. J. Bot. 72: 1939-1943.

GORNALL, R.J. and BOHM, B.A. (1984). Breeding systems and relationships among species of Boykinia and related genera (Saxifragaceae). Can. J. Bot. 62: 33-37.

GORSUCH, T.T. (1959). Radiochemical investigations on the recovery for analysis of trace elements in organic and biological materials. Analyst 84: 135-173.

GORSUCH, T.T. (1970). The Destruction of Organic Matter. Oxford: Pergamon Press Ltd. pp. 1-144.

GREENWOOD, J.S. and BEWLEY, J.D. (1984). Subcellular distribution of phytin in the endosperm of developing castor bean: a possiblity for its synthesis in the cytoplasm prior to deposition within protein bodies. Planta 160: 113-120.

GREENWOOD, J.S., GIFFORD, D.J. and BEWLEY, J.D. (1984). Seed development in Ricinus communis cv. Hale (castor bean). II. Accumulation of phytic acid in the developing endosperm and embryo in relation to the deposition of lipid, protein and phosphorus. Can. J. Bot. 62: 255-261.

GRIFFITHS, D.W. and THOMAS, T.A. (1981). Phytate and total phosphorus content of field beans (Vicia faba L.). J. Sci. Food Agric. 32: 187-192.

GUARDIOLA, J.L. and SUTCLIFFE, J.F. (1971). Mobilization of phosphorus in the cotyledons of young seedlings of the garden pea (Pisum sativum L.). Ann. Bot. 35: 809-823.

GUARDIOLA, J.L. and SUTCLIFFE, J.F. (1972). Transport of materials from the cotyledons during germination of seeds of the garden pea (Pisum sativum L.). J. Exp. Bot. 23: 322-337.

GULDAN, S.J. and BRUN, W.A. (1985). Relationship of cotyledon cell number and seed respiration to soybean seed growth. Crop Sci. 25: 815-819.

GUTTRIDGE, C.G. (1982). Calcium deficiencies. Grower, March 11, pp. 17-18.

HANSON, J.B. (1984). The functions of calcium in plant nutrition. In Advances in Plant Nutrition . vol. 1. (ed. P.B. Tinker and A. Lauchli). New York: Praeger Publishers. pp. 149-208.

HARA, I. and MATSUBARA, H. (1980). Pumpkin (Cucurbita sp.) seed globulin. V. Proteolytic activities involved in globulin degradation in ungerminated seeds. Plant and Cell Physiol. 21: 219-232.

HARA, I., WADA, K. and MATSUBARA, H. (1976a). Pumpkin (Cucurbita sp.) seed globulin II. Alterations during germination. Plant and Cell Physiol. 17: 815-823.

HARA, I., WADA, K. WAKABAYASHI, S. and MATSUBARA, H. (1976b). Pumpkin (Cucurbita sp.) seed globulin. I. Purification, characterization, and subunit structure. Plant and Cell Physiol. 17: 799-814.

HARA-NISHIMURA, I., NISHIMURA, M. and AKAZAWA, T. (1985). Biosynthesis and intracellular transport of 11S globulin in developing pumpkin cotyledons. Plant Physiol. 77: 747-752.

HARA-NISHIMURA, I., NISHIMURA, M., MATSUBARA, H. and AKAZAWA, T. (1982). Suborganellar localization of proteinase catalyzing the limited hydrolysis of pumpkin globulin. Plant Physiol. 70: 699-703.

HARNLY, J.M., KANE, J.S. and MILLER-IHLI, N.J. (1982). Effects of air-acetylene flame parameters on simultaneous multielement atomic absorption spectrometry. Appl. Spec. 36: 637-643.

HARPER, R.J., GILKES, R.J. and ROBSON, A.D. (1982). Biocrystallization of quartz and calcium phosphates in plants - re-examination of the evidence. Aust. J. Agric. Res. 33: 565-571.

HARPER, J.L., LOVELL, P.H. and MOORE, K.G. (1970). The shapes and sizes of seeds. Ann. Rev. Ecol. and Syst. 1: 327-356.

HARRISON-MURRAY, R.S. and CLARKSON, D.T. (1973). Relationship between structural development and the absorption of ions by the root system of Cucurbita pepo . Planta 114: 1-16.

HAYMAN, B.I. (1954). The theory and analysis of diallel crosses. Genetics 39: 789-809.

HAYNES, R.J. (1980). A comparison of two modified Kjeldahl digestion techniques for multi-element plant analysis with conventional wet and dry ashing methods. Comm. Soil Sci. Plant Anal. 11: 459-467.

HEISER, C.B. (1979). The Gourd Book . Norman: University of Oklahoma Press. pp. 3-14, 30-47, 231.

HELMS, K. (1971). Calcium deficiency of dark-grown seedling of Phaseolus vulgaris L. Plant Physiol. 47: 799-804.

HEPLER, P.K. and WAYNE, R.O. (1985). Calcium and plant development. Ann. Rev. Plant Physiol. 36: 397-439.

HESLOP-HARRISON, J. (1975). Incompatibility and the pollen-stigma interaction. Ann. Rev. Plant Physiol. 26: 403-425.

HESSE, P.R. (1971). A Textbook of Soil Chemical Analysis. New York: Chemical Publishing Co. Inc. pp. 106-107.

HEYDORN, K. (1984). Neutron Activation Analysis for Clinical Trace Element Research. vol. 1. Boca Raton, Florida: CRC Press, Inc. pp. 83-93, 123-142.

HINTON, J.J.C. (1959). The distribution of ash in the wheat kernel. Cer. Chem. 36: 19-31.

HOAGLAND, D.R. and ARNON, D.I. (1950). The water-culture method for growing plants without soil. University of California Agricultural Experimental Station Circular #347. pp. 30-31.

HOCKING, P.J. (1980). Redistribution of nutrient elements from cotyledons of two species of annual legumes during germination and seedling growth. Ann. Bot. 45: 383-396.

HOCKING, P.J. (1982). Accumulation and distribution of nutrients in fruits of castor bean (Ricinus communis L.). Ann. Bot. 49: 51-62.

HOCKING, P.J. and PATE, J.S. (1977). Mobilization of minerals to developing seeds of legumes. Ann. Bot. 41: 1259-1278.

HOCKING, P.J., PATE, J.S., ATKINS, C.A. and SHARKEY, P.J. (1978). Diurnal patterns of transport and accumulation of minerals in fruiting plants of Lupinus angustifolius L. Ann. Bot. 42: 1277-1290.

HOFFMAN, E.L. (1980). Analytical geochemistry advanced by neutron activation. Can J. Spectroscopy 25: 23A-25A.

HOOD, S.L., PARK, R.Q. and HURWITZ, C. (1944). Mineral contamination resulting from grinding plant samples. Ind. Eng. Chem. (Anal. Ed.) 16: 202-205.

HOOVER, W. L. (1976). Comparison of wet ash and dry ash methods for the digestion of mineral-mix feeds. J. Ass. Offic. Anal. Chem. 59: 937-938.

HOSKING, J.W., OLIVER, K.R. and STURMAN, B.T. (1979). Errors in the atomic absorption determination of calcium by the standard addition method. Anal. Chem. 51: 307-310.

HORWITZ, W. (ed.) (1975). Official Methods of Analysis, of the Association of Official Analytical Chemists. 12 th edition. Washington, D.C.: OAAC.

HUANG, A.H.C. and MOREAU, R.A. (1978). Lipases in the storage tissues of peanut and other oil seeds during germination. Planta 141: 111-116.

HUME, R.J. and LOVELL, P.H. (1981). Reduction of the cost involved in hybrid seed production of pumpkins (Cucurbita maxima Duchesne). N. Z. J. Exp. Agric. 9: 209-210.

INGESTAD, T. (1972). Mineral nutrient requirements of cucumber seedlings. PLant Physiol. 52: 332-338.

ISAAC, R.A. (1980). Atomic absorption methods for analysis of soil extracts and plant tissue digests. J. Assoc. Off. Anal. Chem. 63: 788-796.

ISAAC, R.A. and JOHNSON, W.C. (1975). Collaborative study of wet and dry ashing techniques for the elemental analysis of plant tissues by atomic absorption spectrophotometry. J. Ass. Offic. Anal. Chem. 58: 436-440.

ISAAC, R.A. and JONES, Jr., J.B. (1972). Effects of various dry ashing temperatures on the determination of 13 nutrient elements in five plant tissues. Comm. Soil Sci. Plant Anal. 3: 261-269.

IVERSON, J.L. (1972). Collaborative study of a joint AOAC-OICC method for the determinations of ash in cacao products. J. Ass. Offic. Anal. Chem. 55: 1027-1030.

IUPAC-IUB. (1968). The nomenclature of cyclitols. Eur.J. Biochem. 5: 1-12.

JACKS, T.J., HENSARLING, T.P. and YATSU, L.Y. (1972). Cucurbit seeds: I. Characterization and uses of oils and proteins. A review. Econ. Bot. 26: 135-141.

JANOVA, D. and DVORAK, M. (1976). Conditions of calcium movement by diffusion in plants. Acta Univ. Carolinae Biol. 1-2: 11-30.

JINKS, J.L. (1954). The analysis of continuous variation in a diallel cross of Nicotiana rustica varieties. Genetics 39: 767-787.

JOHNSON, J.W. and COLLINS, F.I. (1973). Low temperature grinding of some high oil content seeds. Agron. J. 65: 995-996.

JOHNSON, L.F. and TATE, M.E. (1969). Structure of "phytic acids". Can. J. Chem. 47: 63-73.

KEISLING, T.C., HAMMONS, R.O. and WALKER, M.E. (1982). Peanut seed size and nutritional calcium requirements. J. Plant Nutr. 5: 1177-1185.

KIHARA, H. (1951). Triploid watermelons. Proc. Amer. Soc. Hort. Sci. 58: 217-230.

KNUDSEN, D., CLARK, R.B., DENNING, J.L. and PIER, P.A. (1981). Plant analysis of trace elements by x-ray. J. Plant Nutr. 3: 61-75.

KWACK, S.N. and FUJIEDA, K. (1985). Pollen tube growth and embryo development in interspecific crosses of Cucurbita . J. Fac. Agr., Kyushu Univ. 30: 1-8.

LABANAUSKAS, C.K. and HANDY, M.F. (1975). Dry-ashing temperature, time, and sample size as variables influencing nutrient concentrations in citrus leaf analysis. HortSci. 10: 386-388.

LAMBERT, M.J. (1976). Preparation of plant material for estimating a wide range of elements. Forestry Commission of N.S.W., Research Note No. 29. pp. 1-51.

LARSON, L.A. (1968). The effect soaking pea seeds with or without seedcoats has on seedling growth. Plant Physiol. 43: 255-259.

LASLEY, S.E. and GARBER, M.P. (1978). Photosynthetic contribution of cotyledons to early development of cucumber. HortSci. 13: 191-193.

LEGOCKA, J., GWOZDZ, E.A. and BRUSKA, B. (1985). The effect of kinetin on the level of proteins and lipids in cucumber cotyledons (Cucumis sativus L. cv. Monastyrski). Acta Physiol. Plant. 7: 85-93.

LEVINE, B.A. and WILLIAMS, R.J.P. (1982). The chemistry of calcium ion and its biological relevance. In The Role of Calcium in Biological Systems. vol. 1. (ed. L. J. Anghileri and A.M. Tuffet-Anghileri). Boca Raton, Florida: CRC Press, Inc. pp. 3-26.

LOEWUS, F. (1971). Carbohydrate interconversions. Ann. Rev. Plant Physiol. 22: 337-364.

LOEWUS, F.A. and LOEWUS, M.W. (1983). myo -Inositol: Its biosynthesis and metabolism. Ann. Rev. Plant Physiol. 34: 137-161.

LOLAS, G.M. and MARKAKIS, P. (1975). Phytic acid and other phosphorus compounds of beans (Phaseolus vulgaris L.). J. Agr. Food Chem. 23: 13-15.

LOLAS, G.M., PALAMIDIS, N. and MARKAKIS, P. (1976). The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. Cer. Chem. 53: 867-871.

LONG, G.L. and BOSS, C.B. (1982a). Depression of calcium, strontium, and barium signals by phosphine in atomic spectrometry. Anal. Chem. 54: 624-629.

LONG, G.L. and BOSS, C.B. (1982b). Phosphine depression of calcium in flame atomic emission spectrometry with a uniform droplet generator. Anal. Chem. 54: 2496-2502.

LOOMIS, E.L. and SMITH, O.E. (1980). The effect of artificial aging on the concentration of Ca, Mg, Mn, K, and Cl in imbibing cabbage seed. J. Amer. Soc. Hort. Sci. 105: 647-650.

LOTT, J.N.A. (1970a). Changes in the cotyledons of Cucurbita maxima during germination. I. General characteristics. Can. J. Bot. 48: 2227-2231.

LOTT, J.N.A. (1970b). Changes in the cotyledons of Cucurbita maxima during germination. III. Plastids and chlorophylls. Can. J. Bot. 48: 2259-2265.

LOTT, J.N.A. (1973). A scanning electron microscope study of Cucurbita maxima seed coat structure. Can. J. Bot. 51: 1711-1714.

LOTT, J.N.A. (1975). Protein body composition in Cucurbita maxima cotyledons as determined by energy dispersive x-ray analysis. Plant Physiol. 55: 913-916.

LOTT, J.N.A. (1980). Protein bodies. In The Biochemistry of Plants, A Comprehensive Treatise. vol. 1. (ed. P.K. Stumpf and E.E. Conn). New York: Academic Press. pp. 589-623.

LOTT, J.N.A. (1984). Accumulation of seed reserves of phosphorus and other minerals. In Seed Physiology . vol. I. (ed. D.R. Murray), London: Academic Press. pp. 139-166.

LOTT, J.N.A. and BUTTROSE, M.S. (1978a). Globoids in protein bodies of legume seed cotyledons. Austr. J. Plant Physiol. 5: 89-111.

LOTT, J.N.A. and BUTTROSE, M.S. (1978b). Thin sectioning, freeze fracturing, energy dispersive x-ray analysis, and chemical analysis in the study of inclusions in seed protein bodies: almond, Brazil nut, and quandong. Can. J. Bot. 56: 2050-2061.

LOTT, J.N.A. and BUTTROSE, M.S. (1978c). Location of reserves of mineral elements in seed protein bodies: macadamia nut, walnut, and hazel nut. Can. J. Bot. 56: 2072-2082.

LOTT, J.N.A. and OCKENDEN, I. (1986). The fine structure of phytate-rich particles in plants. In Phytic Acid: Chemistry and Applications. (ed. E. Graf). Minneapolis: Pilatus Press. pp. 43-55.

LOTT, J.N.A. and VOLLMER, C.M. (1973a). Changes in the cotyledons of Cucurbita maxima during germination. IV. Protein bodies. Protoplasma 78: 255-271.

LOTT, J.N.A. and VOLLMER, C.M. (1973b). The structure of protein bodies in Cucurbita maxima cotyledons. Can. J. Bot. 51: 687-688.

LOTT, J.N.A. and VOLLMER, C.M. (1979). Composition of globoid crystals from embryo protein bodies in five species of Cucurbita . Plant Physiol. 63: 307-311.

LOTT, J.N.A., GREENWOOD, J.S. and VOLLMER, C.M. (1978). Energy-dispersive x-ray analysis of phosphorus, potassium, magnesium, and calcium in globoid crystals in protein bodies from different regions of Cucurbita maxima embryos. Plant Physiol. 63: 984-988.

LOTT, J.N.A., GREENWOOD, J.S. and VOLLMER, C.M. (1982). Mineral reserves in castor beans: the dry seed. Plant Physiol. 69: 829-833.

LOTT, J.N.A., LARSEN, P.L. and DARLEY, J.J. (1971). Protein bodies from the cotyledons of Cucurbita maxima . Can. J. Bot. 49: 1777-1782.

LOTT, J.N.A., RANDALL, P.J., GOODCHILD, D.J. and CRAIG, S. (1985).

Occurrence of globoid crystals in cotyledonary protein bodies of Pisum sativum as influenced by experimentally induced changes in Mg, Ca and K contents of seeds. Austr. J. Plant Physiol. 12: 341-353.

LOTT, J.N.A., SPITZER, E. and VOLLMER, C.M. (1979). Calcium distribution in globoid crystals of Cucurbita cotyledon protein bodies. Plant Physiol. 63: 847-851.

LOVELL, P.H. and MOORE, K.G. (1970). A comparative study of cotyledons as assimilatory organs. J. Exp. Bot. 21: 1017-1030.

MAGA, J.A. (1982). Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agr. Food Chem. 30: 1-9.

MAGILL, W.A. and SVEHLA, G. (1974). Study on the elimination of interferences in the determination of calcium by atomic absorption spectrophotometry. Z. Anal. Chem. 268: 177-180.

MANDAL, N.C. and BISWAS, B.B. (1970). Metabolism of inositol phosphate. I. Phytase synthesis during germination in cotyledons of mung beans, Phaseolus aureus . Plant Physiol. 45: 4-7.

MANN, L.K. (1943). Fruit shape of watermelon as affected by placement of pollen on stigma. Bot. Gaz. 105: 257-262.

MANN, L.K. and ROBINSON, J. (1950). Fertilization, seed development, and fruit growth as related to fruit set in the cantaloupe (Cucumis melo L.). Amer. J. Bot. 37: 685-697.

MANNING, D.C. and CAPACHO-DELGADO, L. (1966). Dissociation and ionization effects in atomic absorption spectrochemical analysis. Anal. Chim. Acta 36: 312-318.

MARGOSHES, M. and VALLEE, B.L. (1956). Instrumentation and principles of flame spectrometry. Anal. Chem. 28:180-184.

MARME, D. (1983). Calcium transport and function. In Inorganic Plant Nutrition. Encyclopedia of Plant Physiology. vol. 15B (ed. A. Lauchli and R.L. Bieleski). New York: Springer Verlag. pp. 599-625.

MARSCHNER, H. (1983). General introduction to mineral nutrition. In Inorganic Plant Nutrition. Encyclopedia of Plant Physiology. vol. 15A. (ed. A. Person and M.H. Zimmerman). New York: Springer Verlag. pp. 5-60.

MARSHALL, P.E. and KOZLOWSKI, T.T. (1974). Photosynthetic activity of cotyledons and foliage leaves of young angiosperm seedlings. Can. J. Bot. 52: 2023-2032.

MARSHALL, P.E. and KOZLOWSKI, T.T. (1975). Changes in mineral contents of cotyledons and young seedlings of woody angiosperms. Can. J. Bot. 53: 2026-2031.

MATHER, K. and JINKS, J.L. (1982). Biometrical Genetics. New York: Chapman and Hall. pp. 1-36, 255-260.

MAZER, S.J., SNOW, A.A. and STANTON, M. (1986). Fertilization dynamics and parental effects upon fruit development in Raphanus raphanistrum : consequences for seed variation. Amer. J. Bot. 73: 500-511.

McGIBBON, R. and WILLIAMS, W. (1981). The accumulation of lipids in developing seeds of white lupin (Lupinus albus L.). J. Sci. Food Agric. 32: 792-796.

McKERSIE, B.D. and SENARATNA, T. (1983). Membrane structure in germinating seeds. In Mobilization of Reserves in Germination. Recent Advances in Phytochemistry. vol. 17. (ed. C. Nozzolillo and P.J. Lea). New York: Plenum Press. pp. 29-52.

MENDEN, E.E., BROCKMAN, D., CHOUDHURY, H. and PETERING, H.G. (1977). Dry ashing of animal tissues for atomic spectrometric determination of zinc, copper, cadmium, lead, iron, manganese, magnesium and calcium. Anal. Chem. 49: 1644-1655.

MENGEL, K. and KIRKBY, E.A. (1982). Principles of Plant Nutrition. Worblaufen-Bern, Switzerland: International Potash Institute. pp. 387-471.

METCALF, R.L., RHODES, A.M., METCALF, R.A., FERGUSON, J., METCALF, E.R. and LU, P.-Y. (1982). Cucurbitacin contents and Diabroticite (Coleoptera: Chrysomelidae) feeding upon Cucurbita spp. Environ. Entomol. 11: 931-937.

MEYER, M. and MEYER, Jr. R.A. (1976). Unsuitability of porcelain crucibles for dry ashing of biological tissues for calcium analysis. Clin. Chem. 22: 1396-1398.

MICHOTTE, Y., MASSART, D.L. and PELSMAEKERS, J. (1976). The effect of low-temperature ashing on the structure and composition of mineral components. Talanta 23: 691-692.

MIDDLETON, G. and STUCKEY, R.E. (1953). The preparation of biological material for the determination of trace metals. I. A critical review of existing procedures. Analyst 78: 532-542.

MIDDLETON, G. and STUCKEY, R.E. (1954). The preparation of biological material for the determination of trace metals. II. A method for the destruction of organic matter in biological material. Analyst 79: 138-142.

MUNTER, R.C., GRANDE, R.A. and AHN, P.C. (1979). Analysis of animal tissue and food materials by inductively coupled plasma emission spectrometry. ICP Inf. Newsl. 5: 368-383.

MURRAY, D.R. (1980). Functional significance of acid phosphatase distribution during embryo development in Pisum sativum L. Ann. Bot. 45: 273-281.

MURRAY, D.R. (1984). Axis-cotyledon relationship during reserve mobilization. In Seed Physiology . vol. 2. (ed. D.R. Murray). Toronto: Academic Press. pp. 247-280.

NAIDENOV, M.B. and RAIKOV, L.P. (1980). Neutron-activation analysis of plant materials. Comptes Rendus de l'Academie Bulgare des Sci. 33: 273-276.

NAKAYAMA, J. SAIO, K. and KITO, M. (1981). Decomposition of phospholipids in soybeans during storage. Cer. Chem. 58: 260-264.

NELSON, H.C. (1932). Development of the foliaceous cotyledons of Cucurbita maxima . Univ. Iowa Stud. Natur. Hist. 14: 3-27.

NELSON, P.V. and BOODLEY, J.W. (1965). An error involved in the preparation of plant tissue for analysis. Amer. Soc. Hort. Sci. 86: 712-716.

NITSCH, J.P. (1953). The physiology of fruit growth. Ann. Rev. Plant Physiol. 4: 199-236.

OKAMOTO, H. (1962). Transport of cations from cotyledon to seedling of the embryonic plants of Vigna sesquipedalis . Plant and Cell Physiol. 3: 84-94.

O'KENNEDY, B.T., REILLY, C.C., TITUS, J.S. and SPLITTSTOESSER, W.E. (1979). A comparison of the storage protein (globulin) of eight species of Cucurbitaceae. Can. J. Bot. 57: 2044-2049.

OKUSANYA, O.T. OLA-ADAMS, B.A. and BAMIDELE, J.F. (1981). Variations in size, leaf morphology, and fruit characters among 25 populations of Luffa aegyptiaca . Can. J. Bot. 59: 2618-2627.

PATE, J.S. and HOCKING, P.J. (1978). Phloem and xylem transport in the supply of minerals to a developing legume (Lupinus albus L.) fruit. Ann. Bot. 42: 911-921.

PATE, J.S. and SHARKEY, P.J. (1975). Xylem to phloem transfer of solutes in fruiting shoots of legumes studied by a phloem bleeding technique. Planta 122: 11-26.

PENNER, D. and ASHTON, F.M. (1966). Proteolytic enzyme control in squash cotyledons. Nature 212: 935-936.

PENNER, D. and ASHTON, F.M. (1967). Hormonal control of proteinase activity in squash cotyledons. Plant Physiol. 42: 791-796.

PEOPLES, M.B., PATE, J.S., ATKINS, C.A. and MURRAY, D.R. (1985). Economy of water, carbon, and nitrogen in the developing cowpea fruit. Plant Physiol. 77: 142-147.

PERRING, M.A. (1981). Calcium and magnesium in the apple fruit: their distribution at harvest and redistribution during storage. J. Sci.

Food Agric. 32: 992-993.

PESIS, E. and NG, T.J. (1986). The effect of seedcoat removal on germination and respiration of muskmelon seeds. Seed Sci. and Technol. 14: 117-125.

PET, G. and GARRETSEN, F. (1983). Genetical and environmental factors influencing seed size of tomato (Lycopersicon esculentum Mill.) and the effect of seed size on growth and development of tomato plants. Euphytica 32: 711-718.

PETERS, D.G., HAYES, J.M. and HIEFTJE, G.M. (1974). Atomic absorption spectroscopy. In Chemical Separations and Measurements. Philadelphia: W. B. Saunders and Co. pp. 688-694.

PHARR, D.M., HUBER, S.C. and SOX, H.N. (1985). Leaf carbohydrate status and enzymes of translocate synthesis in fruiting and vegetative plants of Cucumis sativus L. Plant Physiol. 77: 104-108.

PICHL, I. (1976). Seed globulins of various species of Cucurbitaceae. Phytochem. 15: 717-722.

PICHL, I. (1978). Characterization of albumins isolated from seeds of Cucurbita maxima L. Biochem. Physiol. Pflanzen 172: 61-66.

POOVAIAH, B.W. (1985). Role of calcium and calmodulin in plant growth and development. HortSci. 20: 347-351.

POSTERNAK, T. (1965). The Cyclitols. Paris: Editiones Scientifiques Hermann. pp. 221-241, 301-303.

POWELL, A.A. and MATTHEWS, S. (1978). The damaging effect of water on dry pea embryos during imbibition. J. Exp. Bot. 29: 1215-1229.

PRASAD, M. and SPIERS, M. (1978). Comparative study of ashing techniques for the digestion of horticultural plant samples. J. Agr. Food Chem. 26: 824-827.

PRATT, H.K. (1971). Melons. In The Biochemistry of Fruits and Their Products. vol. 2. (ed. A.C. Hulme). New York: Academic Press. pp. 207-232.

PRINGLE, J.S. (1973). The concept of the cultivar. The Gardens' Bulletin 27: 13-27.

RABOY, V. and DICKINSON, D.B. (1984). Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. Plant Physiol. 75: 1094-1098.

RABOY, V., DICKINSON, D.B. and BELOW, F.E. (1984). Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of Glycine max and G. soja . Crop Sci. 24: 431-434.

RAMAKRISHNA, T.V., WEST, P.W. and ROBINSON, J.W. (1968). The determination

of calcium and magnesium in acetylene flames. Anal. Chim. Acta 40: 347-350.

RASI-CALDOGNO, F. and DE MICHELIS, M.I. (1978). Correlation between oxygen availability, energy change, and protein synthesis in squash cotyledons isolated from germinating seeds. Plant Physiol. 61: 85-88.

RAVEN, J.A. (1977). H^+ and Ca^{2+} in phloem and symplast: relation of relative immobility of the ions to the cytoplasmic nature of the transport paths. New Phytol. 79: 465-480.

REILLY, C.C., O'KENNEDY, B.T., TITUS, J.S. and SPLITTSTOESSER, W.E. (1978). The solubilization and degradation of pumpkin seed globulin during germination. Plant and Cell Physiol. 19: 1235-1246.

RICHARDSON, P.T., BAKER, D.A. and HO, L.C. (1982). The chemical composition of cucurbit vascular exudates. J. Exp. Bot. 33: 1239-1247.

RICHARDSON, P.T., BAKER, D.A. and HO, L.C. (1984). Assimilate transport in cucurbits. J. Exp. Bot. 35: 1575-1581.

ROBERTS, E.H. and ROBERTS, D.L. (1972). Moisture content of seeds. In Viability of Seeds. (ed. E.H. Roberts). Syracuse, N.Y.: Syracuse University Press. pp. 424-429.

ROBINSON, J.W. (1975). Atomic Absorption. New York: Marcel Dekker, Inc. pp. 1-131.

ROBINSON, R.W., MUNGER, H.M., WHITAKER, T.W. and BOHN, G.W. (1976). Genes of the Cucurbitaceae. HortSci. 11: 554-568.

ROCCHICCIOLI, C. and TOWNSHEND, A. (1968). Some interferences in the atomic absorption spectrophotometry of calcium. Anal. Chim. Acta 41: 93-98.

RYAN, B.F., JOINER, B.L. and RYAN, Jr., T.A. (1985). Minitab Handbook. Boston: Duxbury Press. pp. 92-120, 181-258.

SAIF, S.R. (1977). The influence of stage of host development on vesicular-arbuscular mycorrhizae and endogonaceous spore population in field-grown vegetable crops. I. Summer-grown crops. New Phytol. 79: 341-348.

SALIMATH, B.P. and MARME, D. (1983). Protein phosphorylation and its regulation by calcium and calmodulin in membrane fractions from zucchini hypocotyls. Planta 158: 560-568.

SALISBURY, Sir E. (1974). Seed size and mass in relation to environment. Proc. R. Soc. Lond. B. 186: 83-88.

SANSONI, B. and PANDAY, V.K. (1983). Ashing in trace element analysis of biological material. In Analytical Techniques for Heavy Metals in Biological Fluids. (ed. S. Facchetti). New York: Elsevier Science Publishers. pp. 91-131.

SASAKI, K. and TAYLOR, I.E.P. (1984). Specific labeling of cell wall polysaccharides with myo-(2-^{3}H)-inositol during germination and growth of Phaseolus vulgaris L. Plant and Cell Physiol. 25: 989-997.

SCHEERENS, J.C., BEMIS, W.P., DREHER, M.L. and BERRY, J.W. (1978). Phenotypic variation in fruit and seed characteristics of Buffalo gourd. J. Amer. Oil Chemists' Soc. 55: 523-525.

SCOTT, J.J. and LOEWUS, F.A. (1986). Phytate metabolism in plants. In Phytic Acid: Chemistry and Applications. (ed. E. Graf). Minneapolis: Pilatus Press. pp. 23-42.

SHARMA, C.B. and DIECKERT, W. (1975). Isolation and partial characterization of globoids from aleurone grains of Arachis hypogaea seed. Physiol. Plant. 33: 1-7.

SIMON, E.W. (1978). The symptoms of calcium deficiency in plants. New Phytol. 80: 1-15.

SIMON, E.W. (1984). Early events in germination. In Seed Physiology. vol. 2. (ed. D.R. Murray). Toronto: Academic Press. pp. 77-115.

SIMON, E.W. and MATHAVAN, S. (1986). The time-course of leakage from imbibing seeds of different species. Seed Sci. and Technol. 14: 9-13.

SINGH, A.K. (1979). Cucurbitaceae and polyploidy. Cytologia 44: 897-905.

SINGH, B. (1953). Studies on the structure and development of seeds of Cucurbitaceae. Phytomorphol. 3: 224-239.

SINGH, D. and DATHAN, A.S.R. (1972). Structure and development of seed coat in Cucurbitaceae. VI. Seeds of Cucurbita . Phytomorphol. 22: 29-45.

SINNOTT, E.W. (1939). A developmental analysis of the relation between cell size and fruit size in cucurbits. Amer. J. Bot. 26: 179-189.

SLACK, P.T., BLACK, M. and CHAPMAN, J.M. (1977). The control of lipid mobilization in Cucumis cotyledons. J. Exp. Bot. 28: 569-577.

SMETS, B. (1980). Vaporisation interference of sulphate and phosphate anions on the calcium flame atomic absorption signal. Analyst 105: 482-490.

SMITH, D.D. and BROWNER, R.F. (1984). Influence of aerosol drop size on signals and interferences in flame atomic absorption spectrometry. Anal. Chem. 56: 2702-2708.

SMITH, J.H., CARTER, D.L., BROWN, M.J. and DOUGLAS, C.L. (1968). Differences in chemical composition of plant sample fractions resulting from grinding and screening. Agron. J. 60: 149-151.

SNOW, A.A. (1982). Pollination intensity and potential seed set in

Passiflora vitifolia . Oecologia 55: 231-237.

SNOW, A.A. (1986). Pollination dynamics in Epilobium canum (Onagraceae): Consequences for gametophytic selection. Amer. J. Bot. 73: 139-151.

SOMERS, G.F. (1973). The affinity of onion cell walls for calcium ions. Amer. J. Bot. 60: 987-990.

SPAETH, S.C. and SINCLAIR, T.R. (1983). Soybean seed growth. II. Individual seed mass and component compensation. Agron. J. 76: 128-133.

SPITZER, E. and LOTT, J.N.A. (1982). Protein bodies in umbelliferous seeds. II. Elemental composition. Can. J. Bot. 60: 1392-1398.

SPLITTSTOESSER, W.E. (1982). The appearance of phytase and the changes in phytate and inorganic phosphorus during germination and early seedling growth of pumpkin (Cucurbita moschata Poir.). HortSci. 17: 402-403.

SPLITTSTOESSER, W.E. (1983). Effects of embryo removal upon reserve protein degradation in Cucurbita moschata . J. Seed Technol. 8: 25-30.

STANTON, M.L. (1984). Developmental and genetic sources of seed weight variation in Raphanus raphanistrum L. (Brassicaceae). Amer. J. Bot. 71: 1090-1098.

STEPHENSON, A.G. (1981). Flower and fruit abortion: proximate causes and ultimate functions. Ann. Rev. Ecol. Syst. 12: 253-279.

SUZUKI, D.T., GRIFFITHS, A.J.F. and LEWANTIN, R.C. (1981). An Introduction to Genetic Analysis. San Francisco: W.H. Freeman and Co. pp. 807-837.

TAIRA, H., TAIRA, H. and SAITO, M. (1977). Effect of size of seed, variety, and crop year on the chemical composition of soybean seeds. V. Potassium, phosphorus, magnesium, and calcium content. Jap. J. Crop Sci. 46: 483-491.

TEMME, D.H. (1986). Seed size variability: a consequence of variable genetic quality among offspring? Evol. 40: 414-417.

THORNE, J.H. (1980). Kinetics of ^{14}C-photosynthate uptake by developing soybean fruit. Plant Physiol. 65: 975-979.

THORNE, J.H. (1985). Phloem unloading of C and N assimilates in developing seeds. Ann. Rev. Plant Physiol. 36: 317-343.

TSUYUKI, H. ITOH, S. and YAMAGATA, K. (1985). Lipid and tryacylglycerol compositions of total lipids in pumpkin seeds. Nippon Skokuhin Kogyo Gakkaishi 32: 1-15.

TYLER, K.B. and LORENZ, O.A. (1964). Nutrient absorption and growth of four muskmelon varieties. Proc. Amer. Soc. Hort. Sci. 84: 364-371.

TYSON, J.F. (1984). A critical look at calibration procedures for flame atomic-absorption spectrometry. Analyst 109: 313-317.

VAN DE GEIJN, S.C. and PETIT, C.M. (1979). Transport of divalent cations. Plant Physiol. 64: 954-958.

VAUGHAN, J.G. (1970). The Structure and Utilization of Oil Seeds. London: Chapman and Hall Ltd. pp. 63-69.

WALSH, A. (1982). Atomic absorption and atomic fluorescence methods of analysis: their merits and limitations. Phil. Trans. R. Soc. Lond. A 305: 485-498.

WANG, C.H., WILLIS, D.L. and LOVELAND, W.D. (1975). Radiotracer Methodology in Biological, Environmental, and Physical Sciences. Englewood Cliffs, New Jersey: Prentice-Hall, Inc. pp. 389-398.

WANNER, G., FORMANEK, H. and THEIMER, R.R. (1981). The ontogeny of lipid bodies (spherosomes) in plant cells. Planta 151: 109-123.

WANNER, G., VIGIL, E.L. and THEIMER, R.R. (1982). Ontogeny of microbodies (glyoxysomes) in cotyledons of dark-grown watermelon (Citrullus vulgaris Schrad.) seedlings. Planta 156: 314-325.

WATSON, M.E. (1981). Interlaboratory comparison in the determination of nutrient concentrations of plant tissue. Soil Sci. Plant Anal. 12: 601-617.

WEBB, M.A. and ARNOTT, H.J. (1981). An ultrastructural study of druse crystals in okra cotyledons. Scanning Electron Microsc./1981/III: 285-292.

WEBER, E. and NEUMANN, D. (1980). Protein bodies, storage organelles in plant seeds. Biochem. Physiol. Pflanzen 175: 279-306.

WEST, A.C. (1964). Effects of aliphatic acids and their salts on the flame spectrometric emission of calcium. Anal. Chem. 36: 310-314.

WEST, A.C., FASSEL, V.A. and KNISELEY, R.N. (1973a). Lateral diffusion interferences in flame atomic absorption and emission spectrometry. Anal. Chem. 45: 1586-1594.

WEST, A.C., FASSEL, V.A. and KNISELEY, R.N. (1973b). Calcium-phosphate solute volatilization interference in flame spectrometry. A lateral diffusion interference effect. Anal. Chem. 45: 2420-2422.

WHITAKER, T.W. (1951). A species cross in Cucurbita . J. Hered. 42: 65-69.

WHITAKER, T.W. and BEMIS, W.P. (1975). Symposium on the biochemical systematics, genetics and origin of cultivated plants. VIII. Origin and evolution of the cultivated Cucurbita . Bull. Torr. Bot. Club 102: 362-368.

WHITAKER, T.W. and DAVIS, G.N. (1962). Cucurbits. Botany, Cultivation and

Utilization. World Crop Books. New York: Interscience Publishers, Inc. pp. 14-36.

WHITING, A.G. (1938). Development and anatomy of primary structures in the seedling of Cucurbita maxima . Bot. Gaz. 99: 497-528.

WICHMANN, H.J. (1940). I. Ash determinations in foods with an alkaline balance. J. Ass. Offic. Agr. Chem. 23: 680-687.

WICHMANN, H.J. (1942a). Ash determinations in foods with an alkaline balance. III. Decomposition curves of ashes from foods. J. Ass. Offic. Agr. Chem 25: 478-484.

WICHMANN, H.J. (1942b). Ash determinations in foods with an alkaline balance. IV. Effects of water on ash weights. J. Ass. Offic. Agr. Chem. 25: 485-494.

WICHMANN, H.J. (1943). Ash determinations in foods with an alkaline balance. V. Reactions of alkali carbonates with calcium phosphates. J. Ass. Offic. Agr. Chem. 26: 522-559.

WICHMANN, H.J. (1953). Ash determinations in foods with an alkaline balance. VI. Reaction of sodium carbonate with calcium phosphates in the ashing of milk. J. Ass. Offic. Agr. Chem. 36: 979-992.

WILEY, L. (1971). The fate of protein bodies in germinating seeds of Cucurbita maxima Duch. PhD. Dissertation, University of California, Davis, California. pp. 100-102.

WILEY, L. and ASHTON, F.M. (1967). Influence of the embryonic axis on protein hydrolysis in cotyledons of Cucurbita maxima . Physiol. Plant. 20: 688-696.

WILLIAMS, R.J.P. (1980). A general introduction to the special properties of the calcium ion and their deployment in biology. In Calcium-Binding Proteins: Structure and Function. (ed. F.L. Siegel, E. Carafoli, R.H. Kretsinger, D.H. MacLennan and R.H. Wasserman). New York: Elsevier North Holland, Inc. pp. 3-10.

WILSON, M.A. and SPLITTSTOESSER, W.E. (1979). Effect of pumpkin seed size on seedling emergence and yield on two soil types. HortSci. 14: 731.

WITZTUM, A. and GERSANI, M. (1975). The role of polar movement of IAA in the development of the peg in Cucumis sativus L. Bot. Gaz. 136: 5-16.

WONG, C.Y. (1941). Chemically induced parthenocarpy in certain horticultural plants, with special reference to the watermelon. Bot. Gaz. 103: 64-86.

WULFF, R.D. (1986). Seed size variation in Desmodium paniculatum. I. Factors affecting seed size. J. Ecol. 74: 87-97.

YOFE, J. and FINKELSTEIN, R. (1958). Elimination of anionic interference in flame photometric determination of calcium in the presence of

phosphate and sulphate. Anal. Chim. Acta 19: 166-173.

ZAR, J.H. (1984). Biostastistical Analysis. Englewood Cliffs, N.J.: Prentice-Hall, Inc. pp. 186-190.

ZASOSKI, R.J. and BURAU, R.G. (1977). A rapid nitric-perchloric acid digestion method for multi-element tissue analysis. Comm. Soil Sci. Plant Anal. 8: 425-436.

ZIEGLER, H. (1963). Verwendung von ^{45}calcium zur analyse der stoffensorgung wachsender fruchte. Planta 60: 41-45.

ZIEGLER, H. (1975). Nature of transported substances. In Transport in Plants. Encyclopedia of Plant Physiology. New Series, vol. 1. (ed. M.H. Zimmerman and J.A. Milburn). New York: Springer Verlag. pp. 84-90.

APPENDIX A

Fruit, Seed and Embryo Measurements and Calcium Levels of *C. maxima*, *C. andreana* and Their Reciprocal Hybrids.

Table A:1

Fruit and Seed Measurements for Field Grown *C. maxima* Fruits.

Fruit Number	Fruit Weight (kg)	Fruit Length (cm)	Fruit Width (cm)	Fruit Circumf. (cm)	Number of Seeds	Total Seed Wt.(g)	Av. Weight/ Seed (g)
1	10.4	37	29	105	351	111.1	0.3165
2	6.1	29	26	87	290	105.5	0.3638
3	6.5	34	24	80	406	128.3	0.3160
4	4.3	30	20	74	530	128.5	0.2424
5	6.5	30	25	82	554	142.8	0.2578
6	8.2	27	24	89	416	130.8	0.3144
7	8.4	43	25	84	365	115.6	0.3167
8	6.8	40	21	77	339	94.5	0.2788
9	6.8	28	28	92	342	106.7	0.3112
10	6.1	35	25	81	350	92.8	0.2651
11	8.2	41	28	86	100	30.9	0.3090
12	5.8	36	24	77	547	129.7	0.2371
13	9.4	43	25	88	483	140.0	0.2899
14	5.8	35	23	78	622	139.4	0.2241
15	5.7	35	23	78	400	92.3	0.2308
16	9.4	42	26	85	521	150.3	0.2885
17	8.0	41	23	91	391	144.1	0.3685
18	6.0	32	24	86	418	129.0	0.3086
19	4.0	30	20	71	300	84.2	0.2867
20	9.5	36	31	100	248	91.5	0.3690
21	4.3	33	20	68	282	84.9	0.3011
22	3.5	30	18	65	319	76.5	0.2398
23	3.7	28	20	64	297	73.5	0.2475
24	5.5	31	23	75	428	117.7	0.2750
25	3.6	26	20	68	315	71.1	0.2257
26	3.0	26	17	62	302	87.3	0.2891
27	4.5	31	22	70	294	71.0	0.2415
28	6.0	33	22	80	308	79.3	0.2575
29	4.8	28	21	73	450	141.2	0.3138
30	10.0	43	33	96	486	140.0	0.2880
31	7.3	26	30	95	385	105.1	0.2730
32	7.5	34	26	94	201	63.3	0.3149
33	9.0	29	31	103	534	156.2	0.2925
34	7.2	40	24	84	397	111.8	0.2816
35	*	38	20	72	165	43.2	0.2618
36	6.0	36	22	73	266	75.0	0.2820
37	5.5	37	23	75	299	83.6	0.2796
38	6.5	38	21	73	337	88.9	0.2638
39	4.0	33	21	62	335	68.8	0.2054
40	9.0	36	28	90	366	75.2	0.2055
41	6.5	31	23	76	259	64.4	0.2486
42	6.0	40	22	74	142	31.3	0.2204
43	5.0	34	20	64	253	63.7	0.2518
44	6.0	35	24	78	253	61.3	0.2423
45	7.0	37	24	77	322	74.6	0.2317
46	9.0	43	24	78	230	51.5	0.2239
47	7.0	39	24	77	428	99.0	0.2313
48	5.0	33	20	68	356	76.7	0.2154
49	6.5	39	24	76	489	103.8	0.2123
50	6.5	34	24	81	203	49.6	0.2443
51	6.0	36	24	77	298	67.1	0.2252
52	10.0	39	26	87	383	99.7	0.2603
53	9.0	42	28	87	228	69.0	0.3026
54	9.0	39	26	85	342	111.4	0.3257
55	7.5	40	25	82	441	126.9	0.2878

TABLE A:2

Embryo Measurements for Field Grown C. maxima.

Fruit Number	Embryo Length (mm)	Embryo Width (mm)	Embryo Weight (g)	Axis Weight (g)	Weight Cotyl. Pair (g)
1	18.8	9.0	0.2612	0.0042	0.2595
2	20.0	10.0	0.2758	0.0038	0.2701
3	17.8	9.3	0.2270	0.0045	0.2218
4	15.5	7.0	0.1817	0.0038	0.1792
5	16.8	8.8	0.1847	0.0041	0.1817
6	17.8	9.0	0.2361	0.0045	0.2332
7	18.5	9.0	0.2294	0.0057	0.2236
8	18.4	8.9	0.1913	0.0051	0.1860
9	16.8	10.3	0.2238	0.0050	0.2198
10	16.5	8.5	0.1877	0.0046	0.1840
11	17.8	9.5	0.2482	0.0054	0.2458
12	15.3	8.9	0.1802	0.0042	0.1780
13	17.4	8.3	0.2115	0.0044	0.2088
14	15.5	8.3	0.1631	0.0038	0.1594
15	15.8	8.3	0.1704	0.0057	0.1669
16	16.8	8.9	0.2083	0.0048	0.2040
17	18.3	9.0	0.2697	0.0044	0.2661
18	17.5	9.3	0.2262	0.0044	0.2241
19	16.5	9.0	0.2118	0.0040	0.2098
20	17.8	10.3	0.2775	0.0061	0.2730
21	18.3	9.0	0.2227	0.0040	0.2204
22	17.3	8.0	0.1787	0.0041	0.1746
23	18.0	8.0	0.1843	0.0044	0.1798
24	16.3	8.5	0.2033	0.0036	0.2011
25	16.8	8.0	0.1695	0.0037	0.1653
26	16.3	7.5	0.1897	0.0041	0.1856
27	16.5	8.5	0.1969	0.0042	0.1935
28	16.8	9.0	0.1796	0.0046	0.1750
29	17.0	9.0	0.2256	0.0046	0.2208
30	17.5	9.3	0.2079	0.0045	0.2031
31	17.0	8.5	0.2084	0.0048	0.2040
32	16.5	9.0	0.2320	0.0046	0.2275
33	17.5	9.0	0.2020	0.0048	0.1972
34	17.8	8.5	0.1973	0.0053	0.1934
35	17.3	9.3	0.2098	0.0038	0.2057
36	16.8	8.8	0.2121	0.0042	0.2077
37	16.8	8.8	0.2084	0.0039	0.2045
38	16.8	8.5	0.1966	0.0042	0.1922
39	15.5	7.8	0.1433	0.0032	0.1397
40	18.5	9.8	0.2027	0.0046	0.1980
41	18.0	8.0	0.1707	0.0039	0.1667
42	17.5	8.0	0.2174	0.0048	0.2125
43	17.0	8.5	0.1951	0.0039	0.1915
44	17.3	8.8	0.1531	0.0039	0.1491
45	16.8	8.8	0.1696	0.0037	0.1658
46	16.5	9.5	0.1382	0.0034	0.1341
47	16.0	9.0	0.1794	0.0040	0.1755
48	16.8	8.3	0.1441	0.0036	0.1408
49	16.0	8.0	0.1713	0.0043	0.1669
50	16.1	7.8	0.1716	0.0041	0.1694
51	15.8	8.5	0.1633	0.0043	0.1592
52	15.8	8.3	0.1883	0.0046	0.1842
53	16.3	8.8	0.2194	0.0046	0.2159
54	18.0	9.0	0.2400	0.0039	0.2354
55	18.3	9.3	0.2173	0.0039	0.2128

TABLE A:3

Calcium Levels in Embryos of Field Grown C. maxima.

Fruit Number	µg Ca^{2+}/g Embryo	µg Ca^{2+} Embryo	µg Ca^{2+}/g Axis	µg Ca^{2+} Axis	µg Ca^{2+}/g Cotyledons	µg Ca^{2+} Cotyl. Pr.
1	229.7	60.0	1599.4	6.70	205.4	53.3
2	165.3	45.6	1355.3	5.12	152.7	41.2
3	182.7	41.5	1551.8	6.98	155.5	34.5
4	162.7	29.6	1233.8	4.69	138.8	24.9
5	181.8	33.6	1385.3	5.68	153.7	27.9
6	179.6	42.4	1508.3	6.79	152.7	35.6
7	204.5	46.9	1281.6	7.31	177.1	39.6
8	145.5	27.8	1089.9	5.35	120.9	22.5
9	325.4	72.8	1824.3	9.12	289.7	63.7
10	441.3	82.8	1915.5	8.81	402.3	74.0
11	308.4	76.5	1956.5	10.57	268.4	66.0
12	262.7	47.3	1597.9	6.71	228.2	40.6
13	246.5	52.1	1736.9	7.64	227.5	47.5
14	227.1	37.0	1667.8	6.34	192.6	30.7
15	233.9	39.9	1589.2	5.88	203.6	34.0
16	234.0	48.7	1686.6	8.10	199.2	40.6
17	223.6	60.3	1804.4	7.94	196.8	52.4
18	213.6	48.3	1619.5	7.13	183.8	41.2
19	240.5	50.9	1735.8	6.94	209.7	44.0
20	234.3	65.0	1628.6	9.93	201.8	55.1
21	178.9	39.9	1478.1	5.91	154.0	33.9
22	182.9	32.7	1329.8	5.45	156.0	27.2
23	137.3	25.3	1243.3	5.47	110.3	19.8
24	185.8	37.8	1655.4	5.96	158.2	31.8
25	230.0	39.0	1515.8	5.61	201.9	33.4
26	284.3	53.9	1607.9	6.59	255.1	47.4
27	304.8	60.0	1554.5	6.53	276.4	53.5
28	203.2	36.5	1350.7	6.21	172.8	30.2
29	142.9	32.2	1232.0	5.67	120.3	26.6
30	149.2	31.0	1075.6	4.84	128.9	26.2
31	187.3	39.0	1292.1	6.20	161.0	32.8
32	196.1	45.5	1664.2	7.66	166.3	37.8
33	195.9	39.6	1660.8	7.97	160.3	31.6
34	200.4	39.5	1166.6	6.18	172.5	33.4
35	310.4	65.1	1931.0	7.34	280.9	57.8
36	264.0	56.0	1914.8	8.04	230.9	48.0
37	240.9	50.1	1722.5	6.72	240.5	50.1
38	248.1	48.8	1880.2	7.90	212.2	40.8
39	271.1	38.9	1801.7	5.77	236.8	33.1
40	229.0	46.4	1274.4	5.86	204.8	40.6
41	154.9	26.4	770.4	3.00	140.6	23.4
42	230.9	50.2	1702.0	8.17	197.8	42.0
43	218.1	41.8	1485.7	5.79	187.9	36.0
44	232.9	35.7	1380.5	5.38	203.0	30.3
45	214.9	36.5	1301.5	4.82	190.8	31.6
46	238.5	33.0	1473.4	5.01	208.4	28.0
47	202.7	36.4	1360.2	5.44	176.2	30.9
48	223.0	32.1	1446.1	5.41	191.2	26.9
49	243.5	41.7	1731.1	7.44	205.4	34.3
50	304.1	52.2	1714.8	7.03	266.5	45.2
51	210.7	34.4	1254.9	5.40	182.2	29.0
52	167.0	31.5	1214.5	5.59	140.4	25.9
53	290.3	63.7	1896.4	8.72	254.6	55.0
54	290.9	69.8	1932.0	7.53	264.6	62.3
55	203.3	44.2	1410.0	5.50	181.7	38.7

TABLE A:4

Fruit and Seed Measurements for *C. maxima* Grown in the Greenhouse and Growth Chambers.

Fruit Number	Fruit Weight (kg)	Fruit Length (cm)	Fruit Width (cm)	Fruit Circumf. (cm)	Number of Seeds	Total Seed Weight(g)	Av. Weight/ Seed (g)	Embryo Length (mm	Embryo Width (mm)
1*	1.1	15.0	15.0	*	135	*	0.1730	14.5	7.8
2	0.5	15.0	10.5	34.0	182	40.3	0.2210	15.0	7.8
3	1.0	30.0	14.0	43.5	130	32.1	0.2470	16.5	9.3
4	1.3	19.0	15.0	47.0	309	33.0	0.1869	14.8	8.0
5	1.2	18.5	15.0	47.0	126	28.5	0.2262	15.5	8.0
6	0.7	16.0	12.0	37.0	60	9.0	0.1585	12.7	6.8
7	0.8	17.5	13.0	39.0	187	33.3	0.1781	12.8	6.8
8	1.1	21.5	14.5	46.0	122	24.5	0.2008	16.0	8.0
9	2.7	30.0	20.0	58.0	238	64.4	0.2706	16.5	8.5
10	1.3	21.0	16.0	47.0	54	14.0	0.2593	13.3	6.5
11	0.8	17.0	13.0	39.0	126	21.0	0.1667	14.5	7.3
12	0.8	19.0	14.0	42.0	110	20.1	0.1827	14.5	7.0
13	1.1	22.0	14.0	44.0	81	19.1	0.2358	15.3	8.5
14	1.5	21.0	16.0	48.0	168	31.7	0.1887	14.3	8.5
15	0.9	18.0	12.0	41.0	145	33.0	0.2276	15.8	9.0
16	1.4	20.0	14.0	48.5	65	13.2	0.2031	14.5	8.5
17	1.6	20.0	15.5	52.0	171	36.2	0.2117	15.0	8.3
18	2.0	22.0	16.5	53.0	197	47.1	0.2391	16.5	8.5
19	1.3	19.0	13.0	45.0	169	31.4	0.1858	14.0	7.8
20	0.9	21.0	12.0	38.5	150	21.1	0.1407	15.0	7.5
21	0.9	20.0	12.5	41.0	284	26.2	0.1286	14.0	6.0
22	1.7	21.0	16.0	53.0	160	48.6	0.3037	19.0	9.5
23	1.1	19.0	14.0	44.0	183	38.0	0.2077	15.3	7.8
24	1.4	20.0	15.0	49.5	183	48.5	0.2650	18.5	8.5
25	1.0	17.5	14.0	44.0	320	52.6	0.1644	14.8	6.8
26	1.8	23.0	17.5	54.5	172	45.9	0.2669	17.0	9.0
27	2.3	28.0	17.0	54.5	295	91.8	0.3112	17.0	9.0

* Fruits # 1-21 grown in the greenhouse
" #22-27 grown in the growth chambers

* denotes missing values.

TABLE A:5

Embryo Weight and Ca Level of *C. maxima* Grown in the Greenhouse and Growth Chambers.

Fruit Number	µg Ca^{2+}/g Embryo	µg Ca^{2+}/ Embryo	µg Ca^{2+}/g Axis	µg Ca^{2+}/ Axis	µg Ca^{2+}/g Cotyledon	µg Ca^{2+}/Pr. Cotyledons	Embryo Weight(g)	Axis Weight(g)	Weight Cotyl. Pair(g)
1	178.1	18.93	1402.5	4.77	137.6	14.16	0.1063	0.0034	0.1029
2	184.3	27.05	1622.1	6.65	141.7	20.40	0.1468	0.0041	0.1440
3	176.5	31.05	1526.2	6.87	139.8	24.18	0.1759	0.0045	0.1730
4	271.8	38.49	1331.8	4.93	241.3	33.56	0.1416	0.0037	0.1391
5	277.2	47.09	1661.2	6.64	243.8	40.45	0.1699	0.0040	0.1659
6	389.3	43.91	2164.1	7.57	331.9	36.34	0.1128	0.0035	0.1095
7	169.9	22.80	1280.5	4.47	135.4	18.33	0.1383	0.0036	0.1354
8	235.3	30.56	1634.0	6.37	189.4	24.19	0.1299	0.0039	0.1277
9	271.1	60.35	1983.0	8.53	236.1	51.82	0.2226	0.0043	0.2195
10	274.6	37.75	*	*	*	*	0.1149	*	*
11	226.8	31.19	1540.0	5.85	189.5	25.34	0.1375	0.0038	0.1337
12	278.5	33.81	1557.1	5.45	238.9	28.36	0.1218	0.0035	0.1187
13	206.5	31.35	1019.4	5.10	177.5	26.25	0.1518	0.0050	0.1479
14	189.7	29.63	1422.4	5.41	158.9	24.22	0.1562	0.0038	0.1524
15	211.5	36.13	1632.7	5.55	183.0	30.58	0.1708	0.0034	0.1671
16	382.9	59.20	1861.8	7.26	345.1	51.94	0.1546	0.0039	0.1505
17	242.9	37.43	1295.3	4.40	219.3	33.03	0.1541	0.0034	0.1506
18	250.9	44.69	1268.3	5.07	229.3	39.62	0.1781	0.0040	0.1728
19	294.7	41.47	1378.8	3.86	270.6	37.61	0.1407	0.0028	0.1390
20	160.4	17.50	1109.8	2.89	137.1	14.61	0.1991	0.0026	0.1066
21	236.0	27.00	1549.2	5.11	197.0	21.89	0.1144	0.0033	0.1111
22	332.8	76.69	2014.2	6.49	309.3	70.20	0.2301	0.0032	0.2269
23	219.0	35.00	*	*	*	*	0.1598	*	*
24	201.3	41.47	*	*	*	*	0.2060	*	*
25	272.3	34.20	1606.1	4.18	244.5	30.02	0.1256	0.0026	0.1128
26	317.3	67.29	1985.9	8.14	285.5	59.15	0.2121	0.0041	0.2079
27	210.0	50.86	1980.4	7.53	181.0	43.33	0.2422	0.0038	0.3306

TABLE A16

Fruit, Seed and Embryo Measurements and Embryo Ca Levels of *C. maxima* x *C. andreana*.

Fruit Number	Fruit Weight (kg)	Fruit Length (cm)	Fruit Width (cm)	Fruit Circumf. (cm)	Number of Seeds	Total Seed Weight(g)	Av. Weight/ Seed (g)	Embryo Length (mm)	Embryo Width (mm)
1	6.0	36	24	70	462	107.6	0.2329	16.3	8.5
2	6.0	34	21	75	470	122.2	0.2600	17.0	9.5
3	0.5	15	10	34	99	21.7	0.2190	14.8	9.3
4	1.0	17	14	44	131	29.5	0.2254	16.0	7.8
5	1.3	19	16	50	168	35.4	0.2108	16.3	8.8
6	1.8	22	18	51	187	45.4	0.2427	15.0	8.8
7	1.2	16	15	49	144	35.1	0.2679	15.0	9.3
8	2.1	23	19	56	316	72.6	0.2297	16.5	8.3
9	1.5	22	14	48	150	21.4	0.1427	15.5	9.0
10	0.8	15	14	42	105	14.9	0.1423	14.5	9.5
11	1.3	23	15	46	244	41.9	0.1717	14.5	8.0
12	1.8	24	17	54	266	60.3	0.2268	17.3	9.0

Fruit Number	$\mu g\ Ca^{2+}/g$ Embryo	$\mu g\ Ca^{2+}$/ Embryo	$\mu g\ Ca^{2+}/g$ Axis	$\mu g\ Ca^{2+}$/ Axis	$\mu g\ Ca^{2+}/g$ Cotyledon	$\mu g\ Ca^{2+}$/Pr. Cotyledons	Embryo Weight(g)	Axis Weight(g)	Weight Cotyl. Pair(g)
1	340.4	58.8	1397.5	5.17	318.6	53.6	0.1726	0.0037	0.1682
2	269.5	54.3	1926.4	5.97	243.7	48.3	0.2014	0.0031	0.1982
3	493.0	74.5	2040.9	6.33	447.8	68.2	0.1511	0.0031	0.1522
4	307.5	47.8	1876.7	7.32	265.3	40.4	0.1553	0.0039	0.1524
5	201.2	29.3	999.5	4.40	174.3	24.9	0.1457	0.0044	0.1429
6	379.9	72.8	*	*	*	*	0.1917	*	*
7	362.1	64.6	1872.8	7.12	329.2	57.5	0.1784	0.0038	0.1746
8	298.4	53.2	1565.4	7.36	264.2	45.9	0.1784	0.0047	0.1736
9	430.0	58.7	1629.0	5.38	403.3	53.3	0.1365	0.0033	0.1322
10	578.9	56.9	1579.8	6.48	533.7	50.4	0.0983	0.0041	0.0945
11	334.8	43.9	1528.0	4.89	305.2	39.0	0.1311	0.0032	0.1278
12	276.4	47.2	1485.8	5.50	249.5	41.7	0.1709	0.0037	0.1673

Fruits # 1,2 grown in the field
3-8 grown in the greenhouse
9-12 grown in the growth chambers

TABLE A:7

Fruit and Seed Measurements for *C. andreana* x *C. maxima*.

Fruit Number	Fruit Weight (kg)	Fruit Length (cm)	Fruit Width (cm)	Fruit Circumf. (cm)	Number of Seeds	Total Seed Wt.(g)	Av. Weight/ Seed (g)
1	131	8.0	5.0	19.0	208	2.71	0.0130
2	448	13.0	9.0	30.0	636	13.18	0.0207
3	357	10.5	8.5	29.0	684	11.63	0.0170
4	310	10.5	8.0	26.5	357	2.75	0.0077
5	291	10.5	8.0	26.5	437	8.38	0.0192
6	60	6.3	4.5	16.0	196	1.69	0.0086
7	35	4.5	4.0	13.5	59	0.94	0.0159
8	57	6.0	5.0	17.0	149	2.31	0.0155
9	50	6.0	4.5	15.0	106	1.86	0.0175
10	100	7.5	5.5	19.0	169	3.26	0.0193
11	200	9.0	7.5	24.0	346	6.57	0.0190
12	181	7.0	6.0	20.0	144	2.64	0.0183
13	100	7.0	5.5	18.5	128	3.10	0.0242
14	206	10.0	7.5	21.5	280	7.05	0.0252
15	113	8.0	5.5	18.0	218	4.45	0.0204
16	145	9.5	6.0	19.0	303	5.40	0.0178
17	173	10.0	7.5	22.0	444	9.51	0.0214
18	167	9.5	7.5	22.0	496	9.95	0.0201
19	86	7.0	6.0	18.0	255	4.75	0.0186
20	211	11.0	7.5	24.0	188	4.20	0.0223
21	230	11.0	8.0	24.0	460	10.72	0.0233
22	161	9.5	7.5	22.0	467	9.11	0.0195
23	161	8.5	7.5	22.0	279	7.80	0.0280
24	137	8.5	7.0	21.0	108	3.00	0.0278
25	155	10.0	7.0	22.0	269	7.20	0.0268
26	135	8.0	7.0	21.5	154	4.40	0.0286
27	110	8.0	6.5	20.5	43	1.00	0.0233
28	141	9.0	7.0	20.5	406	8.40	0.0207
29	120	7.5	6.0	20.0	227	4.15	0.0183
30	89	7.0	6.0	19.0	73	1.20	0.0164
31	89	6.5	6.0	18.0	222	4.50	0.0203
32	91	7.0	5.5	18.0	213	3.59	0.0177
33	89	6.5	5.5	18.0	218	4.20	0.0193
34	96	6.5	5.5	19.0	211	4.59	0.0218
35	79	6.5	5.0	17.0	135	3.15	0.0233
36	85	7.0	6.0	19.0	207	3.25	0.0157
37	140	8.0	6.5	21.0	157	3.74	0.0238
38	94	7.0	6.0	18.0	216	4.82	0.0223
39	110	7.0	6.0	19.0	139	2.95	0.0212
40	154	8.0	7.0	22.0	418	7.30	0.0175
41	113	7.5	6.5	20.0	440	7.72	0.0175
42	90	6.5	6.0	19.0	281	5.01	0.0178
43	83	7.5	5.5	17.0	104	2.75	0.0264
44	134	7.5	6.5	20.5	251	5.58	0.0222
45	50	5.5	5.0	15.0	80	1.52	0.0190
46	99	7.0	5.5	18.5	145	3.73	0.0257

Fruits # 1- 5 - grown in the field
6-12 - grown in the greenhouse
#13-46 - grown in the growth chamber

TABLE A:8

Embryo Measurements for *C. andreana* x *C. maxima*.

Fruit Number	Embryo Length (mm)	Embryo Width (mm)	Embryo Weight (g)	Axis Weight (g)	Weight Cotyl. Pair (g)
1	5.5	3.0	0.0066	0.00045	0.0061
2	6.0	3.5	0.0136	0.00058	0.0129
3	5.8	3.3	0.0114	0.00054	0.0107
4	5.9	3.5	0.0111	0.00050	0.0107
5	6.0	3.3	0.0129	0.00061	0.0123
6	5.3	3.3	0.0081	•	•
7	5.9	3.6	0.0105	•	•
8	5.5	3.5	0.0102	0.00054	0.0096
9	5.7	3.6	0.0113	•	•
10	5.4	3.7	0.0129	0.00052	0.0124
11	5.8	3.8	0.0143	•	•
12	5.8	3.9	0.0136	•	•
13	7.0	4.3	0.0153	0.00060	0.0147
14	7.2	4.4	0.0163	0.00070	0.0156
15	6.7	3.8	0.0136	0.00056	0.0129
16	6.7	3.6	0.0114	0.00054	0.0108
17	6.0	4.0	0.0137	•	•
18	6.0	3.5	0.0136	•	•
19	5.8	3.5	0.0121	•	•
20	6.5	4.0	0.0153	•	•
21	6.5	4.0	0.0142	•	•
22	5.5	4.0	0.0126	•	•
23	5.9	3.5	0.0171	0.00073	0.0162
24	5.9	3.4	0.0167	•	•
25	5.9	3.4	0.0162	0.00075	0.0156
26	5.8	3.6	0.0183	0.00074	0.0175
27	6.0	4.0	0.0146	•	•
28	6.0	3.1	0.0120	0.00058	0.0114
29	6.0	3.3	0.0111	0.00052	0.0107
30	5.8	3.3	0.0119	•	•
31	5.5	3.0	0.0128	0.00059	0.0123
32	5.5	3.5	0.0113	0.00047	0.0107
33	5.8	3.3	0.0130	0.00060	0.0124
34	6.0	3.6	0.0139	0.00062	0.0134
35	6.5	3.7	0.0146	0.00066	0.0139
36	5.0	3.0	0.0116	0.00050	0.0107
37	6.1	3.3	0.0141	0.00064	0.0133
38	6.5	3.6	0.0133	0.00071	0.0127
39	6.0	3.3	0.0123	•	•
40	5.0	3.2	0.0121	0.00060	0.0118
41	5.5	3.3	0.0156	0.00070	0.0151
42	5.4	3.0	0.0121	0.00060	0.0115
43	6.3	3.6	0.0242	•	•
44	6.0	3.2	0.0115	•	•
45	5.3	3.0	0.0109	•	•
46	6.3	3.6	0.0164	•	•

TABLE A:9

Calcium Levels in Embryos of *C. andreana* x *C. maxima*.

Fruit Number	µg Ca^{2+}/g Embryo	µg Ca^{2+}/ Embryo	µg Ca^{2+}/g Axis	µg Ca^{2+}/ Axis	µg Ca^{2+}/g Cotyledons	µg Ca^{2+} Cotyl. Pr.
1	359.7	2.38	1513.9	0.68	280.7	1.70
2	616.9	8.39	1108.3	0.64	600.5	7.75
3	571.1	6.51	1158.6	0.63	547.9	5.88
4	499.5	5.56	1088.8	0.54	469.4	5.02
5	515.5	6.65	1192.9	0.73	481.5	5.92
6	358.9	2.91	•	•	•	•
7	476.6	5.00	•	•	•	•
8	588.2	6.00	1710.1	0.92	529.2	5.08
9	469.5	5.31	•	•	•	•
10	443.4	5.72	1061.4	0.55	417.3	5.17
11	636.5	9.10	•	•	•	•
12	538.2	7.32	•	•	•	•
13	879.9	13.48	2119.4	1.27	829.6	12.21
14	782.5	12.95	1889.9	1.32	758.7	11.63
15	685.5	9.33	1914.2	1.02	636.7	8.31
16	646.1	7.34	1620.2	0.87	599.0	6.47
17	552.6	7.57	•	•	•	•
18	556.7	7.57	•	•	•	•
19	588.7	7.12	•	•	•	•
20	605.4	9.26	•	•	•	•
21	463.0	6.57	•	•	•	•
22	492.5	6.21	•	•	•	•
23	683.0	11.68	1938.0	1.41	634.1	10.27
24	868.7	14.51	•	•	•	•
25	756.2	12.25	2197.5	1.65	679.5	10.60
26	929.5	17.01	1831.7	1.36	894.2	15.65
27	1025.4	14.97	•	•	•	•
28	572.5	6.87	1643.0	0.95	519.5	5.92
29	650.5	7.22	1046.7	0.54	624.3	6.68
30	763.0	9.08	•	•	•	•
31	717.2	9.18	2063.2	1.22	647.2	7.96
32	391.2	6.68	1341.1	0.63	565.6	6.05
33	733.1	9.53	1937.2	1.16	675.1	8.37
34	575.5	8.00	1610.3	1.00	522.2	7.00
35	605.5	8.84	1753.6	1.16	552.6	7.68
36	391.4	4.54	839.9	0.42	384.7	4.12
37	473.0	6.67	1483.9	0.95	429.7	5.72
38	341.4	4.54	931.5	0.66	305.7	3.88
39	501.0	6.16	•	•	•	•
40	536.4	6.49	1120.8	0.67	492.9	5.82
41	675.6	10.54	1681.9	1.18	619.9	9.36
42	544.6	6.59	1477.3	0.89	495.3	5.70
43	501.1	12.13	•	•	•	•
44	402.2	4.63	•	•	•	•
45	559.2	6.10	•	•	•	•
46	358.6	5.88	•	•	•	•

TABLE A:10

Fruit and Seed Measurements for C. andreana.

Fruit Number	Fruit Weight (g)	Fruit Length (cm)	Fruit Width (cm)	Fruit Circumf. (cm)	Number of Seeds	Total Seed Weight(g)	Av. Weight/ Seed (g)	Embryo Length (mm	Embryo Width (mm)
1	283	12.0	6.0	24.0	319	7.60	0.0238	6.0	4.0
2	53	5.5	4.5	16.0	215	3.41	0.0159	5.4	3.2
3	52	6.0	4.5	15.0	135	2.75	0.0204	5.7	3.5
4	104	7.0	6.0	20.0	338	5.98	0.0177	5.6	3.4
5	129	7.0	6.5	22.0	169	3.87	0.0229	6.0	4.0
6	46	5.0	4.5	16.0	151	1.96	0.0129	5.0	3.4
7	69	6.0	5.5	18.0	241	2.73	0.0113	4.9	3.2
8	91	7.0	6.0	18.0	108	2.66	0.0246	6.0	3.9
9	54	6.0	5.0	16.0	112	2.10	0.0187	5.0	3.0
10	109	7.5	6.5	20.0	169	3.65	0.0216	5.8	3.7
11	78	7.0	5.5	18.0	94	2.28	0.0242	5.7	3.7
12	76	7.0	5.5	18.0	189	3.36	0.0178	5.5	3.8
13	46	5.0	4.5	14.5	119	0.93	0.0078	5.0	3.5
14	55	5.5	5.0	16.0	124	1.55	0.0125	5.3	3.5
15	68	6.0	5.5	17.0	201	2.62	0.0130	5.3	3.3
16	99	7.5	6.0	19.0	203	2.64	0.0130	5.8	3.8
17	102	7.5	6.0	19.0	211	2.88	0.0136	5.5	3.5
18	240	11.0	7.5	23.0	461	10.50	0.0228	7.1	4.0
19	126	8.0	5.5	19.5	238	5.40	0.0227	6.7	3.9
20	130	8.5	6.0	19.0	361	7.10	0.0197	6.7	4.1
21	141	8.0	6.0	20.0	437	7.50	0.0172	7.0	3.5
22	144	9.0	7.0	21.0	254	6.30	0.0248	6.5	3.5
23	142	*	*	*	258	5.70	0.0221	6.7	3.9
24	143	*	*	*	379	6.20	0.0164	6.8	3.8
25	118	10.0	6.0	18.5	157	4.30	0.0274	6.6	3.4
26	127	9.0	6.5	20.0	306	6.00	0.0196	5.8	3.0
27	92	7.0	5.5	18.0	299	3.60	0.0120	5.0	2.8
28	74	6.0	5.0	17.0	269	3.00	0.0112	5.0	3.0
29	206	9.5	8.0	25.0	204	5.10	0.0250	6.0	4.0
30	120	7.5	6.5	21.0	173	4.40	0.0252	6.2	3.9
31	180	9.0	7.5	23.0	279	6.70	0.0240	5.8	3.5
32	73	7.0	5.0	16.0	183	2.50	0.0138	5.5	3.3
33	144	8.0	6.5	21.5	307	7.40	0.0242	5.6	3.7
34	92	7.0	5.5	18.5	202	3.46	0.0196	6.0	3.0
35	123	8.5	6.5	20.0	164	3.35	0.0204	5.4	2.6

Fruits
#1 grown in the field
#2-17 grown in the greenhouse
#18-35 grown in the growth chambers

TABLE A:11

Embryo Weight and Ca Level of *C. maxima*.

Fruit Number	µg Ca^{2+}/g Embryo	µg Ca^{2+}/ Embryo	µg Ca^{2+}/g Axis	µg Ca^{2+}/ Axis	µg Ca^{2+}/g Cotyledon	µg Ca^{2+}/Pr. Cotyl. Pr.	Embryo Weight(g)	Axis Weight(g)	Weight Cotyl. Pair(g)
1	679.7	10.74	1795.6	1.45	623.4	9.29	0.0158	0.00081	0.0149
2	536.0	6.11	1313.4	0.81	490.8	5.30	0.0114	0.00062	0.0108
3	576.4	7.72	"	"	"	"	0.0134	"	"
4	633.1	7.47	1371.0	0.81	595.0	6.66	0.0118	0.00059	0.0112
5	583.7	8.93	1654.1	1.24	533.9	7.69	0.0153	0.00075	0.0144
6	735.7	6.69	"	"	"	"	0.0091	"	"
7	577.4	5.37	1416.2	0.58	538.3	4.79	0.0093	0.00041	0.0089
8	558.1	9.21	"	"	"	"	0.0165	"	"
9	523.6	4.92	"	"	"	"	0.0094	"	"
10	481.8	6.89	1003.1	0.69	452.8	6.20	0.0143	0.00069	0.0137
11	617.8	8.77	"	"	"	"	0.0142	"	"
12	551.3	6.45	1162.9	0.66	517.2	5.79	0.0117	0.00057	0.0112
13	643.4	4.70	"	"	"	"	0.0073	"	"
14	752.4	8.88	"	"	"	"	0.0118	"	"
15	675.8	7.70	"	"	"	"	0.0114	"	"
16	702.2	9.55	"	"	"	"	0.0136	"	"
17	760.7	9.51	"	"	"	"	0.0125	"	"
18	741.2	10.77	1673.7	1.19	693.5	9.58	0.0145	0.00071	0.0138
19	753.6	11.38	1913.4	1.34	697.0	10.04	0.0151	0.00070	0.0144
20	663.4	8.12	1489.3	1.00	615.7	7.12	0.0122	0.00067	0.0116
21	653.6	7.15	1570.5	0.75	612.1	6.40	0.0109	0.00048	0.0104
22	554.7	8.82	"	"	"	"	0.0159	"	"
23	695.0	7.09	1646.9	1.02	646.0	6.07	0.0102	0.00062	0.0094
24	797.9	11.57	1697.7	1.10	763.9	10.47	0.0145	0.00065	0.0137
25	774.1	11.07	2029.1	1.66	696.8	9.41	0.0143	0.00082	0.0135
26	693.3	7.21	1567.7	1.02	631.9	6.19	0.0104	0.00065	0.0098
27	852.9	5.97	2130.4	1.09	762.5	4.88	0.0070	0.00051	0.0065
28	686.6	4.60	1468.0	0.56	651.8	4.04	0.0067	0.00038	0.0062
29	673.7	12.53	1804.3	1.44	623.3	11.09	0.0186	0.00080	0.0178
30	634.2	10.21	1930.2	1.45	584.2	8.76	0.0161	0.00075	0.0150
31	690.4	10.77	1962.1	1.63	609.2	9.14	0.0156	0.00083	0.0150
32	549.6	3.68	"	"	"	"	0.0067	"	"
33	634.6	9.90	1570.3	1.24	589.2	8.66	0.0156	0.00079	0.0147
34	543.7	5.93	"	"	"	"	0.0109	"	"
35	632.1	8.09	"	"	"	"	0.0128	"	"

APPENDIX B

DETERMINATION OF THE NUMBER OF SEEDS PER FRUIT NEEDED TO OBTAIN REPRESENTATIVE CALCIUM LEVELS.

Two distinct sources of variability needed to be considered. Within each fruit the degree of seed-to-seed variation in Ca level was different. The second source of variation was the analytical procedure which produced sample-to-sample variation that was considerably less than seed-to-seed variation. Pooling of seeds eliminated the seed-to-seed variation but it was necessary to determine how many seeds needed to be pooled to eliminate most of the variation in seeds from different fruits. Increasing the number of seeds sampled would increase the accuracy but also required longer for preparation of seeds. Thus a balance had to be found between how many seeds should be pooled for analysis per fruit and how many subsamples of the pooled mixture should be analyzed. An accuracy level of $\pm$ 15 µg of Ca at a 95% confidence level was chosen and 50 seeds were selected as a practical number of seeds to analyze per fruit. Experimental details were outlined in Chapter 8. Table B1 shows that due to differences in the variances for the Ca levels for the three fruits of *C. maxima* the number of subsamples needed also varied from 2 to 9 per fruit. To ensure that all seed-to-seed variation was removed by pooling, 9 subsamples were selected for all *C. maxima* fruits. For the small *C. andreana* fruits it was necessary to pool a larger number of seeds to obtain sufficient weight of pooled sample to work with. This resulted in the need for only 5 subsamples per *C. andreana* fruit to yield comparable results to those of *C. maxima*.

TABLE B:1

Number of Subsamples of Pooled Mixtures of Embryos Needed to Give Ca Levels With a 95% Confidence Interval of $\pm$ 15 $\mu g Ca^{2+}$.

Parameter Measured	Embryos of *C. maxima* Fruits		
	Fruit # 1	#2	#3
No. of Individually Analyzed Embryos (n)	50	50	50
Mean $\mu g\ Ca^{2+}$/g Embryo	127.0	237.9	333.5
σ_s	19.7	90.8	105.4
σ_s^2	388.1	8244.6	11,109.2
$\sigma_s^2/50$	7.8	164.9	222.2
No. of Embryos Pooled	50	50	50
Mean $\mu g\ Ca^{2+}$/g Embryo	141.0	229.5	298.6
σ_A	11.1	10.4	16.0
σ_A^2	123.2	108.2	256.0
V_1	130.0	272.3	479.6
m	2.3	4.8	8.5

σ_s - standard deviation of indivually ashed seeds
σ_s^2 - seed-to-seed variation
σ_A - standard deviation from analysis of 5 subsamples of pooled embryos
σ_A^2 - variation due to analytical procedure
V_1 - variance between subsamples of pooled seeds ($V_1 = \frac{\sigma_s^2}{n} + \sigma_A^2$)

m - number of subsamples needed as calculated from the formula for the confidence interval

$$2\sqrt{\frac{V_1}{m}} = 15 \quad \text{or} \quad m = \frac{V_1}{(7.5)^2}$$

APPENDIX C

NUMBER OF AVAILABLE BINDING SITES ON PHYTIC ACID COMPARED TO THE NUMBER OF BINDING SITES NEEDED BY THE MEASURED CONCENTRATIONS OF CATIONS IN C. MAXIMA EMBRYOS.

The number of binding sites for Mg, K and Ca on P have been calculated from the number of atoms of each element present and their ionic valence. Based on Avogadro's Constant ($N=6.022 \times 10^{23}$) one mole of each element has 6.022×10^{23} atoms. A mole of Mg, K, Ca and P is equivalent to the atomic weights of these elements. According to Splittstoesser (1982), 85% of the total embryo P in Cucurbita moschata was present as phytic acid. This same percentage was used to estimate phytic acid P in C. maxima but the calculation of the number of cation binding sites was based on the total cation concentration. The P on phytic acid is present as 6 phosphate groups which appear to have a total of 12 hydrolyzable hydrogens available for replacement by cations (Cosgrove, 1966). Table C:1 summarizes the calculation of binding sites for the cations on the phosphate groups based on mineral concentrations per gram embryo tissue.

TABLE C:1

Cation Binding Sites Needed vs. Binding Sites Available on Phytic Acid.

		Binding Sites Needed by Cations			Binding Sites Available on Phytic Acid
Measured Mineral Concentration	%	Mg^{2+} 0.498	K^{+} 0.659	Ca^{2+} 0.02%	P[a] 1.001
	g/g	4.98×10^{-3}	6.59×10^{-3}	0.2×10^{-3}	10.01×10^{-3}
Molecular Weight (g)		24.31	39.10	40.08	30.97
No. of Atoms[aa]		1.233×10^{20}	1.001×10^{20}	0.072×10^{20}	1.944×10^{20}
No. of Binding Sites Needed per Ion		2	1	2	2 cationic binding sites per phosphate group
Total No. of Binding Sites if All Atoms Ionized		2.466×10^{20}	1.001×10^{20}	0.072×10^{20}	If all of Above Phosphate Exists as Phosphate Groups on Phytic Acid, the Number of Cation Binding Sites = 3.888×10^{20}
Total No. of Binding Sites for All Cations		3.539×10^{20}			

$$\text{Ratio} = \frac{\text{Cation Binding Sites Needed}}{\text{Cation Binding Sites Available}} = \frac{3.519 \times 10^{20}}{3.888 \times 10^{20}} = 0.91$$

[a] based on the estimate that phytic acid P is 85% of the total measured P.

[aa] no. of atoms of each mineral present in one gram of pooled, ground *C. maxima* embryos.

APPENDIX D

Correlations Between Weights of Axes and Cotyledons and Levels of Mg, K, Ca and P.

Table D:1

Correlation Between Each of the Four Minerals and the Weight of Axis and Cotyledons.

	C. maxima	C. maxima x C. andreana	C. andreana x C.maxima	C. andeana
Wt. Axis vs. Mg./Axis	+ve*	+ve	NS	NS
K /Axis	+ve	+ve	NS	NS
Ca /Axis	NS	NS	NS	NS
P /Axis	NS	+ve	NS	+ve
Wt. Axis vs. Mg/g Axis	-ve	NS	NS	+ve
K /g Axis	-ve	NS	NS	NS
Ca/g Axis	NS	NS	NS	NS
P /g Axis	NS	NS	NS	+ve
Wt. Cotyl. Pr. vs.				
Mg/Cotyl.Pr.	+ve	+ve	NS	NS
K/Cotyl. Pr.	+ve	NS	NS	NS
Ca/Cotyl. Pr.	+ve	NS	+ve	NS
P/Cotyl. Pr.	+ve	+ve	NS	NS
Wt. Cotyl. Pr. vs.				
Mg/g. Cotyl.	NS	NS	NS	NS
K/g Cotyl.	NS	NS	NS	NS
Ca/g Cotyl.	NS	-ve	+ve	NS
P/g Cotyl.	NS	-ve	NS	NS
Wt. Cotyl. Pr. vs.				
Mg/Axis	+ve	NS	NS	NS
K/Axis	NS	NS	NS	NS
Ca/Axis	+ve	NS	+ve	NS
P/ Axis	+ve	NS	NS	+ve
Wt. Cotyl. Pr. vs.				
Mg/g Axis	NS	NS	NS	-ve
K/g Axis	-ve	-ve	NS	-ve
Ca/g Axis	NS	NS	+ve	-ve
P /g Axis	NS	NS	NS	-ve

* correlation coefficient (r)
correlation significant at P = 0.05 or less

APPENDIX D:2

Correlation Among Mineral Levels Within Axes and Cotyledons of Parent and Hybrid Embryos.

C. maxima - mineral levels in % (per tissue in parentheses)

	Axis			Cotyledons		
	Mg	K	Ca	Mg	K	Ca
K	+ (+)			0 (+)		
Ca	+ (+)	0 (0)		0 (+)	0 (+)	
P	+ (+)	0 (0)	+ (+)	+ (+)	0 (+)	0 (+)

C. maxima x *C. andreana*

	Axis			Cotyledons		
	Mg	K	Ca	Mg	K	Ca
K	0 (+)			+ (+)		
Ca	+ (+)	0 (0)		+ (0)	0 (0)	
P	0 (+)	0 (+)	0 (+)	0 (0)	0 (0)	+ (0)

C. andreana x *C. maxima*

	Axis			Cotyledons		
	Mg	K	Ca	Mg	K	Ca
K	+ (+)			0 (0)		
Ca	0 (+)	0 (0)		0 (+)	0 (0)	
P	- (0)	- (0)	0 (0)	0 (0)	0 (0)	- (0)

C. andreana

	Axis			Cotyledons		
	Mg	K	Ca	Mg	K	Ca
K	+ (0)			+ (+)		
Ca	+ (0)	+ (-)		- (0)	- (0)	
P	- (+)	- (-)	- (+)	0 (0)	0 (0)	0 (0)

\+ > significant positive and negative correlation at P = 0.05
\- or less

0 no significant correlation

TABLE D:3

Correlation Between Mineral Levels in Axes and Cotyledons of Parent and Hybrid Embryos.

C. maxima (axis)

Cotyl.	Mg	K	Ca	P
Mg	+(+)	0(0)	0(+)	0(+)
K	0(+)	0(0)	0(0)	0(0)
Ca	+(+)	0(0)	+(+)	+(+)
P	0(+)	0(0)	0(+)	0(+)

C. maxima x *C. andreana* (axis)

Cotyl.	Mg	K	Ca	P
Mg	+(0)	0(0)	+(0)	0(0)
K	+(0)	+(0)	0(0)	0(0)
Ca	0(0)	0(0)	0(0)	0(0)
P	0(0)	0(0)	0(0)	0(0)

C. andreana (axis)

Cotyl.	Mg	K	Ca	P
Mg	0(0)	0(0)	0(-)	-(-)
K	0(0)	0(-)	0(-)	-(-)
Ca	0(0)	0(0)	0(0)	+(+)
P	0(0)	0(0)	0(0)	-(+)

C. andreana x *C. maxima* (axis)

Cotyl.	Mg	K	Ca	P
Mg	+(+)	+(+)	0(+)	-(0)
K	+(+)	+(+)	0(0)	0(0)
Ca	0(0)	0(0)	+(+)	0(0)
P	0(0)	0(0)	0(0)	0(0)

\+ correlation coefficient is significant and positive at P = 0.05 or less

\- correlation coefficient is significant and negative at P = 0.05 or less

0 not significant

First correlations are on percent basis; / tissue basis in parentheses