CHANGES IN GLOBOID COMPOSITION DURING EARLY SEEDLING GROWTH

CHANGES IN THE ELEMENT COMPOSITION OF GLOBOIDS FROM *CUCURBITA MAXIMA* AND *CUCURBITA ANDREANA* COTYLEDONS DURING EARLY SEEDLING GROWTH

By

PENNY ELIZABETH BEECROFT, B.ArtsSc., B.Sc.

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AUTHOR: Penny Elizabeth Beecroft, B.ArtsSc. (McMaster University)
B.Sc. (McMaster University)

SUPERVISOR: Dr. John N.A. Lott

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ABSTRACT

The cells of *Cucurbita* embryos contain may protein bodies surrounded by smaller lipid vesicles. Within the protein bodies are discrete spherical bodies called globoids. Globoids are made up of phytin, and are an important store of *myo*-inositol, phosphorus, and cations including K, Mg, Ca, Mn, Fe and Zn. During early seedling growth, the protein bodies fuse together to form aqueous vacuoles, and the globoids are degraded, their mineral nutrient stores used by the growing seedling. The changes in the element composition of globoids during early seedling growth were examined in this study and the influence of light and mineral nutrient conditions on the changes were examined

Energy dispersive X-ray analysis was used to determine the element composition of globoids from the cotyledons of *C. maxima* and *C. andreana* seeds and seedlings at various stages of growth. The stages selected ranged from the mature, dry seed, to an established seedling with an elongated hypocotyl and expanded cotyledons. To investigate the influence of light and mineral nutrient conditions, seedlings were grown under four different sets of growth conditions: in the dark, with deionized water; in the dark, with Hoaglands solution; in the light, with deionized water; in the light, with Hoaglands solution.

During early seedling growth, the element composition of globoids changed. In both species, regardless of growth conditions, the same general trend was observed for each element: P remained relatively constant, K decreased markedly and Mg, Ca, Mn, Fe, and Zn generally increased. As the protein bodies fused and became more aqueous, it appeared that K ions came off the phytate molecule and were replaced by di- and trivalent cations with a

higher affinity for phytic acid.

There were species-to-species differences in globoid composition changes which could be attributed, at least in part, to differences in the Ca content of the mature, dry embryos. In *C. andreana*, which had a higher initial Ca content, there was a large increase in the Ca content of the globoids during seedling growth and no significant increase in Mn. In *C. maxima* globoids there was only a slight increase in Ca, but there was a much larger increase in Fe, Zn and Mn than occurred in *C. andreana*.

Light and dark grown seedlings exhibited distinctly different morphological features, but light conditions alone did not have a significant influence on the changes in globoid composition. In combination with mineral nutrients in the later stages of growth, the presence of light resulted in a more rapid degradation of globoids. Mineral nutrient conditions had some effect on globoid composition, affecting mostly elements which were present in large amounts. The effect of mineral nutrient conditions, and its interactions with light conditions may have been mediated through changes in the mobilization of mineral nutrients out of the cotyledons during seedling growth.

There were very large (5 - 7 μm in diameter) globoid-like particles present in some later stage cotyledon samples of both species. There was no apparent pattern to which samples they were found in. These particles had elemental compositions which were consistent with them being composed of phytin. Such large particles had not previously been found in cucurbit tissues.

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CHAPTER 1: INTRODUCTION

OBJECTIVES OF THE STUDY

Mature seeds generally contain reserves of proteins, lipids, carbohydrates, and mineral nutrients. These stored reserves are used during germination and early seedling growth, providing almost everything, except water and gases, required for successful establishment of the seedling.

The most common mineral nutrient storage compound in seeds is phytin, which is a salt of *myo*-inositol hexa*kis*phosphoric acid (phytic acid). Phytin is a store of phosphorus, *myo*-inositol and a variety of cations, including K, Mg, Ca, Mn, Fe and Zn (Lott, 1980, 1984; Buttrose, 1978; Ogawa *et al.*, 1975). In seed tissues, phytin is localized within protein bodies, and is often concentrated in discrete spherical bodies called globoids (Lott and Vollmer, 1973b; Lott *et al.*, 1971).

During germination and seedling establishment, protein bodies fuse together to form aqueous vacuoles, the globoids are degraded and their mineral nutrient stores utilized by the growing seedling (Lott and Vollmer, 1973a). The changes occurring in globoid composition during germination and early seedling growth have not been previously studied, but it has generally been accepted that the composition of the globoids remains unchanged as the phytin store is broken down. Some unpublished, preliminary work using *Cucurbita maxima* and *Ricinus communis* (castor bean) indicated that this apparently was not the case and thus the research reported here was initiated.

The major goal of this study was to document the changes in the abundance of different elements in globoids from the cotyledons of dry seeds, and seedlings at various stages of growth. Two closely related cucurbit species were used: *Cucurbita maxima*, a common cultivated species, and *Cucurbita andreana*, a South American weed. These two species are so closely related that they can be hybridized and produce fertile seed (Ockenden and Lott, 1988b; Whitaker, 1951), but there are also significant differences in their fruits and seeds. *C. maxima* seeds are much larger than *C. andreana* seeds, yet on a dry weight basis, *C. andreana* embryos contain three times more Ca than *C. maxima* embryos (Ockenden and Lott, 1988a, b, c). Energy dispersive X-ray (EDX) analysis has also shown that traces of Ca are much more common in globoids from *C. andreana* embryos, than in globoids from *C. maxima* embryos (Ockenden and Lott, 1990). EDX analysis was selected for use in this study because it could be used to investigate the element composition of small portions of biological samples, such as individual globoids, and because all elements present in a sample could be analyzed simultaneously.

This study was designed to answer four main questions:

1. What changes take place in the element composition of globoids from *C. maxima* and *C. andreana* cotyledons during germination and early seedling growth?
2. Light and dark grown *C. maxima* seedlings exhibit very different morphological features (Lott, 1970). Are these morphological differences mirrored by variations in globoid composition?
3. Does the addition of a complete mineral nutrient source, such as Hoagland's solution,

to the growth medium have any influence on changes in globoid composition?

4. *C. maxima* and *C. andreana* are very closely related, yet are distinctly different in terms of fruit and seed size, and in the amount of Ca stored in their embryos. Are there species-to-species differences in the changes in globoid composition?

ENERGY DISPERSIVE X-RAY ANALYSIS

A scanning transmission electron microscope (STEM) in conjunction with an X-ray detector allows for a fine beam of high energy electrons to be directed at a very small area of a specimen. This enables one, using EDX analysis, to study the distribution of elements in a specific subcellular location, such as a single globoid inside a protein body.

Electrons are found around atomic nuclei, in shells with discrete energies. When a high energy electron beam from an electron microscope strikes a specimen, a number of different interactions between the electrons and the specimen's atoms are possible. One possible result is that an inner shell electron may be ejected from an atom. Loss of this electron temporarily ionizes the atom, which remains unstable until an electron from a higher shell drops into the vacant spot. As it drops into the lower shell, the electron releases energy equal to the difference in energy between the two shells; this energy may be released as an X-ray. X-rays produced in this way have energies which are characteristic of, and unique to, the atoms giving rise to them and can be plotted as discrete peaks (Chandler, 1972). Since each element gives a unique set of peaks, the spectra produced can be used to identify which elements are present in the specimen where it is interacting with the electron beam. The

beryllium window system used in this study allows the simultaneous detection of all elements with atomic number 11 (Na) and greater.

The number of X-rays in any given peak is proportional to the mass of atoms, of the corresponding element, present in the excited volume of the specimen (Chandler, 1972). The total number of counts in a peak includes a background which is not uniform for all energies (Barbi, 1979). The background is produced mainly by bremsstrahlung X-rays which are emitted by electrons as they are slowed down when they pass within the coulombic fields of atomic nuclei (Bozzola and Russell, 1992). The closer an electron approaches a nucleus, the more it will be slowed down, and the energy of the released X-ray will be higher. Background X-ray counts are subtracted from the total counts in a peak in order to obtain the net counts - the value which is associated with the concentration of the element (Barbi, 1979). In order to account for variations in sample thickness and density, it has been found that using peak-to-background (P/B) ratios, as opposed to the gross peak counts, gives a more significant result (Lott *et al.*, 1978).

Conventional TEM preparation techniques - fixation, dehydration and embedding procedures - have been shown to cause the loss or redistribution of soluble elements in a sample (Lott *et al.*, 1984). In this study of globoid composition it was imperative that this be prevented since phytate may be readily soluble in water depending on its composition (Brown *et al.*, 1961; Crean and Haisman, 1963). As germination proceeded, the element composition of the globoids changed, and accordingly, the solubility of the phytate present might also have changed. Rapidly freezing the samples and then freeze-drying under vacuum reduced the probability, to almost zero, that any redistribution of elements would occur on

a scale of importance to this study. Once dried, the samples were crushed, and the resulting powder spread onto grids. Using a STEM, globoids could be readily identified in the powder because of their spherical shape and natural electron density. Although cell-to-cell differences could not be identified using freeze-dried powders, it did provide a means of analyzing globoids in tissue which had not been chemically manipulated. Reducing the possibility of differential extraction of elements between samples was more important, in this initial study, than determining cell-to-cell differences.

THE CUCURBITACEAE

There are currently, 825 species, in 118 genera recognized worldwide in the Cucurbitaceae (Jeffrey, 1990a). Although they are widely distributed over both temperate and tropical zones, the tropical species predominate and approximately 90% of all Cucurbitaceae species are found in three main areas: Africa and Madagascar, Central and South America, and Southeast Asia and Malaysia (Jeffrey, 1990b). Members of this family produce a number of edible fruits, including pumpkins, squash, melons and cucumbers while many other species produce inedible, but useful gourds. To name just a few applications, gourds have been used as containers, musical instruments, rafts, decorative objects, and as an article of clothing (Heiser, 1979). Other parts of the plant, including roots, shoots and flowers have been used for food, as an ornamental, and as a therapeutic agent for everything from snake-bite to cerebral thrombosis (Yang and Walters, 1992). Although cucurbit fruits contain large numbers of oil rich seeds, they are generally under-exploited as an oil source

(Joshi *et al.*, 1993; Vaughan, 1970).

The genus *Cucurbita* includes pumpkins, squashes and gourds. This genus, part of the Cucurbiteae tribe in the subfamily Cucurbitoideae, is made up of 27 species of which five are cultivated: *C. ficifolia*, *C. maxima*, *C. moschata*, *C. mixta*, and *C. pepo* (Jeffrey, 1990a). Some of the cultivated species appear in the archeological record before 5000 B.C. (Culter and Whitaker, 1961). The domesticated *C. maxima* and the wild *C. andreana* are closely related genetically (Wilson *et al.*, 1992; Decker-Walters *et al.*, 1990; Whitaker and Bemis, 1975), although their fruits and seeds differ in size and appearance. Hybridization of the two species produces fertile seed (Ockenden and Lott, 1988b; Whitaker, 1951). *C. andreana* is thought to be a possible ancestor of *C. maxima* (Whitaker and Davis, 1962). *C. andreana* has recently received attention due to its high levels of bitter, toxic cucurbitacins. These chemicals act as a repellant for most insect species, but they are a powerful attractant for Diabroticite beetles. In an effort to control these destructive beetles, *C. andreana* is being used in tissue culture to produce cucurbitacins, which are then used to attract the beetles to poison laced substances (Halaweish and Tallamy, 1993).

C. MAXIMA AND *C. ANDREANA* - FRUITS, SEEDS AND VEGETATIVE GROWTH

The fruits from *Cucurbita* are classed as pepos, or inferior berries (Whitaker and Davis, 1962) and are fleshy fruits made up of over 90% water and less than 5% sugar (Coombe, 1976). Fruits of *C. maxima* and *C. andreana* are green with light green stripes extending from the stalk to the calyx end. *C. maxima* produces large (≈10 kg), warty fruits

with a soft rind and a thick layer of edible, yellow flesh, while *C. andreana* produces smaller (<1 kg) fruits with a smooth, hard rind and thin, stringy, bitter, white flesh (Ockenden and Lott, 1988a; Whitaker, 1951).

Seeds of the two species are flat and elongate or broadly oval, with a distinct marginal rim which is discontinuous at the micropylar end of the seed (Whiting, 1938). *C. maxima* seeds are dull white, while *C. andreana* seeds are tan and weigh only about 10% as much as *C. maxima* seeds (Ockenden and Lott, 1988a). Within the thick outer seed coat, there is a thin, green, membranous second seed coat (Lott, 1973; Singh and Dathan, 1972). Mature cucurbit seeds contain no endosperm, and the embryo fills the seed almost completely. The embryo is made up of two large, flat, unfolded cotyledons joined by a small, narrow, pointed root-shoot axis at the micropylar end of the seed. The cotyledons are made up of an upper epidermis, 2 or 3 layers of palisade mesophyll, up to 20 layers of spongy mesophyll cells and a lower epidermis (Nelson, 1932). There are usually 5 to 9 provascular strands in each cotyledon, which run mainly through the spongy mesophyll (Whitaker and Davis, 1962; Nelson, 1932).

Rehydration of a seed under appropriate conditions can lead to germination. Germination involves growth of the embryonic axis and is considered to be complete when the radicle emerges through the seed coat. As early seedling growth progresses, the cotyledons break through the soil, the root system develops, and the hypocotyl and cotyledons continue to expand as the seedling becomes more established. Germination of cucurbit seeds can take place under a wide variety of conditions, but they germinate best in the dark (Fritts and Loy, 1981). Germination of cucurbits in the light may be under the

control of phytochrome (Gutterman, 1992). *C. maxima* seedlings germinated in the light and in the dark exhibit distinctly different morphological features (Lott, 1970). It is subsequent to germination, as the shoot system continues to develop, that the effects of etiolation can be observed. In light germinated plants, the cotyledons turn green and become greatly enlarged, and the hypocotyl is relatively short compared to that of the etiolated seedlings. Etiolated plants develop less expanded, yellowish, spoon-shaped cotyledons, and very long, spindly hypocotyls. Unlike the cotyledons of many non-endospermic legumes, which undergo autolysis as soon as their reserves have been depleted (McKersie and Senaratna, 1983), the cotyledons of light grown *Cucurbita* cotyledons become fully photosynthetic (Lott, 1970) and may persist for several weeks (Lasley and Garber, 1978).

The mature plants of both species are similar; they have a strong tap root system and a stem consisting of 3 to 8 prostrate branches. The leaves are green and have a roundish shape, and *C. andreana*'s leaves have white spots between the veins. Both species are monoecious, and have large yellow flowers on male and female plants (Esau, 1977; Whitaker and Davis, 1962).

STORAGE RESERVES IN CUCURBITS

In mature cucurbit seeds, the reserves are stored as oil, protein and phytin; starch is not present (Lott and Vollmer, 1973b).

Oil reserves:

The oil reserves, which are located in lipid bodies, make up from 30 - 50% of the embryo weight (Jacks, 1990; Tsuyuki *et al.*, 1985; Lazos, 1986). Lipid bodies are about 1 μm in diameter and are surrounded by a half unit membrane. The oils are predominately unsaturated, and are generally edible with oleic, linoleic, palmitic and stearic acids as the main oil components (Tsuyuki *et al.*, 1985). Although there has been considerable debate on the origin and development of lipid bodies, the majority of evidence indicates that lipid synthesis and lipid body formation are associated with plastids and the endoplasmic reticulum (Murphy, 1993; Bewley and Black, 1985).

Protein reserves:

Protein reserves, which are found inside protein bodies, account for about 35% of the embryo weight, and are made up of albumins and globulins. Cucurbitin, a salt-soluble globulin, accounts for 70 - 90% of the total protein, while water-soluble albumins are present in low concentrations (Jacks, 1990; O'Kennedy *et al.*, 1979; Pichl, 1978, 1976; Hara *et al.*, 1976b). Cucurbit storage proteins are rich in arginine, aspartic acid and glutamic acid and are deficient in lysine and sulfur containing amino acids (Jacks, 1986). Seed storage proteins are synthesized on the rough endoplasmic reticulum during seed development, and are transported, via the Golgi complex, to the vacuoles which subdivide to form the protein bodies (Monma *et al.*, 1992; Greenwood and Chrispeels, 1985). The protein bodies, which range from 5 to 12 μm in diameter, are made up of a proteinaceous matrix bound by a single membrane. Within the proteinaceous matrix there are one or more protein crystalloids and

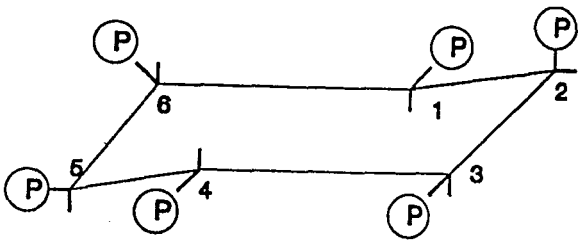
one or more globoids. Protein crystalloids are 4 to 10 μm in diameter and are crystalline deposits of storage protein (mostly cucurbitin). Globoids are naturally electron-dense, spherical bodies made up of phytin (Lott, 1980; Lott and Vollmer, 1973b; Lott *et al.*, 1971).

Phytin/mineral nutrient reserves:

Cellulosic cell wall materials and phytin make up the bulk of the carbohydrate in cucurbit seeds; free sugars are sparse and starch is absent (Jacks, 1990). Phytin, a mixed salt of *myo*-inositol hexa*kis*phosphoric acid (phytic acid), constitutes from one to several percent of the seed's dry weight (Henderson *et al.*, 1986; Lott, 1984), and although it is present in relatively minor amounts, compared to proteins and lipids, it is an important storage compound. It is the major reserve of phosphorus, various cations, and *myo*-inositol in seeds (Ockenden and Lott, 1990; Scott and Loewus, 1986), and accounts for 85% of the total P in cucurbit embryos (Splittstoesser, 1982). Some of the additional biological roles that have been suggested for phytate in seeds include: an energy store (Kikunaga *et al.* 1991; Biswas and Biswas, 1965); an activator of dormancy (Sobolev and Rodionova, 1966); and an antioxidant (Graf *et al.*, 1987).

Phytin is an important mineral nutrient store. Studies indicate that the bulk of K, Mg and Ca in seed tissues is stored in the phytin globoids (Ockenden and Lott, 1990). The six phosphate groups of the phytic acid (Figure 1.1) carry strong negative charges which can attract and hold cations. The exact conformation of the phytic acid molecule *in vivo*, and how may cations are bound to it, has been the subject of much debate (Loewus, 1990; Maga, 1982; Cosgrove, 1966; Johnson and Tate, 1969). It is generally accepted, however, that there

Figure 1.1 Structure of *myo*-inositol hexa*kis*phosphoric acid (phytic acid).



From Greenwood, 1989. pp.110.

are 12 potentially hydrolysable protons on the molecule, based on a 2:1 ratio of bound Na to P for Na-phytate (Cosgrove, 1966). Eight of these protons are highly acidic, two are weakly acidic, and two are very weakly acidic (Siddiqui *et al.*, 1993; Johnson and Tate, 1969). The binding of cations to the phytate is dependent on pH conditions as well as on the relative concentrations of cations and phytate present (Nolan and Duffin, 1987).

In plant tissues, the phytic acid binding sites are occupied by K, Mg, Ca and other cations including Fe, Zn and Mn (Lott, 1984). The relative proportions of each cation and the relative strengths of *in vivo* binding of the cations has not been defined. It is clear, however, that monovalent phytates (ie. K-phytate, Na-phytate) are readily water soluble, whereas phytates containing mainly polyvalent cations are relatively insoluble in water (Brown *et al.*, 1961; Crean and Haisman, 1963). It is also known that Mg, Mn, Fe and Zn all have a higher affinity for phytic acid than does Ca (Xu *et al.*, 1992; Nolan and Duffin, 1987; Graf, 1986).

In seeds, phytin reserves are found almost exclusively inside protein bodies, often concentrated in the globoids (Lott, 1984). How the phytin molecules are arranged within the spherical globoids is not yet known. There are indications that globoid size may be related to their composition, and therefore, to the solubility of the phytate present. In tissues where globoids have lower (Mg + Ca):K ratios, each protein body appears to have numerous, small globoids; in tissues where globoids have higher (Mg + Ca):K ratios, each protein body has only one or two large globoids (Lott *et al.*, 1994; Lott *et al.*, 1985).

The process of phytin biosynthesis and deposition is not yet fully understood (Raboy, 1990; Greenwood, 1989; Scott and Loewus, 1986). Phytic acid is believed to be synthesized,

in the tissue in which it will be stored, by the stepwise addition of phosphate groups to *myo*-inositol. There are several possible biochemical pathways proposed for the synthesis of phytic acid, but no single one has been shown to actually function *in vivo*. Evidence from castor bean studies suggests that phytic acid is synthesized in the cytoplasm in association with the cisternal ER, and transported, as membrane bound particles, to the vacuole which divides to form the protein bodies as seed development continues. Once released into the vacuole/developing protein body, the phytin particles eventually condense into globoids (Greenwood and Bewley, 1984).

Studies with rice and castor bean indicate that during seed development, there is some change in the elemental composition of the globoids. Early in seed development, globoids tend to contain mainly P, Mg and lower levels of Ca and Zn, but little, if any, K. The globoids of mature seeds, however, tend to contain predominantly P, Mg and K, and low or trace amounts of the other metals (Lott, 1984).

Patterns of reserve deposition:

In developing seeds, the deposition of oil and protein reserves follows a similar pattern and occurs at approximately the same rate. The rate of their accumulation is approximately equal to the rate of increase in embryo dry weight (Greenwood *et al.*, 1984; Hocking and Pate, 1977). In whole castor beans, the accumulation of phytin occurs at a slightly slower rate than oil and protein (Greenwood *et al.*, 1984), although phytin and protein accumulate simultaneously in protein bodies of individual cells (Greenwood and Bewley, 1985). The accumulation of mineral reserves in developing seeds depends on both their acquisition by the

plant as well as their transport to the fruits and seeds. These and other factors cause the rate and extent of deposition of different minerals to vary. In *Glycine* sp. higher concentrations of seed phytic acid are related to higher levels of available soil P (Raboy and Dickinson, 1993). In developing barley grains, the accumulation of iron is much more rapid than the accumulation of Mn (Duffus and Rosie, 1976). P, K and Mg are deposited in much higher levels than Ca in *C. maxima* and *C. andreana* embryos, and Ca is deposited in much higher concentrations in the small *C. andreana* embryos than in the larger *C. maxima* embryos (Ockenden and Lott, 1988a, c). In legumes, individual elements may accumulate either faster, slower, or at the same rate as the embryo dry matter (Hocking and Pate, 1977). In xerophytic *Cucurbita* species, oil and protein reserves reach optimal levels at the same time, although the time needed to reach this point varies for different species (Bemis *et al.*, 1977).

ELEMENT COMPOSITION OF GLOBOIDS FROM *CUCURBITA* TISSUES

Initial EDX studies of globoids in *C. maxima* cotyledons showed that globoids contain P, K, Mg and sometimes traces of Ca (Lott, 1975). Trace amounts of Fe, Mn and Zn, have also occasionally been detected. Subsequent studies have shown that the distribution of Ca in globoids is not random. Ca is more commonly found in globoids from the root-shoot axis than in those from the cotyledons (Lott *et al.*, 1978, 1979). Within *C. maxima* and *C. mixta* cotyledons, globoids from the bulk of the mesophyll cells do not contain Ca, while globoids from future epidermal cells and from provascular cells and the adjacent layer of mesophyll cells, are more likely to contain Ca (Lott *et al.*, 1979). Small seeded species, such as *C.*

andreana, *C. foetidissima* and *C. pepo*, have a more even distribution of Ca; calcium is frequently found in the globoids of all cell types (Ockenden and Lott, 1990; Lott and Vollmer, 1979). In soybeans, as well, it has been shown that the Ca level increases as seed size decreases (Taira *et al*, 1977). In cucurbits, P, K and Mg are more evenly distributed in globoids from all cell types from both large and small seeded species. Most EDX analysis studies have shown that all globoids from a single cell have very similar elemental composition (Lott, 1984).

BIOLOGICAL ROLE OF MINERAL NUTRIENTS IN PLANTS

Although mineral nutrients make up less than 5% of the cucurbit embryo dry weight (Ockenden, 1987), the regular appearance of mineral nutrient stores in seed tissues suggests that these elements are vital for germination and seedling establishment. A summary of the biological roles of the mineral nutrients found in *Cucurbita* globoids follows.

Phosphorus is available to the plant principally as the orthophosphate. In this form, P is a vital structural component of biological compounds such as ATP, NADP, membrane phospholipids and nucleic acids, and of metabolic intermediates such as sugar and alcohol phosphates. The principal role of P is as a source of chemical energy in the form of ATP and its related esters. Phosphate also serves a linking function, as in the sugar-phosphate-sugar chains of nucleic acids. In membrane phospholipids, inorganic P provides a polar end to the molecule, a feature that is essential for membrane functioning. (Bieleski, 1973; Mengel and Kirkby, 1982; Glass, 1989)

Potassium is an enzyme activator; at least 60 enzymes, many of which are involved in photosynthesis and respiration, are known to be activated by K. This element is also required in the maintenance of enzyme structure and in protein synthesis through its role in binding mRNA to ribosomes. K plays a key role in maintaining the osmotic potential of cells and is essential for turgor regulation, stomatal opening, cell expansion, anion neutralization and assimilate conduction. (Glass, 1989; Hsiao and Läuchli, 1986; Blevins, 1985)

Calcium is required inside cells in micromolar amounts, while the optimum extracellularly is millimolar (Hanson, 1984; Marme, 1983). It has a wide variety of distinct intracellular and extracellular roles. Much of the Ca in plants is localized in the cell walls as calcium pectate, which serves to increase cell wall rigidity. Ca is an activator for enzymes including α-amylase and some membrane ATPases, and it is also involved in cell division and in the assembly of microtubules. Ca is essential for membrane form and function; it maintains membrane integrity, and acts as a mediator of selective ion uptake. (Glass, 1989; Hanson, 1984; Marme, 1983). Perhaps as its most ubiquitous function, Ca acts as a messenger in transducing many physical and hormonal signals including: touch, wind, gravity, light, cold, auxin, GA, ABA, salt, and fungal elicitors (Pooviah and Reddy, 1993, 1987; Jackson and Hall, 1993). In the response to some stimuli, such as gravity, it is apparently involved in all three steps of the response process: signal perception, signal transduction, and the physiological response (Pooviah and Reddy, 1993).

Magnesium is found in plant tissues in association with anions, such as malate, citrate, oxalate and pectate. It is also found complexed with much of the cellular ATP and ADP. The most well known role of Mg is as the centre of the chlorophyll molecule, which

accounts for only 15 to 20% of the total plant Mg. Mg is a co-factor in almost all enzymes which activate phosphorylation processes; it forms a bridge between the pyrophosphate structure of ATP or ADP and the enzyme molecule. Mg is necessary for maintaining the integrity of ribosomes, and is an activator for some dehydrogenases, enolases and ribulose 1,5-bisphosphate carboxylase. (Mengel and Kirkby, 1982; Glass, 1989)

Manganese is an activator of several enzymes, including malic enzyme and some decarboxylases and dehydrogenases involved in the TCA cycle. It is essential for the photolysis of water, where it is probably involved via redox changes converting Mn^{2+} to $Mn^{3+}+e^-$. In some enzyme reactions, Mn may be a substitute for Mg. (Glass, 1989; Mengel and Kirkby, 1982)

Iron functions in plants by participating in redox reactions and by acting as a chelator. It participates in redox reactions principally at the centre of the porphyrin molecule; such haem complexes form the prosthetic groups of several important enzymes including the cytochromes, peroxidase, catalase and cytochrome oxidase. Fe is also present in ferridoxin, which are a component of the photosynthetic electron transport chain. The majority of Fe in plant leaves is stored as phytoferritin (a ferric phosphoprotein). (Glass, 1989; Mengel and Kirkby, 1982)

Zinc serves as a co-factor, forming stable complexes with many important enzymes including: several dehydrogenases, aldolases, phosphatases, DNA and RNA polymerases, enolase, and carbonic anhydrase. There are over 80 metallo-enzymes known that involve Zn. Zn is closely involved in the nitrogen metabolism of plants, is required for the synthesis of tryptophan, and may be involved in starch formation and chlorophyll biosynthesis. (Glass,

1989; Mengel and Kikby, 1982).

MOBILIZATION OF STORAGE RESERVES

General:

During seedling establishment, the storage reserves are broken down and utilized by the growing plant. The mobilization of stored reserves begins after radicle elongation (Bewley and Black, 1985). As the reserves are mobilized, they are converted into a readily transportable form, and delivered to various parts of the growing plant where they will be used in the production of energy and in the synthesis of necessary compounds (Simon, 1984). In cereals, the breakdown of storage reserves is clearly under the control of growth hormones and the axis is required for reserve mobilization (Bewley and Black, 1985; Ashford and Gubler, 1984). In dicots, the control mechanisms are less clear. In most cases, the presence of the axis is required for maximum mobilization of reserves from the cotyledons (Pino *et al.*, 1991; Davies and Slack, 1981). It has been proposed, that this is not due to the presence of growth hormones, but that the axis stimulates mobilization by acting as a metabolic sink (Davies and Slack, 1981). The evidence remains contradictory and in some species, neither the source-sink, nor the hormonal control mechanism is sufficient on its own to explain the observed results (Garcia-Agustin, 1992; Pino *et al.*, 1991). It appears that either one or both of the proposed mechanisms may actually be involved in regulating reserve mobilization depending on the species, and the stage of growth. In cucurbits, as in most other species, how reserve mobilization is controlled is unclear. Studies with different cucurbits variously

show: that the cotyledon-axis relationship is that of a source-sink (Chapman and Galleschi, 1985; Davies and Chapman, 1979b, 1980); that the axis can be replaced by cytokinin (Legocka *et al.*, 1985; Penner and Ashton, 1967); and that the axis has no effect at all on reserve mobilization (Splittstoesser, 1983).

The breakdown of storage reserves is influenced to a great extent by the testa. Little degradation of either protein or lipid will occur while the seed coat remains on the cotyledons (Davies and Chapman, 1979a; Slack *et al.*, 1977), because the seed coat restricts oxygen uptake by the embryo (Pesis and Ng, 1986). To ensure that the seed coat is removed at the appropriate time, cucurbits have a protuberance, called a peg, in the root-shoot transition area. As the seedling emerges from the soil, the peg presses on the lower half of the seed coat which allows the elongating, hooked hypocotyl to pull the cotyledons out of the seed coat (Nelson, 1932).

Mobilization of oil reserves:

During reserve mobilization the oil reserves are hydrolysed and the lipid bodies gradually decrease in size and disappear (Bewley and Black, 1985). The disappearance of the lipid bodies is parallelled by the appearance of glyoxysomes (Wanner *et al.*, 1982). The initial step in the mobilization of lipids is their hydrolysis to fatty acids and glycerols, which are then converted to sucrose by the gluconeogenic pathway (Huang and Moreau, 1978). By the seventh day after imbibition in *Cucumis*, about 75% of the lipid reserves have been degraded (Davies and Chapman, 1979a).

Mobilization of storage protein:

During germination and early seedling growth, the protein bodies fuse to form aqueous vacuoles and the globoids are degraded (Lott and Vollmer, 1973a; Hara and Matsubara, 1980). In *C. maxima*, by the fourth day after imbibition, protein bodies have become a series of aqueous vacuoles containing small amounts of residual protein (Lott and Vollmer, 1973a). Fusion and vacuolation of the protein bodies begins earlier in cells closer to the vascular bundles, epidermis and roots (Hara and Matsubara, 1980). Initial mobilization of reserves is from the provascular areas while stores in the palisade mesophyll furthest from the provascular area are mobilized last (Davies and Chapman, 1981; Nelson, 1932). Within the protein bodies (Hara-Nishimura *et al.*, 1982), the water insoluble globulins are initially degraded to more soluble components (Hara *et al.*, 1976a). These more soluble components are then further hydrolysed to peptides and amino acids (Hara and Matsubara, 1980), which may be used in the cotyledons, or transported to the growing axis (Bewley and Black, 1985).

Mobilization of phytin and mineral nutrients:

The degradation of phytin is a step-wise process which results in the release of inorganic phosphorus (P_i), *myo*-inositol, and a series of phosphoric esters of *myo*-inositol (Hayakawa *et al.*, 1990). This degradation is facilitated by one, or more, of a group of enzymes known as phytases. The *myo*-inositol released by phytin degradation has a variety of uses in the developing seedling. It is a key intermediate in cell wall biogenesis (Saski and Taylor, 1984), phospholipid synthesis (Loewus and Loewus, 1983) and the production of a number of isomeric inositols and their derivatives (Loewus, 1990). Evidence suggests that

certain inositol polyphosphates may even function as second messengers in plant tissues (Loewus, 1990).

There is much that remains unclear about phytases and the mobilization of phytin (Gibson and Ullah, 1990; Loewus *et al.*, 1990; Scott and Loewus, 1986). In all species studied to date, there is a marked increase in phytase, and a corresponding decrease in phytic acid, which accompany germination and early seedling growth (Kikunga *et al.*, 1991; Gibson and Ullah, 1990). There are, however, definite species and cultivar differences in phytate synthesis, phytate degradation and phytase characteristics. It is unclear, in most cases, whether the rise in phytase activity during seedling growth is due to the activation of pre-existing phytase, or *de novo* synthesis of the enzyme. In many cases, it appears to be a combination of the two mechanisms, while in some species only one or the other seems to apply. Studies with wheat indicate that phytase is synthesized in the embryo early in germination (Laboure *et al.*, 1993; Bianchetti and Sartirana, 1967). In peas only one form of phytase is found in the dry seed, while two are present in the developing seedling (Kuvaeva and Kretovich, 1978). In soybeans, although there is a small amount of phytase present in the dormant seed, there is a marked increase in the amount of the enzyme present as the seed germinates (Gibson and Ullah, 1988). In dry pumpkin seeds, no phytase activity is detected (Splittstoesser, 1982), but in germinating cotyledons of both *C. moschata* and *C. maxima* multiple forms of phytase are detected (Splittstoesser, 1982; Goel and Sharma, 1979). These findings indicate that in cucurbits the increase in phytase activity is due, at least in part, to *de novo* synthesis of the enzyme.

The site of phytase activity in seeds is not known. Studies of phytase in seeds has

almost exclusively involved isolation of the enzyme from whole, crushed seedlings or cotyledons. In pollen, however, which also contains phytin as globoid-like inclusions inside of membrane bound organelles, at least one phytase is anchored to the inside of the membrane, degrading phytin within the organelle (Loewus *et al.*, 1990).

It has been proposed that phytate degradation by phytase is controlled by a feedback inhibition mechanism. Exogenous P_i applied to isolated wheat embryos inhibits the synthesis of phytase (Bianchetti and Sartirana, 1967), and the breakdown of phytate by phytase is strongly inhibited in the presence of P_i, although whether it is the synthesis or activity of phytase which is inhibited is not known (reviewed in Scott and Loewus, 1986). Both findings support the idea of some form of feedback inhibition, since P_i is one of the products of phytase action. Phytate degradation also appears to be under the control of certain growth regulators, including gibberellic acid, but the mechanism is not yet understood (Kikunga *et al.*, 1991; Scott and Loewus, 1986).

The cations bound to phytate are released during germination and early seedling growth. In legumes, a linear relationship exists between the export of mineral nutrients and the decrease in dry matter of the cotyledons (Hocking, 1980; Collins and Sutcliffe, 1977; Guardiola and Sutcliffe, 1972). In legumes and lupins, individual minerals are not mobilized from the cotyledons at the same rate, nor to the same extent; the mobilization of Ca is lower than the mobilization of P, K and Mg (Hocking, 1980; Ferguson and Bollard, 1976). The degree of mobilization of a mineral is thought to be related to its mobility in phloem (Guardiola and Sutcliffe, 1972). Cotyledons occasionally act as sinks during seedling growth, especially for Ca (Hocking, 1980).

Ockenden and Lott (1988d), studied the mobilization of P, K, Mg and Ca out of the cotyledons of *C. maxima* and *C. andreana* seedlings during germination and early seedling growth. They reported that only a small percentage of the stored minerals was moved out of the cotyledons of etiolated seedlings grown in distilled water, and the percentage mobilized was even lower in light grown seedlings. Seedlings grown in the light, in complete mineral nutrient solution, actually imported mineral nutrients into the cotyledons. In conditions where mineral nutrients were mobilized out of the cotyledons, a higher percentage of K was lost than any other element. The percentage of minerals lost from the cotyledons to the root-shoot axis was about twice as high in *C. andreana*, as in *C. maxima*. Work with several dicot species also found that there was little or no movement of minerals out of the cotyledons during germination and seedling establishment (Rao and Deosthale, 1983; Reddy *et al.*, 1978).

There has been no comprehensive study done to determine how the element composition of globoids changes during the period of reserve mobilization. Preliminary studies on castor bean have shown that there is some change in globoid composition during germination and early seedling growth. In that species, K levels in globoids from both cotyledon and endosperm tissues decreased and Ca levels increased (Lott *et al.*, unpublished). This thesis describes a study in which the elemental composition of globoids from the cotyledons of *C. maxima* and *C. andreana* seeds and seedlings at different stages of growth was determined. The effect of conditions of light and mineral nutrients on globoid composition at the various stages was also examined.

CHAPTER 2: MATERIALS AND METHODS

SEEDS

All seeds were from fruits grown in the McMaster University greenhouse. *C. maxima* seeds were pooled from five different fruits, and the seeds had a mean weight of 0.30 g. *C. andreana* seeds were pooled from ten different fruits and had a mean weight of 0.019 g. Seeds for planting and analysis were selected randomly from the pooled samples.

GROWTH CONDITIONS

Seeds were planted in glass trays in a 1:1:0.7 mixture of perlite, vermiculite and either distilled water or full strength Hoagland's solution (Hoagland and Arnon, 1950). In each tray, either 25 to 30 *C. maxima* seeds, or 45 to 50 *C. andreana* seeds, were planted. Each planted tray was covered by an inverted glass tray to prevent excessive moisture loss, while facilitating gas exchange. Seedlings were kept moist with either distilled water or half strength Hoagland's solution (100 ml/day for light grown, 50 ml/day for dark grown). Immediately after planting, trays of "light grown" samples were placed in a Conviron E7 growth chamber, and illuminated constantly (80 $\mu mol \cdot s^{-1}m^{-2}$); trays of "dark grown" samples were immediately placed in a ventilated, light-tight box. The dark box was ventilated by pumping air directly from the growth chamber into the box, which was vented to allow air to escape. The air temperature in both growing chambers was maintained at 31°C. Once the cotyledons protruded above the surface of the growth medium, the seed coats were removed to allow for even expansion of the cotyledons.

Two trays of seeds were exposed to each of the four different sets of growth conditions: dark/no nutrients, light/no nutrients, dark/with nutrients, and light/with nutrients. The terms "with nutrients" and "no nutrients" refer, respectively, to those samples grown with Hoagland's solution and those grown with distilled water. The distinction refers only to mineral nutrients applied in the growth medium, and not to any description of mineral nutrient stores in the seeds themselves.

STAGES OF GROWTH

Globoid crystals from seeds and seedlings at various stages of early growth were analyzed. The time periods chosen corresponded to changes in morphological features (Table 2.1). Stage I was the dry seed, before planting. Stage II corresponded to the emergence of the radicle through the seed coat (the end of germination). Stage III was the point at which the cotyledons appeared above the growth medium; in the light, the cotyledons were starting to green and were slightly expanded; in the dark, the cotyledons were white and the hypocotyls were longer than those in the light. At Stage IV, the first foliage leaves were not yet apparent. In the light, the cotyledons were fully green and much expanded, and were supported on thick short hypocotyls. In the dark, the cotyledons were yellow and spoon shaped, and the hypocotyls were long and thin.

The number of days after planting which corresponded to the different morphological stages were used to determine when the *C. maxima* samples would be harvested. *C. maxima* is a cultivated species and its seedling growth rate was very consistent. Even so, seedlings were screened to ensure that they showed the appropriate morphology for each stage. *C.*

Table 2.1 Morphological characteristics used to define the stages of seedling growth.

Stage	Morphological Characteristic		Days after Planting	
	Light	Dark	*C.max*	*C.and*
I	- Dry seed.	- Dry seed.	0	0
II	- Radicle protruding.	- Radicle protruding.	2	≈ 4
III	- Cotyledons above the growth medium. - Cotyledons starting to green and expand.	- Cotyledons well above the growth medium. - Cotyledons remain white.	4	≈ 8
IV	- Cotyledons fully green and expanded. - Thicker, shorter hypocotyls. - No visible epicotyl elongation.	- Cotyledons yellow and spoon-shaped. - Extremely long hypocotyls. - No visible epicotyl elongation.	5	≈ 10

Notes: • *C.max* = *C. maxima*, *C.and* = *C. andreana*
• The times given for *C. andreana* are approximate; seedlings were selected for study solely on the basis of their morphology since germination time and growth rate were inconsistent for this species.

andreana, however, is a wild species, and its germination and growth rate were erratic. The days after planting corresponding to the various stages, given in Table 2.1, are only approximate for this species. *C. andreana* samples were selected for analysis strictly on the basis of their morphology, regardless of the number of days which had passed since the seeds were planted.

Each combination of a morphological stage with a set of growth conditions was referred to in this work as a "treatment" ie. Stage II, in the dark, with nutrients was a single treatment. A total of 13 treatments for each species were considered:

4 conditions X 3 stages (ie. *C. maxima* Stage II, III, IV)	= 12
Stage I = Day 0 = dry seeds	= 1
	13

SAMPLING

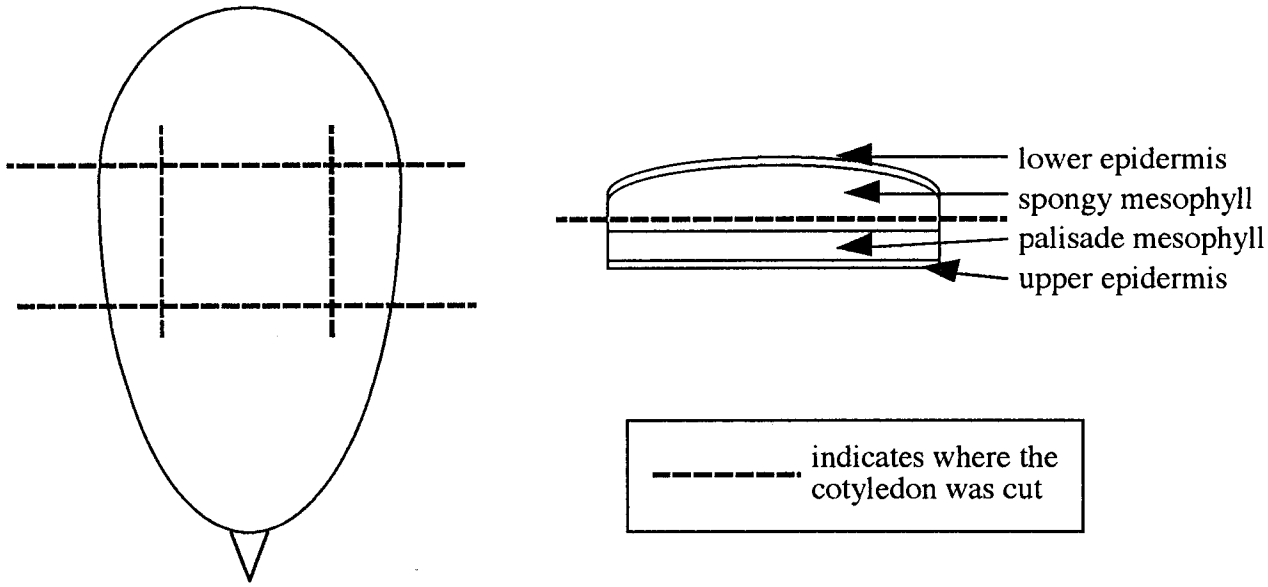
Ten dry seeds of each species were processed; these were the "Stage I" samples. At each subsequent stage, 8 to 10 seedlings were randomly selected from each growth condition, for each species. The dark grown samples were harvested in the dark, without exposing the collected samples, or those remaining in the tray, to any light. In the dark treatments, the stage of growth was determined by touch.

The hypocotyl length and length of the cotyledons were noted for each seedling, and the average values for each treatment were calculated. The cotyledons were removed from the seedling, samples of tissue were excised from the centre of the cotyledon, and then these sections were cut along their length to remove the upper epidermis and most of the palisade mesophyll (Fig.2.1). The remaining tissue was made up mostly of spongy mesophyll, which

Figure 2.1 Isolation of spongy mesophyll samples from the cotyledons of *Cucurbita* seeds and seedlings.

a) Samples of tissue were excised from the center of the cotyledon.

b) These sections were cut along their length to isolate the spongy mesophyll.



has more numerous, larger globoid crystals than other cotyledon cells (Lott *et al.*, 1979). Samples were frozen in nitrogen slush and subsequently freeze-dried. Dried samples were stored in sealed vials over calcium sulfate.

The dark grown samples were exposed to light as little as possible during the above procedure, but some illumination was necessary for measuring, cutting and transferring the samples into nitrogen slush. These procedures were carried out under dim, white light and took only minutes to complete.

To determine whether or not the freeze drying procedure influenced the elemental composition of the globoids, samples from dry *C. maxima* seeds (Stage I) were also prepared without freeze-drying. The results from this set of nonfreeze-dried samples was compared to those from the freeze-dried Stage I samples.

EDX ANALYSIS PROCEDURE

Using an alumina mortar and pestle, small pieces of dried cotyledon from each seed/seedling were crushed, one at a time, into powders and spread on to carbon-Formvar coated copper grids. From each seed, 10 globoids were analyzed , and the approximate diameter of each globoid was noted. Globoids normally had diameters between 1 and 2 μm. The total number of spectra collected was 10 spectra X 8 or 10 seeds X 13 treatments X 2 species, or approximately 2600 spectra.

EDX analysis was carried out for 60 s at an accelerating voltage of 80 kV with a JEOL 1200 EX-II scanning transmission electron microscope connected to a PGT model IMIX-II microanalysis system. P, K, Mg, Ca, Mn, Fe, and Zn were all measured. X-ray

counts for the measured elements were obtained by integrating peaks at the following window widths: P, 1905.3-2120.7 eV; K, 3193.7-3432.3 eV; Mg, 1153.7-1354.3 eV; Ca, 3568.6-3813.4 eV; Mn 5758.5-6037.5 eV; Fe, 6359.9-6546.1 eV; Zn, 8478.9-8795.1 eV. Detector distance, aperture, spot size and tilt were kept the same for all analyses.

The background was subtracted from each peak and the total number of counts in each element window before and after background subtraction were used to calculate peak-to-background (P/B) ratios (Stewart *et al.*, 1988). Ratios of each element to P (ie.(K P/B)/(P P/B)) were also calculated. For each element, mean P/B and element/P values were calculated for each seed, and for each treatment.

The Ca K_α peak is overlapped by the K K_β peak. In order to calculate the counts for Ca a correction factor of 8.8% of the X-ray counts in the K K_α peak was subtracted from the net counts collected in the Ca K_α window. Similarly, the Fe K_α is overlapped by the Mn K_β peak, and the Zn K_α peak is overlapped by the Cu K_β peak. Fe peak counts were corrected by subtracting 11.6% of the net Mn K_α counts, and the Zn peak counts were corrected by subtracting 2% of the net Cu K_α counts (net = total - background). These correction factors were derived, as shown in Appendix A, from the analysis of K, Mn and Cu salts as suggested by Barbi (1979).

For elements present in trace amounts some spectra gave negative values, and others very small positive values. In order to take into account this variation about 0, which was caused by the background modelling, cut-off limits were set, below which the element was said to be not present. These limits were determined based on the lowest negative P/B ratio produced for that element. Cut-off limits were determined separately for each species. The

P/B cut-off limits for *C. maxima* were: Ca, 0.20; Mn, 0.12; Fe, 0.12; Zn, 0.10. Cut-off limits for *C. andreana* were: Ca, 0.10; Mn, 0.20; Fe, 0.07; and Zn, 0.10.

STATISTICAL ANALYSIS

Statistical analyses were carried out using single and multifactor analyses-of-variance (ANOVA's) and Tukey tests using Minitab Statistical Software version 7.2. Table 2.2 outlines which analyses were done. ANOVA's were used to determine statistically significant sources of variation. Tukey tests were used when there was significant variation to ascertain which segments of the analyzed group were different from each other. Separate analyses were carried out for each element from each species.

Table 2.2 Statistical analyses carried out.

Test	Data Analyzed	Information Gathered
one-way ANOVA Tukey test	1. cotyledon lengths 2. hypocotyl lengths	The effect of growth conditions on seedling morphology.
four-way ANOVA (crossed and nested mixed model)	All Stage I, II and III P/B data for each element.	Are any of the following factors significant sources of variation in the P/B ratios for each element? - seed-to-seed variation - stage of development - light conditions - mineral nutrient conditions
one-way ANOVA Tukey test	P/B data from seeds of each individual treatment.	Seed-to-seed variation within a treatment.
one-way ANOVA Tukey test	P/B data from all stages for each set of growth conditions.	Change in P/B over time for each set of growth conditions.
one-way ANOVA	*C. maxima*, Stage I P/B ratios; freeze-dried vs. not freeze-dried.	Effect of freeze-drying on globoid composition.

CHAPTER 3: RESULTS

MORPHOLOGICAL CHANGES

For the two species, the mean hypocotyl and cotyledon lengths were calculated for each treatment (Tables 3.1a and b). As the seedlings passed through the various stages, they progressed from dry seed, to imbibed seed with the radicle protruding, to a seedling with enlarged cotyledons. In the light, the cotyledons greened and became greatly expanded, the hypocotyls increased in length. In the dark, the cotyledons turned a yellowish-white, and expanded slightly, taking on a spoon-like shape. The hypocotyls of etiolated plants were much longer and thinner than those of light grown plants. The presence or absence of mineral nutrients in the growth medium did not affect the length of hypocotyls or cotyledons of plants grown under the same light conditions, except in the Stage IV *C. maxima* samples. In that case, seedlings supplied with nutrients had significantly longer cotyledons than the seedlings grown with deionized water.

The cotyledons of the dry *C. maxima* seeds (Stage I) were about three times as long as those from *C. andreana* seeds, and this size difference was maintained throughout the study period. Although the *C. maxima* seedlings were larger than the *C. andreana* plants both species showed approximately the same relative increase in cotyledon length from Stage I to Stage IV (Table 3.1c).

Table 3.1a Mean (±SD) length (mm) of cotyledons and hypocotyls from *Cucurbita maxima* seeds and seedlings, at various stages of growth, grown under different conditions of light and mineral nutrients.

Growth Conditions	Stage					
	I	II	III		IV	
	cotyledons	cotyledons	cotyledons	hypocotyl	cotyledons	hypocotyl
dark/no nutrients	15.5 ± 0.8	16.1 ± 0.9 a	21.3 ± 1.5 b	59.4 ± 7.3 i	22.9 ± 1.9 f	105.1 ± 17.4 k
dark/with nutrients	15.5 ± 0.8	16.3 ± 0.8 a	22.9 ± 1.8 bc	67.2 ± 16.9 i	24.6 ± 3.8 f	121.1 ± 14.6 k
light/no nutrients	15.5 ± 0.8	16.6 ± 0.9 a	25.3 ± 2.0 d	22.8 ± 2.7 j	35.5 ± 2.5 g	32.0 ± 3.3 m
light/with nutrients	15.5 ± 0.8	16.4 ± 1.0 a	24.6 ± 1.4 cd	22.7 ± 1.8 j	43.2 ± 4.4 h	30.8 ± 5.6 m

Notes: • For all samples except Stage I, N=12. For Stage I, N=40
• Stage I data is replicated for each set of growth conditions to facilitate comparisons.
• Values in the same column followed by the same letter are not significantly different at $P > 0.05$.

Table 3.1b Mean (±SD) length (mm) of cotyledons and hypocotyls from *Cucurbita andreana* seeds and seedlings, at various stages of growth, grown under different conditions of light and mineral nutrients.

Growth Conditions	Stage					
	I	II	III		IV	
	cotyledons	cotyledons	cotyledons	hypocotyl	cotyledons	hypocotyl
dark/no nutrients	5.1 ± 0.5	5.8 ± 0.4 a	6.6 ± 0.5 bc	21.0 ± 6.4 h	7.1 ± 0.8 f	67.9 ± 10.6 k
dark/with nutrients	5.1 ± 0.5	5.8 ± 0.5 a	6.1 ± 0.8 b	16.7 ± 4.0 hi	7.8 ± 0.8 f	65.2 ± 18.4 k
light/no nutrients	5.1 ± 0.5	6.0 ± 0.5 a	7.7 ± 0.9 d	8.1 ± 2.7 j	12.2 ± 2.3 g	23.2 ± 4.8 m
light/with nutrients	5.1 ± 0.5	6.0 ± 0.4 a	7.5 ± 1.4 cd	13.6 ± 4.1 ij	14.0 ± 3.3 g	19.6 ± 2.8 m

Notes:
- For all samples except Stage I, N=12. For Stage I, N=40
- Stage I data is replicated for each set of growth conditions to facilitate comparisons.
- Values in the same column followed by the same letter are not significantly different at $P > 0.05$.

Table 3.1c Total change in mean cotyledon length from Stage I to Stage IV, of *C. maxima* and *C. andreana* seedlings, measured as a percent of the mean cotyledon length at Stage I.

	Growth Conditions			
	dark/ no nutrients	dark/ with nutrients	light/ no nutrients	light/ with nutrients
C. maxima	48	59	129	179
C. andreana	39	53	131	175

SUBCELLULAR CHANGES

The observed changes were the same for both species. In the dry seed (Stage I) there were abundant globoids, mostly with diameters between 1 and 2 μm, all located within protein bodies (Fig.3.1a and b). At Stage II, globoids were still easily located, and most globoids were still surrounded by protein. By Stage III, it was more difficult to locate globoids, and those that were present were not surrounded by protein. In addition to the 1 - 2 μm globoids, some Stage III and IV samples also contained an occasional very large (5-7 μm), round, electron-dense, particle that was globoid-like (Fig.3.1c). By Stage IV, globoids were very difficult to locate, and in the Stage IV, light/with nutrients samples, no globoids were detected all. In Stages III and IV, it was more difficult to locate globoids in the *C. maxima* samples than in the *C. andreana* samples.

CHANGES IN GLOBOID ELEMENT COMPOSITION

Spectra:

C. maxima:

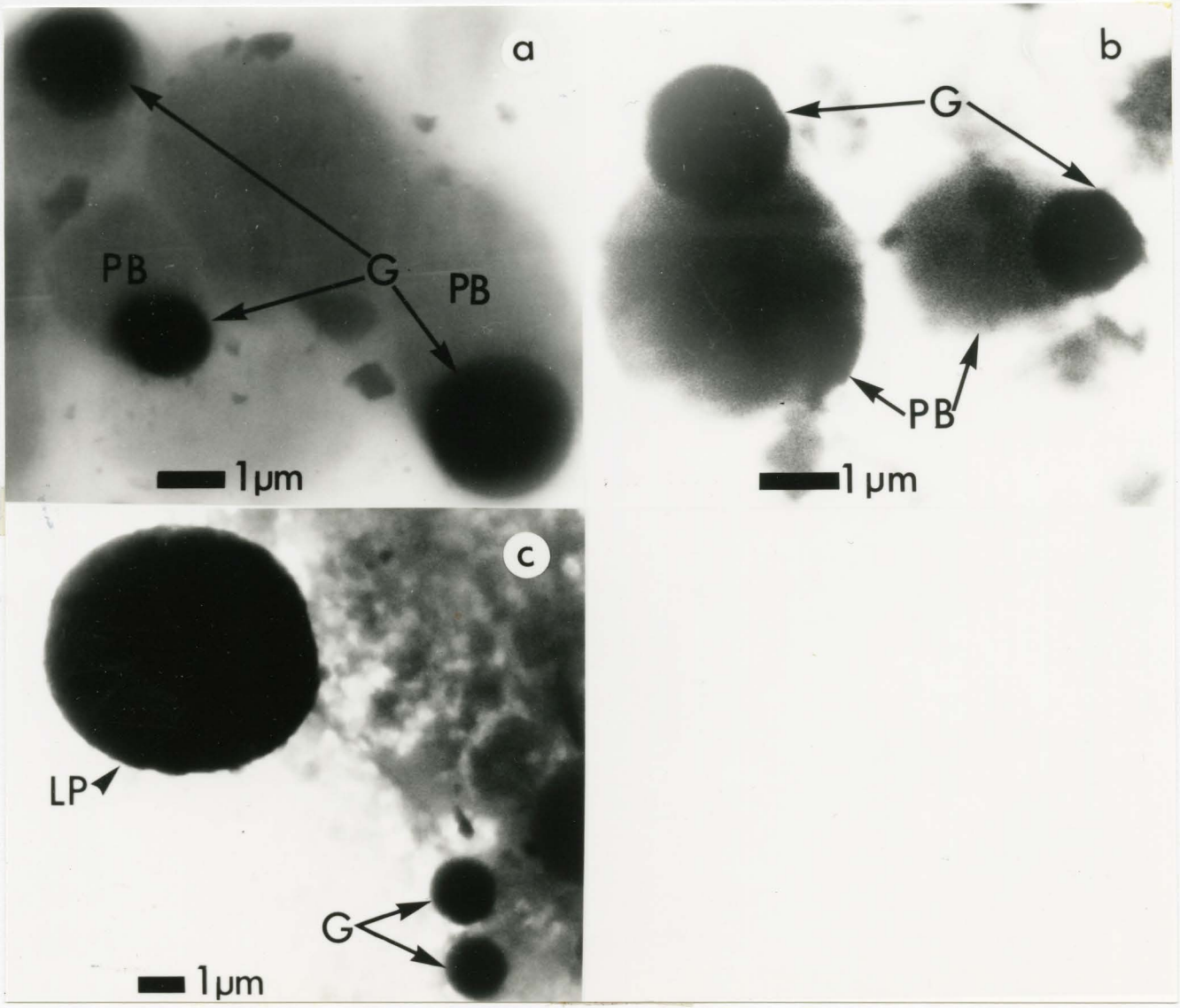
The EDX analysis spectra collected from globoids in dry *C. maxima* seeds (Stage I) had high P, K and Mg peaks, much smaller Fe and Zn peaks (Fig.3.2a). A small percentage of Stage I spectra also had small Ca and Mn peaks. From the height of peaks on the spectra, even before any peak-to-background ratios were calculated, it was apparent that globoid composition was changing as germination progressed. The K peak decreased, whereas Fe,

Figure 3.1 STEM micrographs of *C. maxima* and *C. andreana* cotyledon tissue prepared for EDX analysis by freeze-drying and then crushing chemically untreated samples.

a) Crushed tissue from a Stage I *C. maxima* seed. The globoids (G) are contained within protein bodies (PB).

b) Crushed tissue from a Stage I *C. andreana* seed.

c) Crushed tissue from a Stage III "light/with nutrients" sample. The globoids (G) are no longer surrounded by protein, but are approximately the same size as in Stage I. Also present in the sample is a large, round, electron-dense particle (LP).


a
PB
G
PB
1µm
b
G
PB
1µm
c
LP
G
1µm

Mn, and Zn increased (Compare Fig.3.2a with Fig.3.2b). The very large, electron-dense particles, found in Stage III and IV samples, produced spectra that were essentially the same as spectra collected from normal sized globoids in the same samples (Fig.3.2c).

C. andreana:

Spectra from Stage I samples showed that, in the dry seed, *C. andreana* globoids contained appreciable amounts of P, K, Mg and Ca (Fig.3.3a). As germination and early seedling growth progressed, changes in globoid composition were apparent from the peaks on the spectra. It was evident that K decreased while Ca increased (Compare Fig.3.3a with Fig.3.3b). In the later stages, Cl was also evident in many spectra (Fig.3.3c). The large, electron-dense particles which were found in Stage III and IV samples produced spectra similar to those of normal globoids from the same samples.

Peak-to-Background Ratios:

For each element, mean P/B ratios and element/P ratios were calculated for globoids from each seed from each treatment, and the overall mean values for each treatment were calculated (Appendix B). Mean P/B ratios for *C. maxima* and *C. andreana* are summarized in Tables 3.2a and 3.2b, respectively. Standard deviations were high, indicating a great deal of variation in globoid composition both between seeds from a single treatment and within the same seed. Only a few large, electron-dense particles were analyzed, therefore, no P/B data is presented for these particles.

Calculations and analyses were carried out using both P/B and element/P data. The element/P data did not provide any information significantly different from that given by the

Figures 3.2 and 3.3

Spectra produced by EDX analysis of globoids found in freeze-dried and crushed samples of *C. maxima* and *C. andreana* cotyledon tissue. The apparent Ca peak is caused by the K K_β peak overlapping the Ca K_α peak. The Cu peak is background from the copper grid.

Figure 3.2 Typical EDX analysis spectra produced by *C. maxima* globoids:

a) Stage I.

b) Stage IV, dark/with nutrients.

c) a large, electron-dense particle from the same sample as (**b**).

Figure 3.3 Typical EDX analysis spectra produced by *C. andreana* globoids:

a) Stage I.

b) Stage IV, light/no nutrients.

c) Stage IV, light/no nutrients. Note the small Cl peak.

Figure 3.2 ***Cucurbita maxima***

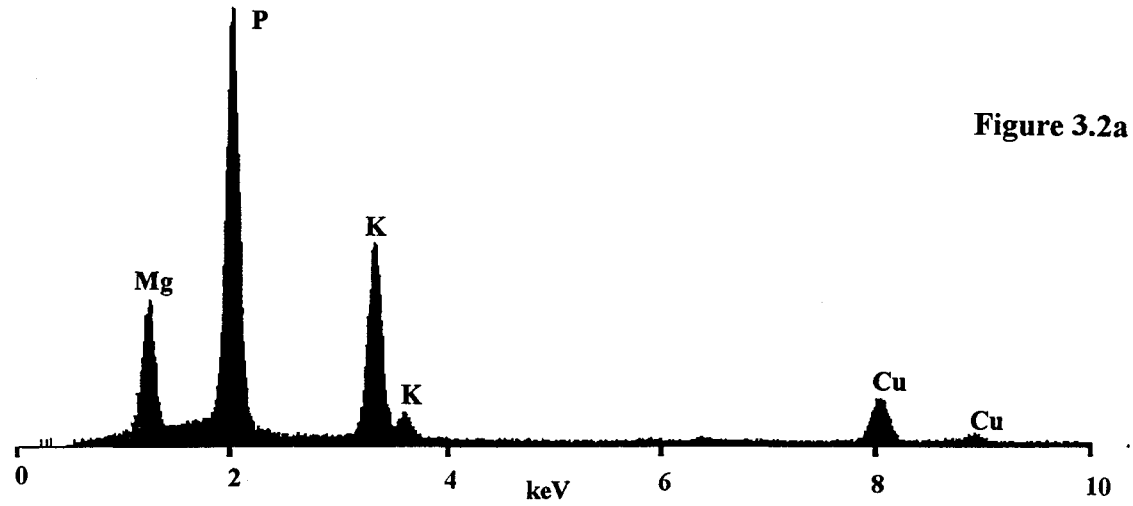


Figure 3.2a

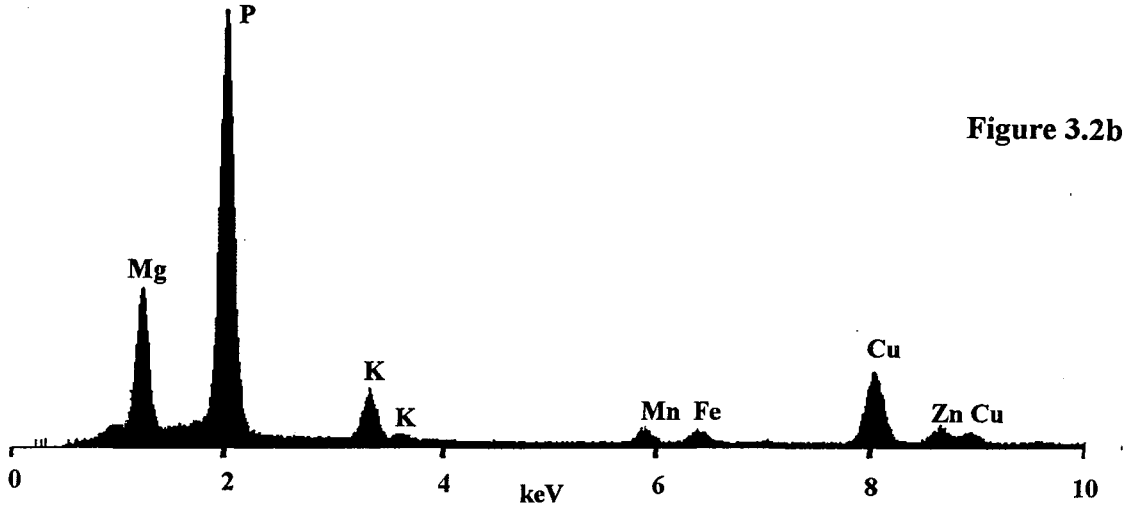


Figure 3.2b

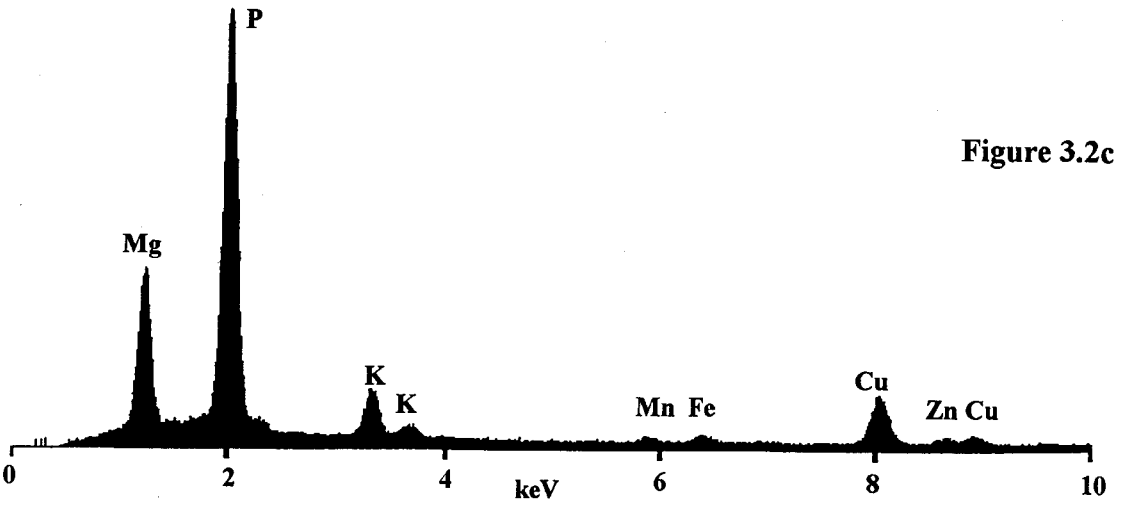


Figure 3.2c

Figure 3.3 ***Cucurbita andreana***

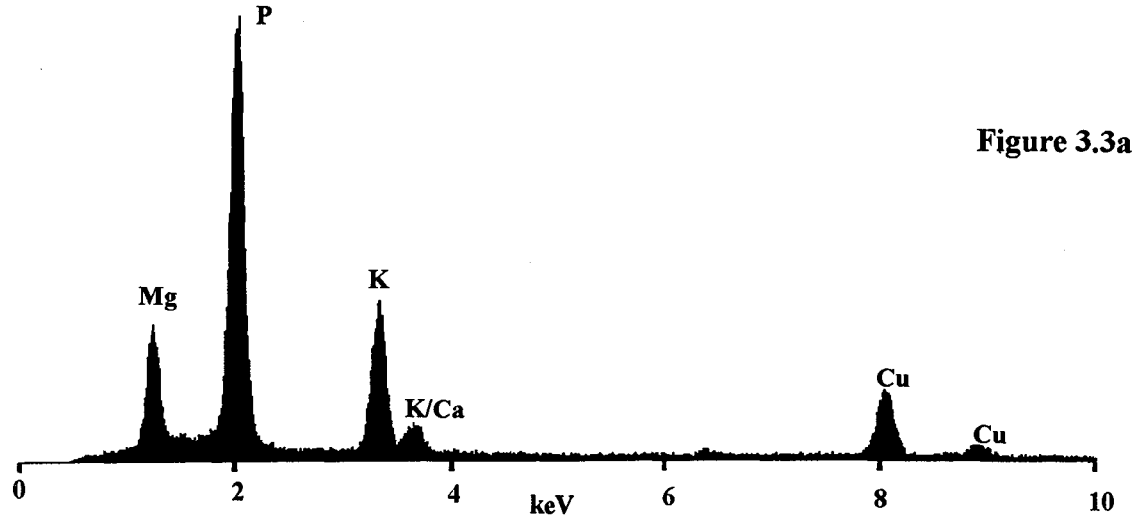


Figure 3.3a

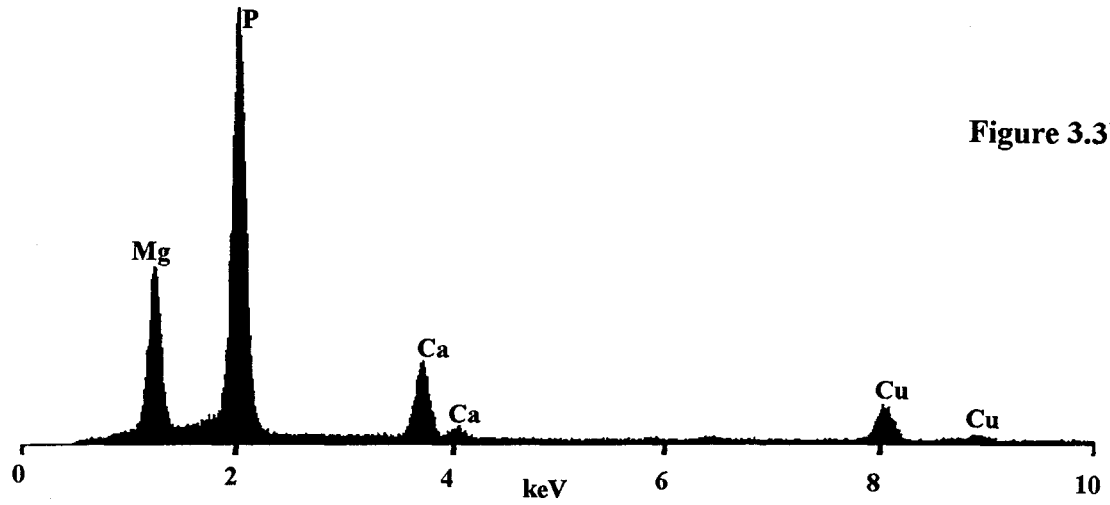


Figure 3.3b

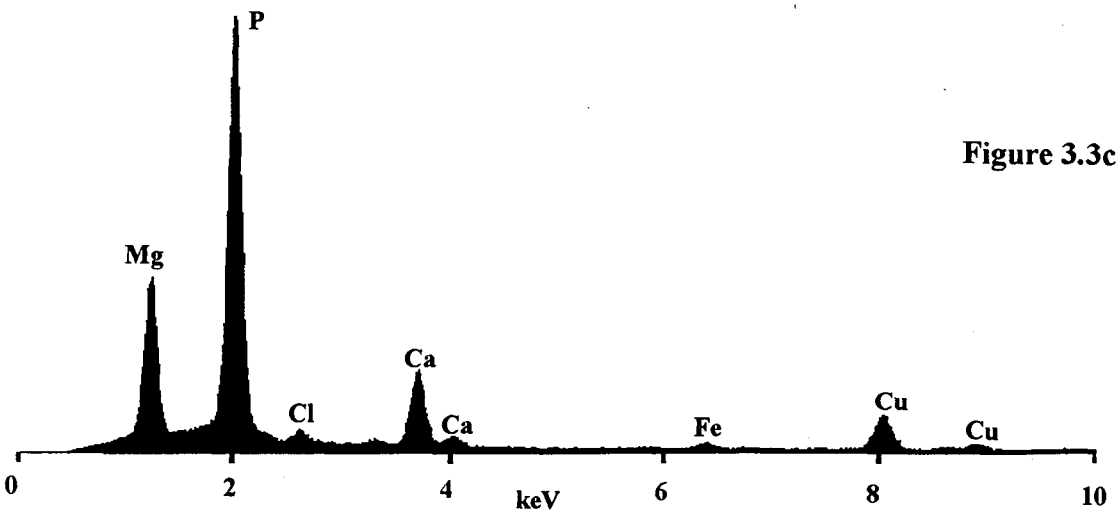


Figure 3.3c

P/B data. Therefore, only the P/B information has been reported and discussed.

The effect of the freezing, and freeze-drying on globoid composition was tested. Globoids from Stage I samples which had not been freeze-dried did not have significantly different P/B ratios, for any of the elements measured, than globoids from Stage I samples which had been rapidly frozen in nitrogen slush and then freeze-dried (Table 3.3).

Stage I globoids from the two species contained comparable levels of most of the elements; they had high P, K and Mg peaks, lesser peaks of Fe and Zn, and no Mn (Tables 3.2a and b). But, globoids from dry seeds of the two species contained very different levels of Ca: *C. andreana* globoids had considerable Ca, whereas the mean *C.maxima* Ca P/B was below the cut-off limit.

The element composition of the globoids (as measured by changes in mean P/B ratios) changed over time; some elements increased, some decreased, and others remained basically unchanged. For each species, the changes over time for each element followed the same general trend regardless of light and mineral nutrient conditions (Tables 3.2a and b). For each element, the mean P/B ratio for each treatment was plotted, and all four sets of growth conditions were plotted on the same graph.

Phosphorus: The P P/B remained relatively constant over the course of the study in both species (Fig.3.4a). In *C. andreana*, there was no significant overall change from Stage I to Stage IV, for any of the sets of growth conditions (Table 3.2b). The total change from Stage I was between 0 and 5 percent. For *C. maxima*, three was a slight, significant increase over time (Table 3.2a), but the total change was still only around 10%.

Potassium: Over time, K decreased, in excess of 80% in many cases, for both species

Table 3.2a Mean (± SD) peak-to-background ratios derived from EDX analysis of globoids from the cotyledons of *C. maxima* seeds and seedlings at various stages of growth, grown under different conditions of light and mineral nutrients.

Light	Mineral	Stage	N	P	K	Mg	Ca	Mn	Fe	Zn
dark grown	no nutrients	I	100	12.18 ± 1.28a	13.01 ± 2.14a	4.09 ± 0.88a	NS a	NS a	0.27 ± 0.15a	0.21 ± 0.16a
		II	100	12.15 ± 1.27a	9.35 ± 2.24b	3.97 ± 0.95a	NS a	NS a	0.24 ± 0.10a	0.26 ± 0.14a
		III	100	12.56 ± 1.62a	4.64 ± 2.49c	5.24 ± 1.19b	NS a	0.35 ± 0.34b	0.48 ± 0.36b	0.61 ± 0.44b
		IV	100	13.43 ± 1.32b	4.04 ± 2.80c	5.82 ± 1.16c	0.58 ± 0.99b	0.49 ± 0.36c	0.60 ± 0.58b	0.99 ± 1.05c
	with nutrients	I	100	12.18 ± 1.28p	13.01 ± 2.14p	4.09 ± 0.88p	NS p	NS p	0.27 ± 0.15p	0.21 ± 0.16p
		II	80	12.43 ± 1.19p	10.29 ± 2.11q	4.49 ± 0.99p	NS p	NS p	0.34 ± 0.20p	0.21 ± 0.16p
		III	80	13.11 ± 1.31q	4.25 ± 2.64r	5.64 ± 1.10q	NS p	0.41 ± 0.31q	0.76 ± 0.67q	0.95 ± 0.91q
		IV	80	13.16 ± 1.37q	2.89 ± 1.41s	5.66 ± 1.13q	0.27 ± 0.59q	0.63 ± 0.39r	0.78 ± 0.52q	1.46 ± 1.09r
light grown	no nutrients	I	100	12.18 ± 1.28t	13.01 ± 2.14t	4.09 ± 0.88t	NS t	NS t	0.27 ± 0.15t	0.21 ± 0.16t
		II	100	11.78 ± 1.32t	8.33 ± 1.78u	3.81 ± 1.08t	NS t	NS t	0.27 ± 0.14t	0.22 ± 0.13t
		III	97	12.76 ± 1.14u	3.59 ± 2.38v	5.26 ± 1.08u	0.25 ± 1.14t	0.45 ± 0.46u	0.65 ± 0.57u	1.10 ± 1.21u
		IV	64	13.78 ± 1.44v	1.76 ± 1.58w	5.74 ± 1.40u	0.59 ± 0.63u	1.25 ± 0.92v	1.35 ± 0.85v	2.70 ± 1.86v
	with nutrients	I	100	12.18 ± 1.28x	13.01 ± 2.14x	4.09 ± 0.88x	NS x	NS x	0.27 ± 0.15x	0.21 ± 0.16x
		II	80	12.40 ± 1.37x	9.89 ± 2.12y	4.44 ± 0.92x	0.32 ± 0.70y	NS x	0.35 ± 0.31xy	0.24 ± 0.16x
		III	80	13.07 ± 1.46y	6.93 ± 2.58z	5.36 ± 1.28y	0.33 ± 0.68y	0.25 ± 0.15 y	0.42 ± 0.22y	0.67 ± 0.54y
		IV		No globoids detected						

Notes:
- N = number of spectra collected
- NS = mean P/B ratiois not greater than cut-off limit
- Stage I data is replicated in each treatment block to facilitate comparisons.
- Values in the same column followed by the same letter are not significantly different at $P > 0.05$. Only values from the same set of growth conditions were compared.

Table 3.2b Mean (±SD) peak-to-background ratios derived from EDX analysis of globoids from the cotlyedons of *C.andreana* seeds and seedlings at various stages of growth, grown under different conditions of light and mineral nutrients.

Light	Mineral	Stage	N	P	K	Mg	Ca	Mn	Fe	Zn
dark grown	no nutrients	I	80	13.01 ± 1.30 a	10.14 ± 2.70 a	5.08 ± 1.09 a	1.13 ± 0.78 a	NS	0.31 ± 0.17 a	0.24 ± 0.18 a
		II	80	12.80 ± 1.90 a	2.26 ± 2.68 b	5.60 ± 1.32 b	1.39 ± 1.23ab	NS	0.33 ± 0.26 a	0.30 ± 0.30 a
		III	80	13.23 ± 1.48 a	2.50 ± 1.94 c	7.08 ± 1.03 c	1.92 ± 1.34 b	NS	0.49 ± 0.35 b	0.57 ± 0.57 b
		IV	80	13.04 ± 1.38 a	1.40 ± 1.11 d	6.54 ± 1.10 d	3.16 ± 2.38 c	NS	0.43 ± 0.35ab	0.51 ± 0.36 b
	with nutrients	I	80	13.01 ± 1.30pq	10.14 ± 2.70 p	5.08 ± 1.09 p	1.13 ± 0.78 p	NS	0.31 ± 0.17 p	0.24 ± 0.18 p
		II	80	12.64 ± 1.22 p	4.99 ± 2.57 q	5.70 ± 1.07 q	1.60 ± 1.17pq	NS	0.34 ± 0.16 p	0.22 ± 0.13 p
		III	80	12.66 ± 1.25 p	2.08 ± 1.46 r	6.39 ± 0.91 r	2.23 ± 1.71 q	NS	0.44 ± 0.25 q	0.40 ± 0.21 q
		IV	80	13.31 ± 1.13 q	1.30 ± 1.19 r	7.06 ± 0.76 s	4.58 ± 2.20 r	NS	0.43 ± 0.19 q	0.73 ± 0.54 r
light grown	no nutrients	I	80	13.01 ± 1.30 t	10.14 ± 2.70 t	5.08 ± 1.09 t	1.13 ± 0.78 t	NS	0.31 ± 0.17tu	0.24 ± 0.18 t
		II	80	12.07 ± 1.88 u	4.56 ± 2.87 u	5.71 ± 1.37 u	1.48 ± 1.06 t	NS	0.25 ± 0.16 t	0.31 ± 0.23tu
		III	80	13.95 ± 1.23 v	1.40 ± 1.22 v	7.36 ± 0.96 v	2.67 ± 1.84 u	NS	0.40 ± 0.30uv	0.49 ± 0.67uv
		IV	80	13.47 ± 1.24vt	0.39 ± 0.38 w	6.97 ± 1.27 v	6.53 ± 3.65 v	NS	0.49 ± 0.38 v	0.55 ± 0.55 v
	with nutrients	I	80	13.01 ± 1.30 x	10.14 ± 2.70 x	5.08 ± 1.09 x	1.13 ± 0.78 x	NS	0.31 ± 0.17 x	0.24 ± 0.18 x
		II	80	12.71 ± 1.36 x	5.83 ± 3.10 y	5.59 ± 0.88 y	1.03 ± 0.91 x	NS	0.27 ± 0.16 x	0.27 ± 0.17 x
		III	80	12.90 ± 1.28 x	2.05 ± 2.21 z	6.45 ± 0.99 z	3.16 ± 2.54 y	NS	0.49 ± 0.41 y	0.37 ± 0.25 y
		IV		No globoids detected						

Notes:
- N = number of spectra collected.
- NS = mean P/B ratio is not greater than the cut-off limit.
- Stage I data is replicated in each treatment block to facilitate comparisons.
- Values in the same column followed by the same letter are not significantly different at $P > 0.05$. Only values from the same set of growth conditions were compared.

Table 3.3 Mean (±SD) P/B ratios derived from EDX analysis of globoids from the cotyledons of dry *C. maxima* seeds (Stage I) prepared with and without freeze drying.

	N	P	K	Mg	Ca	Mn	Fe	Zn
not freeze-dried	50	12.05 ± 1.44	13.10 ± 1.92	4.10 ± 0.84	0.07 ± 0.25	0.10 ± 0.13	0.24 ± 0.14	0.23 ± 0.17
freeze-dried	50	12.32 ± 1.10	12.93 ± 2.35	4.07 ± 0.93	0.09 ± 0.26	0.08 ± 0.13	0.30 ± 0.16	0.20 ± 0.15

Note: Values in the same column are not significantly different at $P > 0.05$.

(Fig.3.4b). Statistically significant drops in K P/B usually occurred at each stage (Tables 3.2a and b).

Magnesium: The Mg P/B increased over time, with significant increases noted in all cases by Stage III (Tables 3.2a and b). The increase was of approximately the same magnitude for both species (Fig.3.4c).

Calcium: In the dry seed stage, there was significantly more Ca present in *C. andreana* globoids than in *C. maxima* globoids, and the extent of change over time was different in the two species (Fig.3.4d). In *C.andreana* there was a very definite increase in Ca P/B over time, whereas the increase in *C.maxima* was much less notable. In *C.maxima* the increase was from no Ca, to a small, but significant peak. In *C. andreana*, the Ca P/B increased over time, with significant increases occurring by Stage III and then again by Stage IV (Table 3.2b).

Manganese: In both species, the mean Mn P/B was below the cut-off limit in Stages I and II (Fig.3.4e). In *C.andreana*, although Mn was detected in a small percentage of the globoids analyzed, the mean P/B remained below the cut-off limit throughout the course of the study. In *C.maxima* there was an increase in Mn over the study period; Mn increased significantly by Stage III and again by Stage IV (Table 3.2a).

Iron: The mean Fe P/B ratio for Stage I was not significantly different in the two species. In both species, the Fe P/B ratio increased over the study period, showing a significant increase in all cases by Stage III (Tables 3.2a and b). The total increase from Stage I to Stage IV was much greater in *C. maxima*, than in *C. andreana* (Fig.3.4f).

Zinc: Stage I globoids from the two species, contained similar levels of Zn. Over time, the Zn P/B increased in both species (Fig.3.4g). The overall Zn increase in *C. maxima*

Figure 3.4 Changes in mean P/B ratios derived from EDX analysis of globoids from the cotyledons of *C. maxima* and *C. andreana* seeds and seedlings germinated for varying times under different conditions of light and mineral nutrients.

The light/with nutrients plot ends at Stage III because there were no globoids present in the Stage IV light/with nutrients samples. The dotted horizontal lines represent the detectable cut off limit for each trace element.

Changes in mean P/B ratios for:

a) phosphorus.

b) potassium.

c) magnesium.

d) calcium.

e) manganese.

f) iron.

g) zinc.

Figure 3.4a: Phosphorus peak-to-background ratios.

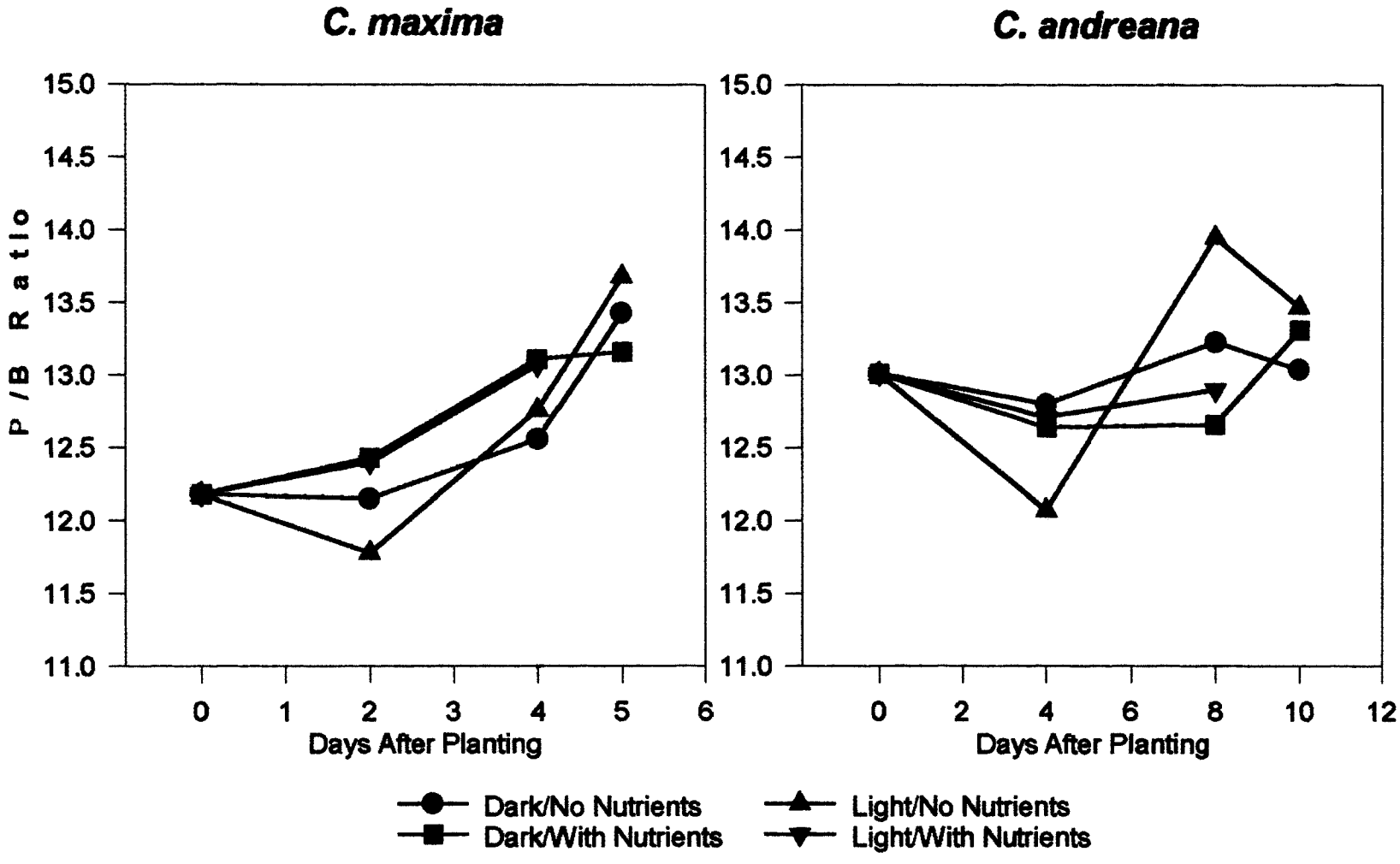


Figure 3.4b: Potassium peak-to-background ratios.

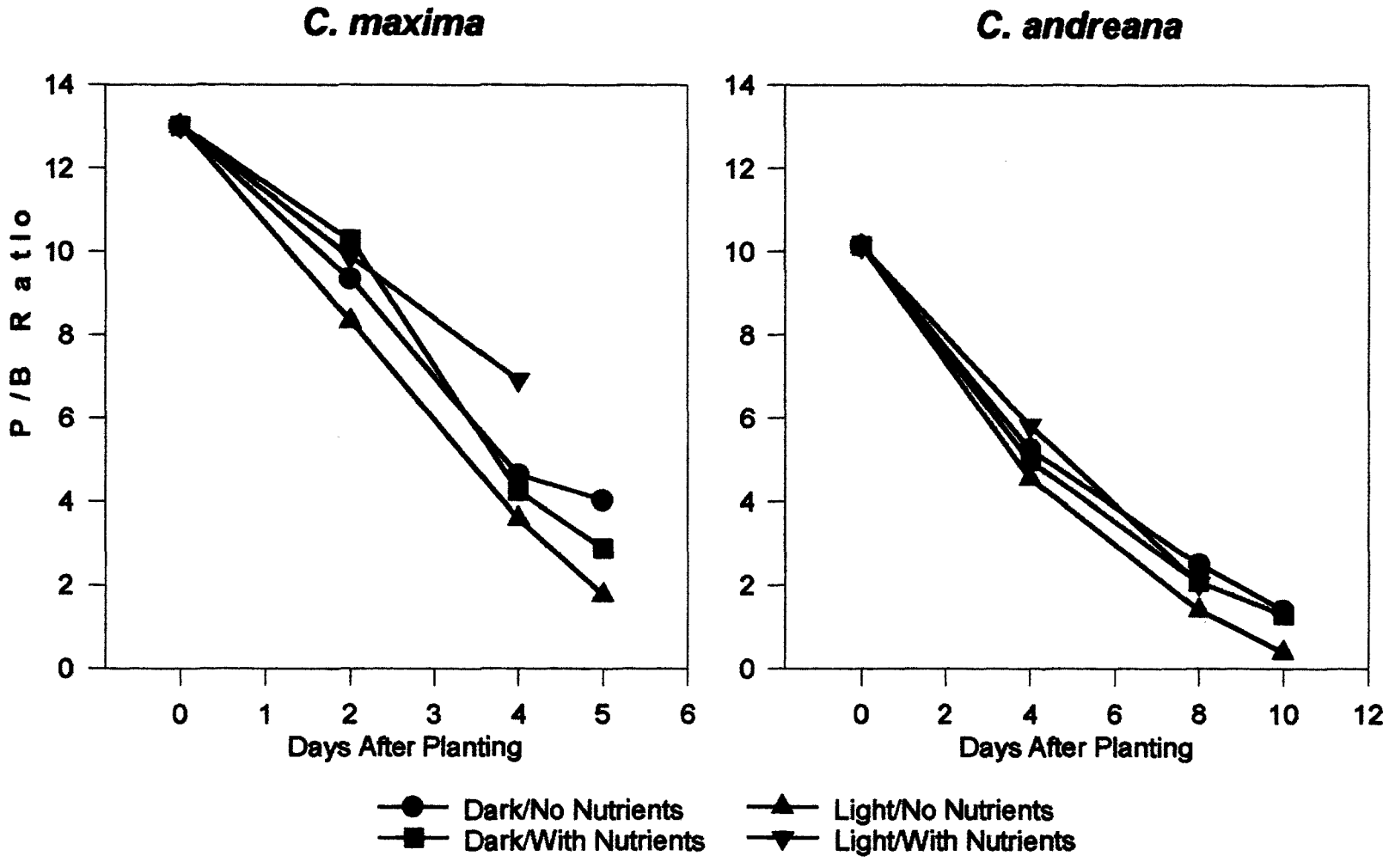


Figure 3.4c: Magnesium peak-to-background ratios.

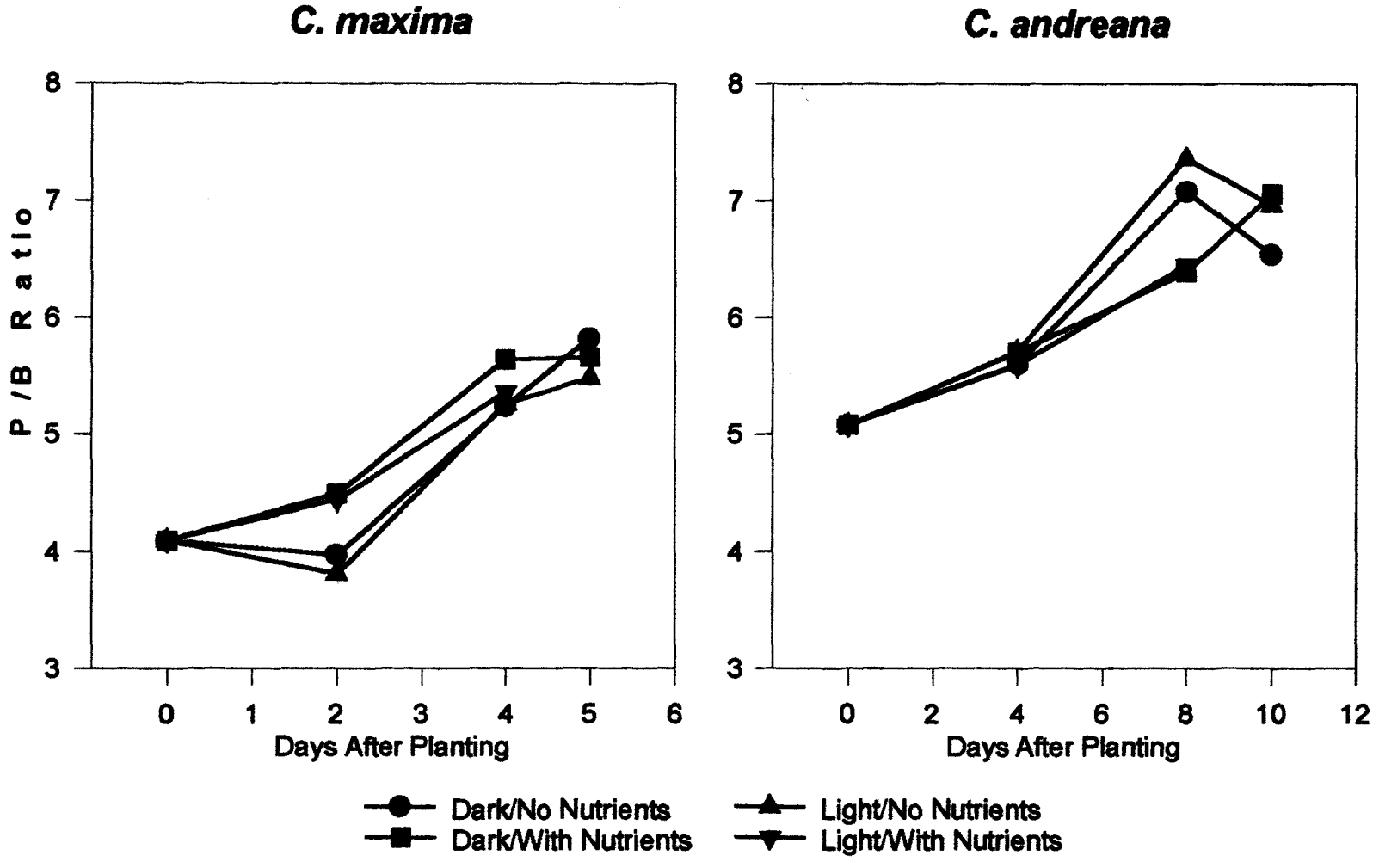


Figure 3.4d: Calcium peak-to-background ratios.

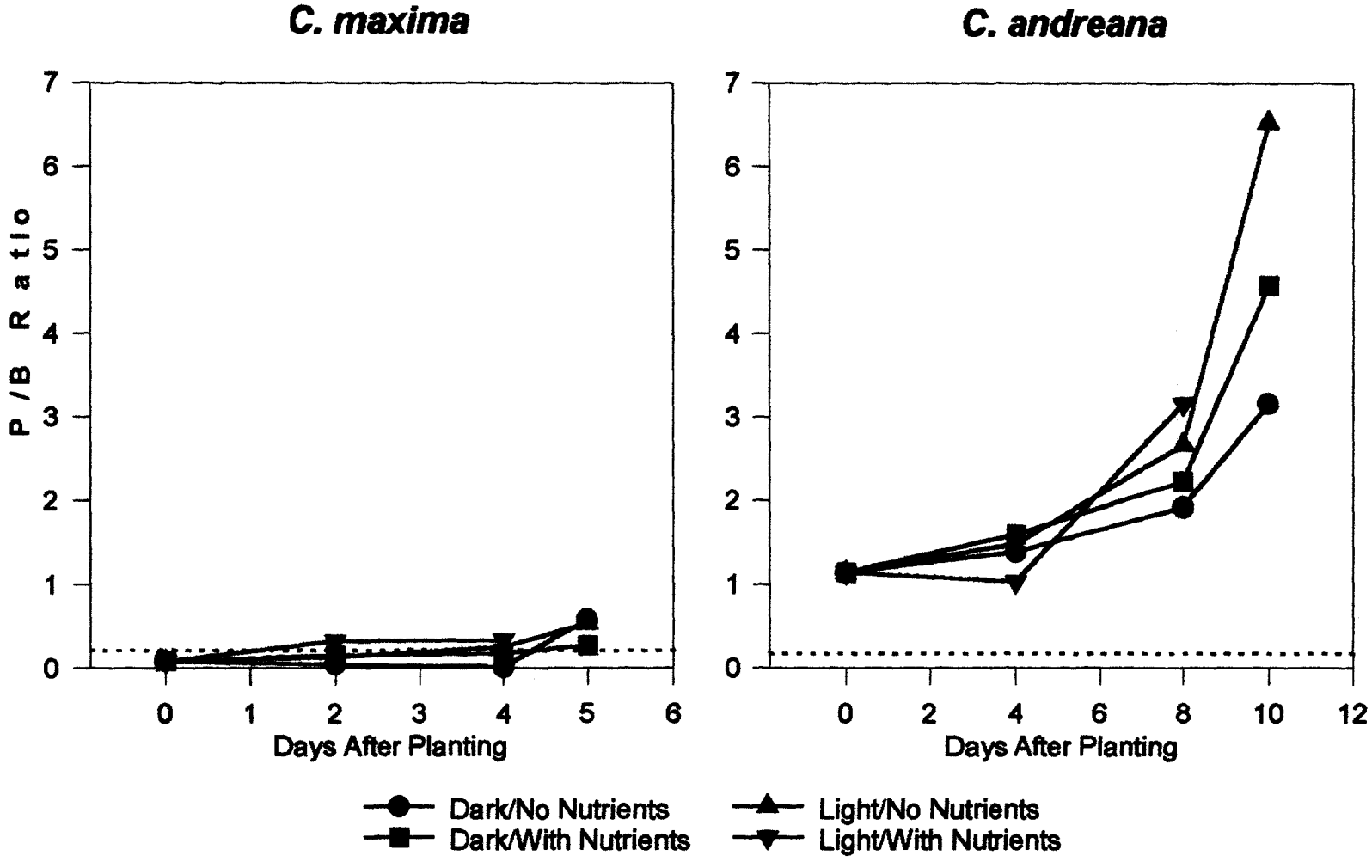


Figure 3.4e: Manganese peak-to-background ratios.

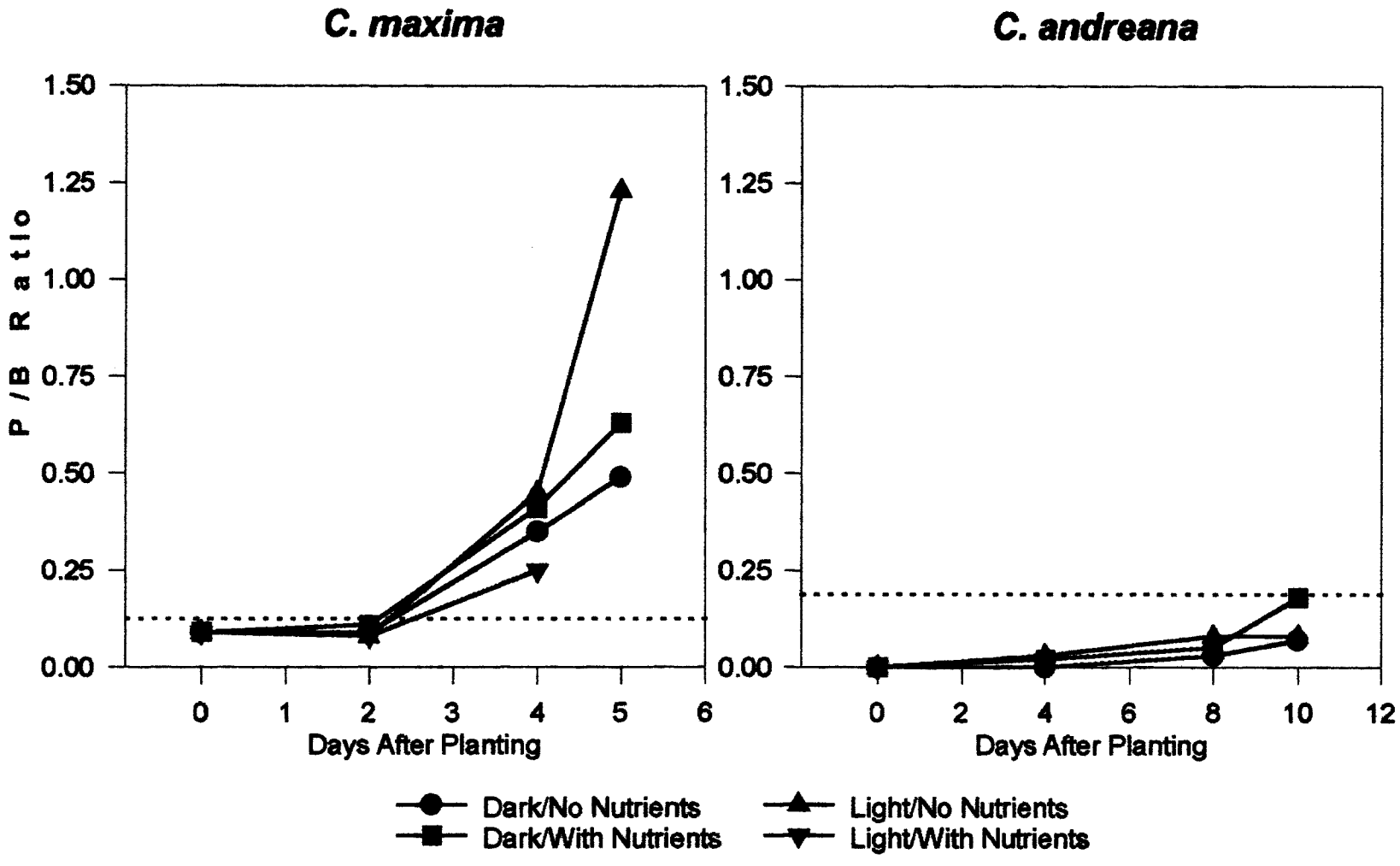


Figure 3.4f: Iron peak-to-background ratios.

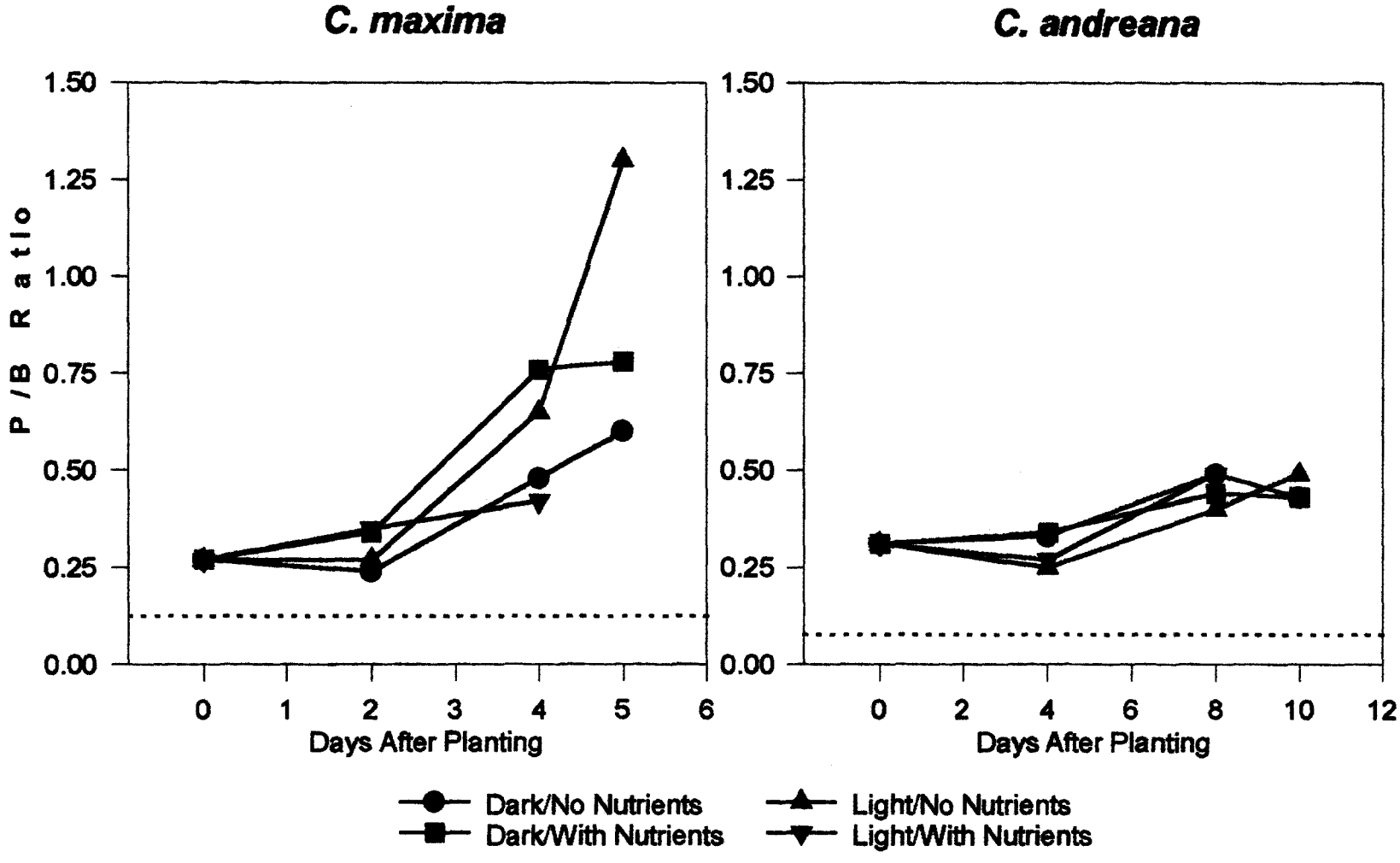
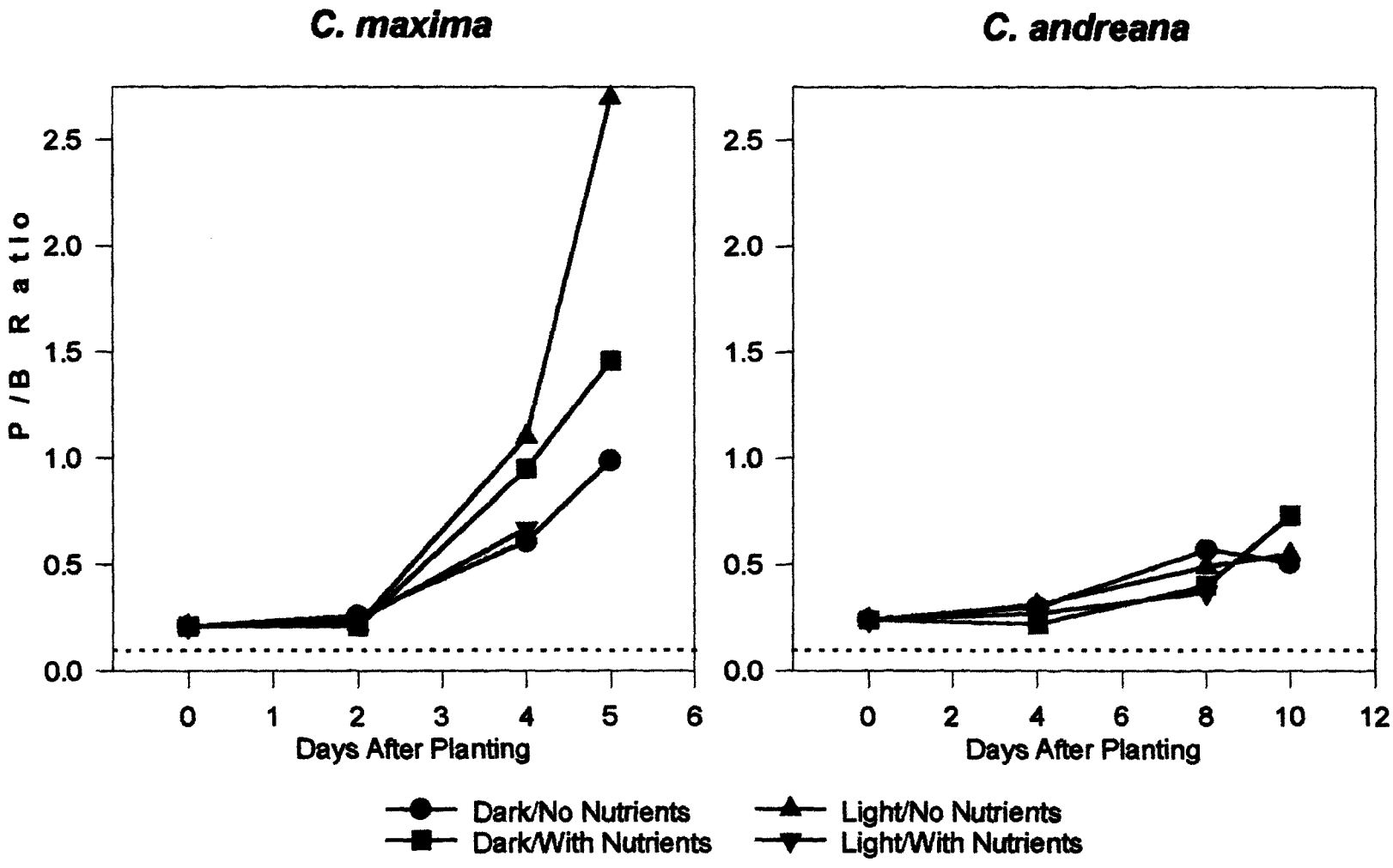


Figure 3.4g: Zinc peak-to-background ratios.



was considerably greater than that observed in *C. andreana*.

Occurrence of Trace Elements:

In order to determine if the increase in the P/B ratios of the trace elements was due in part to an increase in the number of globoids with detectable levels of an element, the percentage of globoids containing each trace element was calculated for each treatment (Table 3.4a and b). A globoid was determined to contain levels of an element if the P/B for that element was greater than the cut-off limit. For example a spectrum with a Ca P/B of 0.09 would be counted as not having Ca since it was below the cut-off limit.

Calcium: In *C. maxima* the percentage of globoids containing Ca was highly variable, between treatments but it always showed an overall increase from Stage I to Stage IV. The no nutrients treatments, which showed a greater increase in mean Ca P/B (Table 3.2a) , also showed a larger increase in the percentage of globoids which had Ca. In *C. andreana* 100% of the globoids analyzed at Stage I contained Ca.

Manganese: In *C. maxima*, the percentage of globoids with Mn increased from 30% at Stage I to at least 92% at Stage IV. In *C. andreana*, only 7.5% of the Stage I globoids analyzed contained Mn. This value increased over time, to a maximum of 35% in the Stage IV, light/with nutrients samples. The Mn P/B values were very low and the mean P/B for all treatments was below the cut-off limit.

Iron: In both species, a high percentage of Stage I globoids (89-95%) contained Fe, and the percentage remained high throughout all stages.

Zinc: In *C. maxima*, the percentage of globoids which had Zn showed an increase

Table 3.4a Percentage of globoids analyzed from the cotyledons of *C. maxima* seeds and seedlings which contained Ca, Mn, Fe and Zn.

Light	Mineral Nutrients	Stage	N	Ca	Mn	Fe	Zn
dark grown	no nutrients	I	100	21	30	89	74
		II	100	10	35	91	86
		III	100	4	87	98	96
		IV	100	68	92	97	92
	with nutrients	I	100	21	30	89	74
		II	80	26.2	38.8	95	76.2
		III	80	25	81.2	100	98.8
		IV	80	28.8	97.5	97.5	98.8
light grown	no nutrients	I	100	21	30	89	74
		II	100	23	24	96	84
		III	97	15.5	93.8	96.9	96.9
		IV	64	64	96.9	100	100
	with nutrients	I	100	21	30	89	74
		II	80	33.8	25	88.8	82.5
		III	80	36.2	80	97.5	97.5
		IV		No globoids detected.			

Notes: • Stage I data is replicated in each treatment block to facilitate comparisons.
• A globoid contained an element if the mean P/B for that element was greater than the cut-off limit. Cut-off limits were set to take into account slight variations about "0" caused by the background modelling.

Table 3.4b Percentage of globoids analyzed from the cotyledons of *C. andreana* seeds and seedlings which contained Ca, Mn, Fe and Zn.

Light	Mineral Nutrients	Stage	N	Ca	Mn	Fe	Zn
dark grown	no nutrients	I	80	100	7.5	95	85
		II	80	96.2	8.8	95	85
		III	80	95	12.5	95	97.5
		IV	80	90	13.8	96.2	100
	with nutrients	I	80	100	7.5	95	85
		II	80	97.5	6.2	100	86.2
		III	80	93.8	10	98.8	95
		IV	80	96.2	35	98.8	98.8
light grown	no nutrients	I	80	100	7.5	95	85
		II	80	97.5	7.5	95	91.2
		III	80	96.2	17.5	98.8	98.8
		IV	80	100	22.5	100	98.8
	with nutrients	I	80	100	7.5	95	85
		II	80	92.5	3.8	97.5	88.8
		III	80	95	6.2	98.8	93.8
		IV		No globoids detected.			

Note: • Stage I data is replicated in each block/condition to facilitate comparisons
• A globoid contained an element if the mean P/B for that element was greater than the cut-off limit.

from 74% at Stage I to around 100% at Stage IV. The change was approximately the same for all growth conditions. In *C. andreana*, the percentage of globoids containing Zn increased from 85% at Stage I to as high as 100% at Stage IV.

INFLUENCE OF LIGHT AND MINERAL NUTRIENT CONDITIONS:

Multifactor ANOVA's were carried out to determine whether the conditions of light and mineral nutrients had any influence on the changes in globoid composition. Separate analyses were done for each species. That there were no globoids present in the Stage IV, light/with nutrients samples made it impossible to do a complete analysis of all the P/B data collected. The most complete analysis possible was a four-way ANOVA on all of the P/B data for Stages I, II and III, assessing the effects of the stage of growth, light conditions and mineral nutrient conditions (Table 3.5a and b). This analysis also tested for seed-to-seed variation within a treatment. Three-way ANOVA's were also carried out on various portions of the P/B data to examine the effect of individual factors under limited conditions (Appendix C).

From these analyses it was found that the stage of development and mineral nutrient conditions were significant sources of variation in the P/B ratios of some elements. It was also found that various interactions between the experimental factors influenced globoid composition. The results showed that, for most elements, there was significant seed-to-seed variation within most treatments (Appendix B).

Stage of growth had a significant effect on all of the elements analyzed, except for P

in *C. andreana*. The apparent discrepancy of this statment with Table 3.5b is due to the limited scope of the analysis summarized in Table 3.5b. Table 3.5b reported the result of an analysis of data from Stages I, II and III only. There was a large fluctuation in the mean P P/B ratio between Stage II and III in the light/no nutrients samples, and this caused Stage to appear to be significant in Table 3.5b. But, the overall change from Stage I to Stage IV, as reported in Table3.2b, was not significant for any growth conditions. The effect of the stage of growth has already been examined as the change in globoid composition over time. Over the study period, light conditions alone did not have a significant influence on the P/B ratios for any of the elements. Mineral nutrient conditions often affected elements which were present in the globoids in high amounts; specifically P, K and Mg. Up to Stage III, P, K and Mg in *C. maxima* were generally higher in the with nutrients samples and in *C. andreana* Mg was lower in the with nutrients samples. There was no clear trend to the effect of the various interactions between stage, light conditions and mineral nutrient conditions. For the two species, different interactions were significant sources of variation in the P/B ratios of various elements.

Table 3.5a The effect of stage, light conditions and mineral nutrient conditions on the elemental composition of globoids from *C. maxima* cotyledons as determined using a multifactor analysis-of-variance. The significance of interactions between the three factors was also assessed.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	NA	SE	SE	SE
Light Conditions (LC)				NA			
Mineral Nutrient Conditions (MN)	SE	SE	SE	NA			
S * LC		SE		NA			
S * MN		SE		NA			
LC * MN		SE		NA	SE	SE	
S * LC * MN				NA	SE	SE	SE

Notes:
- This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from Stages I, II and III. Stage IV data was not included in this analysis due to the absence of globoids in the Stage IV light/with nutrients samples. Variability between seeds in the same treatment was also considered.
- SE = the factor or interaction had a significant effect on the mean P/B ratio for that element at $P \leq 0.05$.
- NA indicates that the mean P/B ratios were too low to allow for accurate analysis of any effects.
- _ * _ designates the interaction between two factors such as stage and light conditions (S * LC).

Table 3.5b The effect of stage, light conditions and mineral nutrient conditions on the elemental composition of globoids from *C. andreana* cotyledons as determined using a multifactor analysis-of-variance. The significance of interactions between the three factors was also assessed.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	SE	NA	SE	SE
Light Conditions (LC)					NA		
Mineral Nutrient Conditions (MN)			SE		NA		SE
S * LC				SE	NA		
S * MN	SE		SE		NA		
LC * MN					NA		
S * LC * MN					NA		

Notes:
- This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from Stages I, II and III. Stage IV data was not included in this analysis due to the absence of globoids in the Stage IV light/with nutrients samples. Variability between seeds in the same treatment was also considered.
- SE = the factor or interaction had a significant effect on the mean P/B ratio for that element at $P \leq 0.05$.
- NA indicates that the mean P/B ratios were too low to allow for accurate analysis of any effects.
- _ * _ designates the interaction between two factors such as stage and light conditions (S * LC).

CHAPTER 4: DISCUSSION

MORPHOLOGICAL AND SUBCELLULAR CHANGES

As germination and seedling growth progressed the influence of light on seedling morphology became apparent. Light grown seedlings developed large, fully expanded, photosynthetic cotyledons and the etiolated seedlings had less expanded, yellowish cotyledons and long, spindly hypocotyls. Although *C. andreana* seedlings were much smaller than those of *C. maxima*, the percent increase in mean cotyledon length from Stage I to Stage IV was the same for seedlings of both species grown under similar conditions. The relative increase in mean hypocotyl length from Stage III to Stage IV was greater in *C. andreana* than in *C. maxima*. Similar differences in growth patterns have previously been noted for these two species (Ockenden and Lott, 1988d).

Mineral nutrient conditions did not significantly influence cotyledon expansion, except in the Stage IV, light grown samples where the with nutrients cotyledons were significantly longer than the no nutrients cotyledons. This suggests that the mineral nutrients stored in the seed were sufficient to meet the needs of the growing seedling until it became well established. Between Stages III and IV, the cotyledons of the light/with nutrients seedlings expanded more rapidly than did the cotyledons of the light/no nutrients seedlings. The stored reserves in the light/with nutrients samples were also more extensively mobilized than they were under any other set of growth conditions, as indicated by the absence of globoids from the Stage IV light/with nutrients samples.

In cucurbits, as early seedling growth progresses, the protein bodies fuse together, forming large aqueous vacuoles containing irregular shaped pieces of protein and globoids, which are eventually degraded (Hara and Matsubara, 1980; Lott and Vollmer, 1973a). The observations made in this study were in accordance with this sequence of events. In the dry seed, globoids were found inside protein bodies, and as seedling growth progressed, this surrounding protein was no longer apparent. It was impossible to determine in crushed tissue where the globoids were in the cell, and what had happened to the surrounding protein. From the sequence of events described in earlier works (Hara and Matsubara, 1980; Lott and Vollmer, 1973a) the following is a probable explanation of the observed changes. First, the proteinaceous region became less dense as proteinases began hydrolysing the storage protein, making it more likely that the globoids would be separated from the protein when the tissue was freeze-dried and then crushed. As seedling growth continued, the proteinaceous region was broken down into discrete particles randomly scattered throughout the vacuole. The remaining globoids, no longer surrounded by protein, were also scattered in the growing vacuole. As seedling growth continued, the globoids were completely digested, as indicated by their absence from the Stage IV, light/with nutrients samples.

As seedling growth progressed, it became increasingly difficult to locate globoids in the crushed samples, especially in those from light grown seedlings. A combination of two factors was probably responsible for this. First, as germination progressed, it was very possible that some globoids were degraded earlier than others, leaving fewer globoids in each cotyledon at each successive stage. Secondly, although the expansion of cotyledons was due to both cell division and cell expansion, expansion of the spongy mesophyll region was the

result of cell expansion only. Spongy mesophyll cells do not divide during cotyledon expansion (Nelson, 1932). The same volume of spongy mesophyll would then contain fewer cells, and correspondingly fewer globoids, as the cotyledons expanded. For each species, the size of the tissue sample was kept approximately the same for all stages, therefore, later stage samples would have included a lower percentage of the total cells in the cotyledon. This also explains, at least in part, why globoids were more difficult to locate in light grown samples than in etiolated samples at the later stages, since the light grown cotyledons expanded much more than the etiolated cotyledons.

CHANGES IN GLOBOID COMPOSITION

Stage I globoids:

Cotyledonary globoids from the dry seeds (Stage I) of both *C. maxima* and *C. andreana* contained high levels of P, K and Mg; *C. andreana* globoids also regularly contained Ca. These findings were in agreement with earlier EDX analysis studies on the element composition of cucurbit globoids (Ockenden and Lott, 1990; Lott *et al.*, 1979; Lott and Vollmer, 1979). Globoids of the two species contained roughly equal concentrations of P as measured using P/B ratios. *C. maxima* globoids had a slightly higher mean K P/B and a slightly lower mean Mg P/B than *C. andreana* globoids. Globoids from the two species also contained trace amounts of Fe and Zn and very occasionally Mn. *C. andreana* globoids were almost 5 times as likely as *C. maxima* globoids to contain Ca, and the mean Ca P/B ratio for those globoids which contained Ca was much higher in *C. andreana* than in *C. maxima*

(1.13 vs. 0.45). Although previous studies mentioned the appearance of trace amounts of Fe, Zn and Mn in the globoids of some species, there are no published reports of the P/B ratios for these elements in *Cucurbita* embryos. Globoids from *C. maxima* were 4 times more likely to contain Mn than those from *C. andreana*, but in both species, the proportion of globoids containing Mn was small and the level of Mn in each globoid was very low. In both species the mean Mn P/B ratio was below the cut-off limit. Approximately equal percentages of *C. maxima* and *C. andreana* globoids contained Fe (≈90%) and Zn (≈80%) and the mean P/B for each element was approximately equal in the two species. The standard deviations for the mean P/B ratio for each seed, and for the overall mean P/B ratio for each element were high, indicating a great deal of variability in globoid composition both within a seed, and between different seeds of the same species. This high variability has been previously reported in EDX analysis studies of globoid composition, and it has been shown that although globoids from the same cell have very similar element composition, there can even be a large difference even between globoids from adjacent cells (Lott, 1984).

Change over time:

The element composition of globoids in *C. maxima* and *C. andreana* cotyledons changed significantly during early seedling growth. In both species, the general trends were the same regardless of growth conditions: P remained relatively constant, K decreased markedly and Mg, Ca, Mn, Fe and Zn generally increased. These changes were comparable to those which occurred in the cotyledons and endosperm of germinating castor beans, where K decreased while Ca, Fe and Zn increased (Lott *et al.*, unpublished). Although the general

trends were the same, there were distinct species-to-species differences in the extent to which some elements changed. P increased slightly in *C. maxima*, but not in *C. andreana*. Ca, which was initially present in *C. andreana* globoids in much higher levels than in *C. maxima* globoids, increased to a much greater extent in *C. andreana* than in *C. maxima*. Mn, Fe and Zn, which were present in globoids from Stage I globoids in roughly equal levels in the two species, increased much more in *C. maxima* than in *C. andreana*. The observed changes were essentially the reverse of the changes in globoid composition which occurred during seed development in rice and castor bean. In those two species, globoids contained mainly P and Mg, lower levels of Ca and Zn and little, if any, K, early in seed development, whereas globoids in mature seeds contained mainly P, K and Mg, and little or none of the other elements (Lott, 1984).

It appears that as the protein bodies began to fuse, and the storage proteins were broken down, the K^+ ions, which were bound to the phytate in the dry seed, came off as the environment became more aqueous and were replaced by cations of other elements including Mg, Ca, Mn, Fe and Zn. K-phytate is readily water soluble whereas phytate salts of di- and trivalent ions are less soluble (Crean and Haisman, 1963; Brown *et al.*, 1961). The more aqueous the vacuole became, the more K^+ came off of the phytate and the more binding sites became available for other cations to occupy. Once bound to the phytic acid, di- and trivalent cations would be less likely to come back off, and this would have resulted in the increasing P/B levels of these elements in the globoids. Changes in globoid composition were most likely strictly the result of increasing water potential in the protein body/vacuole. Cation binding is also a function of pH. Changes in pH may have occurred as the vacuole became

more aqueous. How,or if, the pH of the vacuole was altered during early seedling growth, and how this may have influenced the observed changes in globoid composition is unknown.

As the phytate became increasingly interlinked by di- and trivalent cations, its solubility would go down. There were possible benefits to maintaining at least some of the globoids as growth progressed. As long as globoids were present, the seedling had a continued reserve of mineral nutrients. Additionally, Fe, Mn, and Zn can be toxic to the seedling when present in too high a concentration (Mengel and Kirkby, 1982). Binding these elements to phytic acid may have provided a safe means of storing them until they were required for growth. Just what purpose was served by sequestering these elements in a relatively inaccessible form until the seedling was well established is not clear. An indication of what proportion of the mineral nutrients were still bound to globoids at each stage would give some idea of just how important this sequestering was.

The exchange of cations was almost certainly a direct result of the increasingly aqueous nature of the globoids' environment, and not due to the action of phytase. Some phytase activity might, however, have resulted in a change in the arrangement of phytate molecules in the globoid such that more cation binding sites were accessible. This would help to account for the continuing exchange of cations as seedling growth progressed. Since the arrangement of phytin molecules in the globoids of dry seeds has not been elucidated, any inferences about changes to this arrangement are tentative at best. The phosphorus P/B ratio remained relatively constant, indicating that the phytate structure of the globoid was not being greatly altered or degraded. The slight increase over time in the mean P P/B in *C. maxima* which was also observed in castor beans grown in de-ionized water (Lott *et al.*, unpublished),

is not readily explainable. It would be remarkable if phytate became more concentrated during seedling growth when reserves were being broken down.

If one accepts the idea that the changes in globoid element composition occurred as K ions were exchanged for other metal cations under aqueous conditions, two important questions remain: Where did the K go? and Where did the other cations come from? Previous work showed that, in *C. maxima* and *C. andreana*, there was little mobilization of K out of the cotyledons during germination and early seedling growth (Ockenden and Lott, 1988d). This suggests that, although some of the released K may have been transported to the axis, most remained within the cotyledons. In the cotyledons, there are a variety of places that the K could have gone. Some undoubtedly remained in the vacuole since K is essential for turgor regulation and maintaining osmotic potential (Glass, 1989). K is also involved in a wide variety of enzyme reactions, in almost every part of the cell (Glass, 1989), and some K may have moved out of the vacuole and into the cytoplasm and various organelles including chloroplasts and mitochondria. K is also important in regulating stomatal opening, and some K may have been moved out of the spongy mesophyll cells to the guard cells developing in the epidermis. The wide ranging functions of K in plant cells suggest that in the cotyledons, the liberated K may remain in the vacuole, may be moved to other parts of the cell, and may be moved between cells or even between tissues.

There were several possible sources of Mg, Ca, Mn, Fe and Zn: they could have come from the degradation of proteins and globoids from different protein bodies in the same cell; they could have moved into the developing vacuole from other parts of the same cell; alternatively, they could have been transported or moved passively in from other cells or other

tissues. Evidence suggests that most of the minerals in a dry seed are stored in the globoid (Ockenden and Lott, 1990), and that little, if any Mn, Fe, Zn or Mg are found in the proteinaceous region of *Cucurbita* protein bodies (Lott, 1975); essentially ruling out the storage proteins as an important source of these elements. Globoids from different protein bodies in the same cell were possibly broken down at different rates, with some globoids being completely degraded by phytase before the protein bodies fused. The cations liberated by the degradation of those globoids would then be free in the vacuolar solution, available for binding to phytic acid in intact globoids. However, most of the elements that increased in the globoids were present in mesophyll cell globoids only in trace amounts, whereas the changes in globoid composition represented fairly sizable increases in these elements. It seems likely, then, that some of the cations were transported into the developing vacuole.

Using freeze-dried powders there was no way to determine where these elements came from so any postulation on their origin is only tentative. Elements could have been carried into the vacuole from other parts of the seed in the imbibitional water; soluble compounds in the seed could have been dissolved as the water moved into the cells and subsequently into the developing vacuole. But had this been the case, there would have been a large change in globoid composition between Stages I and II, when imbibition was taking place, and then little change thereafter. Another possibility is that the cations could have arisen from mobilization of reserves in other tissues in the cotyledon. In cucurbits, proteins are mobilized first from cells closer to the vascular bundles and epidermis (Davies and Chapman, 1981; Hara and Matsubara, 1980; Nelson, 1932) and globoid digestion is thought to occur at the same time as protein degradation (Hara and Matsubara, 1980; Lott and

Vollmer, 1973a). The elements liberated by the complete degradation of globoids in some cotyledon cells earlier than in others would have provided one possible source of cations, since there was very little net mobilization of mineral nutrients out of the cotyledons during seedling growth (Ockenden and Lott 1988d). Ca distribution has been examined in several cucurbits, and in species where there was differential distribution of Ca, it was more commonly found in the epidermal and provascular regions (Lott *et al.*, 1979; Lott and Vollmer, 1979). In other species, globoids from areas where reserve mobilization occurred first, often had higher concentrations of trace elements such as Ca, Mn and Fe than did globoids in the mesophyll cells (Lott, 1984). The distribution of Mn, Fe and Zn in *Cucurbita* globoids has not been reported. Perhaps the techniques used in past studies were not sensitive enough to detect the small amounts of these elements present in the globoids.

The species-to-species differences in globoid composition changes were explicable, at least in part, based on the difference in the Ca content of their embryos. At pH 7, Mn, Fe and Zn all have a higher affinity for phytic acid than does Ca, but the solubilities of different phytic acid salts depends on the concentration of the elements present as well as on pH conditions (Xu *et al.*, 1992; Nolan and Duffin, 1987; Graf, 1986). In *C. maxima* there was a relatively low amount of Ca present in the embryo compared to *C. andreana* (Ockenden and Lott, 1988a). Therefore, in *C. maxima* as the K ions came off, the available binding sites were more likely to be filled by the cations with the higher affinity for phytic acid, namely Mn, Fe, and Zn. In *C. andreana*, Ca was present in far higher concentrations than Mn, Fe, and Zn, and would have out-competed these others for binding sites on the phytic acid. In both species, Mg increased to approximately the same extent. Mg was present in mature embryos

in far greater concentrations than Ca, Mn, Fe, and Zn (Ockenden and Lott, 1988a), and thus would have bound to phytic acid in the same proportions in the two species, regardless of the Ca concentration.

Increases in the mean P/B of trace elements was usually due to an increase in the amount of an element in each globoid. In both species, Fe and Zn increased substantially between Stage I and Stage IV. These elements were present in a high percentage of the Stage I globoids, therefore the increase in mean P/B had to be the result of an increase in the amount of Fe and Zn in each globoid rather than an increase in the proportion of globoids containing these elements. The same holds true for Ca in *C. andreana*. In *C. maxima*, the small, but significant, increase in the mean Ca P/B was the result of both an increase in the percentage of globoids which contained Ca, as well as an increase in the level of Ca in each Ca-containing globoid. The increase in the mean Mn P/B in *C. maxima* globoids was also due to a combination of an increase in the percentage of globoids containing Mn and an increase in the amount of Mn in each globoid with that element.

The observed species differences for the trace elements could not be attributed to differences in the cut-off limits set. Even had a single cut-off limit been set for each element and applied to both species the result would have been the same. In each case the higher cut-off limit would have been selected, since limits were based on the lowest negative P/B value obtained for each species. For Ca, the *C. maxima* cut-off limit would have been selected, and all the *C. andreana* mean Ca P/B values would still have been above the limit. For Mn, the *C. andreana* limit would have been selected, and in *C. maxima* the mean Mn P/B ratios for all the growth conditions would still have increased above the limit by Stage III. For both

species, all mean Fe P/B ratios would still have been above the cut-off limit regardless of which limit was used. For Zn, the same limit was used for both species. Changing the cut-off limits would not have altered any of the mean P/B ratios because the actual P/B values for each globoid were used in all calculations and analyses; values below the cut-off limits were not set equal to zero.

Some of the later stage *C. andreana* globoids from all of the growth conditions produced spectra with small Cl peaks. There was no apparent selective distribution of globoids containing Cl. Cl has been reported in globoids of some species (Lott, 1980), and may have acted as a counter-ion to the di- or trivalent cations which was also bound to a phytate molecule. The function of Cl in plant cells is relatively unclear but it is thought to be involved in maintaining the electrostatic balance as a counter-ion to K (Glass, 1989). Why Cl appeared only in *C. andreana* globoids is unclear.

By Stage II there had not been much change in globoid composition in either species; K had decreased, but there had been little change otherwise. Stage II represented the point at which the radicle protruded through the seed coat, germination had just been completed. Since reserve mobilization is a post-germinative event (Bewley and Black, 1985), it was to be expected that protein bodies were still intact and globoid composition was relatively unchanged at this point. K had probably been lost from the globoids due to its high solubility; the increase in water content in the cell accompanying germination apparently was sufficient to result in some K^+ coming off the phytate molecules. As the protein body was further broken down, and mobilization of reserves continued, other cations became present in the vacuole, available for binding to the phytic acid.

Two areas should be kept in mind as one interprets the data reported here. The first is that only those globoids which remained intact at each stage could be EDX analyzed. Whether the observed changes held true for those which were degraded at a faster rate is unknown. The second is that, using these methods, there was no way of telling if the globoids analyzed had been present in the dry seed, or if they had been newly synthesized during seedling growth. In castor bean it was shown that as seedling growth continued the cotyledons could synthesize new phytate when the initial reserve had been depleted (Organ et al., 1988). While there is no evidence to suggest that cucurbit cotyledons synthesize new phytate, there is no way, using the methods employed in this study, to rule out the possibility that the globoids were made up of new phytate.

Influence of light and mineral nutrient conditions:

It was expected that globoids from light grown samples would have lower levels of Fe and Mg, due to the importance of these elements in photosynthesis, but this was not the case. Up to Stage III, light conditions had no significant influence on the changes in globoid composition. In Stages III and IV, globoids were more difficult to locate in light grown samples than in the corresponding dark grown samples, so perhaps a higher proportion of the globoids had been broken down in the light grown seedlings to provide the additional mineral nutrients required for photosynthesis. It was also possible that there was a difference in globoid composition in light and dark grown samples, but the exposure to light, required for measuring and cutting the samples, was sufficient to alter the physiology of the cells, so that the dark grown samples became equivalent to light grown. This was not very likely since the

exposure time was very short, and the samples were frozen immediately after the exposure; any change in globoid composition would have had to be almost instantaneous. Movement of mineral nutrients out of the cotyledons, to the growing axis was influenced by light conditions. In *C. maxima* and *C. andreana* seedlings grown in distilled water in the light, there was a lower percentage of nutrients moved out of the cotyledons than in etiolated seedlings grown in the same conditions; in the light there was actually some movement of P, K and Mg into the cotyledons (Ockenden and Lott, 1988d). In the light, the cotyledons would have had a larger demand for mineral nutrients, to support increased expansion as well as the transition to photosynthesis. This was not reflected in globoid composition. Movement of mineral nutrients in and out of the cotyledons may have maintained the concentration of mineral nutrients in the vacuoles of the cotyledon cells, regardless of light conditions, resulting in similar globoid composition in both light and dark grown samples.

Mineral nutrient conditions had some influence on the changes in globoid composition. When it had an effect it was usually on elements that were present in the globoids in high amounts. In *C. maxima*, the with nutrients samples tended to have higher mean P/B ratios for P, K and Mg, whereas in *C. andreana*, the with nutrients samples generally had a lower Mg P/B value than the no nutrients samples. These effects were statistically significant when all the P/B data for Stages I, II and III was considered. When these results were compared with the graphs and some of the partial analyses, the effect of mineral nutrients became less clear, and in some cases was even reversed. Given the large standard deviations of all the mean P/B data, it was difficult to say whether these statistically significant effects were actually relevant in terms of their biological significance. These effects were most likely

mediated by movement of mineral nutrients in and out of the cotyledons. In *C. maxima*, P, K, Mg and Ca were all moved into the cotyledons of seedlings grown in mineral nutrient solution (Ockenden and Lott, 1988d), and this could have increased the concentrations of these elements in the vacuoles. There was no similar movement of elements into *C. andreana* cotyledons (Ockenden and Lott, 1988d), and this could help explain the species-to-species difference in effect. In general, the mineral nutrient stores in the dry seed seemed to be more than sufficient to allow for successful seedling establishment.

Various interactions were statistically significant sources of variation for different elements in the two species. The combination of growth in the light, with mineral nutrients was definitely significant at Stage IV. Globoids were absent from the Stage IV light/with nutrients samples, indicating that in this case mobilization had proceeded much further than under any other set of growth conditions.

LARGE PARTICLES

One of the most intriguing findings of this study was the presence of very large (5 - 7 μm in diameter) electron-dense particles in some Stage III and IV samples of both species. These particles were present in only a small percentage of the samples, and there was no apparent pattern to their appearance in some samples and not in others. Such particles were not found in any Stage I or II samples, and their presence had not been noted before in any species in which globoids have been studied. However, most studies of globoids have been done with dry seeds. These particles were similar in many ways to the normal sized (1 -2 μm

diameter) globoids found in the same samples. Globoids were spherical, naturally electron dense particles made up of phytin. The large particles were also spherical and naturally electron dense. Whether they were composed of phytin is not known. The large particles did produce spectra which were almost identical to those generated by globoids from the same samples, greatly increasing the likelihood that these large particles were in fact, very large globoids.

Nothing is known about these particles, and there are many questions that need to be addressed. How were they formed? Were they localized to any particular cells or regions in the cotyledons? Were they more likely to form under certain conditions? It is unlikely that they were formed simply by a clumping together of globoids, as this would most likely have resulted in a structure resembling a cluster of grapes. It is also not probable that they resulted from the expansion of smaller globoids. If they were smaller globoids just expanded in size by some change in the phytin structure, they would have been less dense, and had lower count rates than typical globoids. The large particles analyzed in this study, however, had count rates that were just as high, or higher than those of the typical globoids. An intriguing finding, these globoid-like particles present a puzzle that should be further examined.

KEY FINDINGS

Previous research has: examined the composition of globoids in the mature, dry embryos of many species, including *C. maxima* and *C. andreana* (Ockenden and Lott, 1990; reviews in Lott, 1980, 1984); documented the mobilization of storage reserves from

cotyledons during early seedling growth (Ockenden and Lott, 1988d; Bewley and Black, 1985); investigated changes in the structure of protein bodies and globoids during germination and seedling development (Hara and Matsubara, 1980; Lott and Vollmer, 1973a); and looked at changes in phytate and phytase levels in seedlings during early seedling growth (Gibson and Ullah, 1990; Loewus *et al.*, 1990; Loewus, 1986). The research presented here was the first comprehensive study of changes in the element composition of globoids during germination and seedling establishment.

The element composition of globoids did change during early seedling growth. Regardless of the species studied and the growth conditions used, the general trend for each element was the same; P remained relatively constant, K decreased markedly, and Mg, Ca, Mn, Fe and Zn generally increased. As the protein bodies fused and became more aqueous, it is likely that K came off the phytate molecule and was replaced by di- or trivalent cations with a higher affinity for phytic acid.

There were species-to-species differences in globoid composition changes which could be attributed, at least in part, to differences in the Ca content of their mature, dry embryos. In *C. andreana*, which had a higher initial concentration of Ca in the embryo and in the globoids, there was a large increase in the Ca content of the globoids, and no significant increase in Mn. In *C. maxima* globoids, which only occasionally contained trace amounts of Ca in the dry embryo, there was a slight increase in Ca, and a much larger increase in Fe, Zn and Mn than occurred in *C. andreana* globoids.

Light conditions alone did not have a significant influence on the changes in globoid composition. In combination with mineral nutrients, in the later stages of growth, the

presence of light resulted in a more rapid degradation of globoids. Mineral nutrient conditions had some influence on globoid composition, affecting mostly elements which were present in large amounts. The effect of mineral nutrient conditions, and its interactions with light conditions may have been mediated through changes in the mobilization of mineral nutrients out of the cotyledons during seedling growth.

There were very large globoid-like particles present in some later stage samples of both species. Such large particles had not previously been found in cucurbit tissues. What these particles were and how they were formed is unknown.

FUTURE DIRECTIONS

This study has provided answers to the questions posed at the beginning of the work, but it has also raised many questions for which answers need to be found. Some of the potential work, which could be done to develop a more detailed picture of what occurs when globoids are broken down during early seedling growth, is listed below:

1. Light and electron microscope studies of sectioned or freeze-fractured tissues could be done examining samples of cotyledons at the same stages used here. This work could look at the entire cotyledon in cross section, identifying differences between cell types. Some of the information that could be gathered includes:

a) Are globoids degraded sooner in one part of the cotyledon than in another? Does globoid degradation occur at the same time, and the same rate as storage protein mobilization?

b) Are all globoids in a single cell degraded at the same time? If not, is there a pattern to which are degraded first?

c) Where are the large globoid-like particles found? Are they located in any specific tissue type? When do they appear? When do they disappear? Are they real? Are they composed of phytin?

2. Freeze-fracture studies, combined with light microscope and thick section work could be done to determine the physical changes which occur as globoids are degraded. Lott and Vollmer (1973a) found that they were digested either by the formation of internal pitted regions, or through external digestion. No evidence of either of these forms of digestion were observed in this study, but their presence could have been masked when the globoids were viewed in STEM mode. In STEM mode the globoids appeared consistently electron dense and smoothly spherical in shape.

3. There are differences in the element composition of globoids in cereals and dicots (Lott, 1981). EDX analysis studies similar to this one could be done on various monocot and dicot species. Differences in the changes in globoid element composition during seedling growth might provide information on differences in reserve mobilization in monocots versus dicots.

4. The changes in levels of phytic acid, inorganic phosphorus and total phosphorus in the seedlings and cotyledons of *C. maxima* and *C. andreana* grown to the different stages would

provide additional information on the changes occurring during seedling growth. It could give some idea of how much of the total population of globoids had been degraded at each successive stage.

5. EDX analysis studies of thick sectioned *C. andreana* tissue could be used to follow the appearance of Cl in the globoids. When does it appear? Is it localized in a particular tissue or cell type? Is it found more often in seedlings grown in any particular conditions?

REFERENCES CITED

Ashford, A.E. and Gubler. F. 1984. Mobilization of polysaccharide reserves from endosperm.**In** Seed Physiology. Vol.2. Edited by D.R. Murray. Academic Press, Toronto. pp.117-162.

Barbi, N.C. 1979. Quantitative methods in biological x-ray microanalysis. Scanning Electron Microsc. 11: 658-672.

Bemis, W.P., Schreens, J.C., Berry, J.W., Dreher, M.L. and Weber, C.W. 1977. Accumulation of crude protein and oil contents. J. Amer. Oil Chemist's Soc. 454: 537-538.

Bewley, J.D. and Black, M. 1985. Seeds, Physiology of Development and Germination. Plenum Press, New York. pp.10-25, 253-327.

Bianchetti, R. and Sartirana, M.L. 1967. The mechanism of the repression by inorganic phosphate of phytase synthesis in the germinating wheat embryo. Biochim. Biophys. Acta 145: 485-490.

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

Biswas, S. and Biswas, B.B. 1965. Enzymatic synthesis of guanosine triphosphate. Biochem. Biophys. Acta 108: 710-713.

Blevins, D.G. 1985. Role of potassium in protein metabolism in plants. **In** Potassium in Agriculture. ASA-CSSA-SSSA, Madison, Wisconsin. pp.413-424.

Bozzola, J.J. and Russel, L.D. 1992. Electron microscopy: Principles and techniques for biologists. Jones and Bartlett Publishers, Boston.

Brown, E.C., Heit, M.L. and Ryan, D.E. 1961. Phytic acid: An analytical investigation. Can. J. Chem. 39: 1290-1297.

Buttrose, M.S. 1978. Manganese and iron in globoid crystals of protein bodies from *Avena* and *Casuarina*. Aust. J. Plant Physiol. 5: 631-639.

Chandler, J.A. 1972. An introduction to analytical electron microscopy. Micron 3: 85-92.

Chapman, J.M. and Galleschi, L. 1985. The control of reserve mobilization in seeds of *Cucumis sativus* L. VI. The production of starch. Ann. Bot. 55: 29-34.

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.

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

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

Crean, D.E and Haisman, D.R. 1963. The interaction between phytic acid and divalent cations during the cooking of dried peas. J. Sci. Food Agric. 14: 824-833.

Culter, H.C. and Whitaker, T.W. 1961. History and distribution of the cultivated cucurbits in the Americas. Antiquity. 26: 469-485.

Davies, H.V. and Chapman, J.M. 1979a. The control of food mobilization in seeds of *Cucumis sativus* L. I. The influence of the 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 mobilization 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 mobilization 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 mobilization in seeds of *Cucumis sativus* L. IV. The pattern of protein degradation. Z. Pflanzenphysiol. Bd. 101S: 347-353.

Davies, H.V. and Slack, P.T. 1981. The control of food mobilization in seed of dicotyledonous plants. New Phytol. 88: 41-51.

Decker-Walters, D.S., Walters, T.W. and Posluszny, U. 1990. Geneology and gene flow among annual domesticated species of *Cucurbita*. Can. J. Bot. 68:782-789.

Duffus, C.M and Rosie, R. 1976. J. Agric. Sci. 87: 75-79.

Esau, K. 1977. Anatomy of Seed Plants. John Wiley and Sons, New York. pp.429-473.

Ferguson, I.B. and Bollard, E.G. 1976. The movement of calcium in germinating pea seeds. Ann. Bot. 40: 1047-1055.

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.

Garcia-Agustin, P., Benaches-Gastaldo, M.J. and Primo-Millo, E. 1992. Lipid mobilization in *Citrus* cotyledons during germination. J. Plant Physiol. 140: 1-7.

Gibson, D.M. and Ullah, A.B.J. 1988. Purification and characterization of phytase from cotyledons of germinating soybean seeds. Arch. Biochem. Biophys. 260: 503-513.

Gibson, D.M. and Ullah, A.B.J. 1990. Phytases and their action on phytic acid. **In** Inositol Metabolism in Plants. Edited by D.J. Morre, W.F. Boss and F.A. Loewus. Wiley-Liss Inc., New York. pp.77-92.

Glass, A.D.M. 1989. Plant Nutrition: An Introduction to Current Concepts. Jones and Bartlett Publishers, Boston. pp.163-202.

Goel, M. and Sharma, C.B. 1979. Multiple forms of phytase in germinating cotyledons of *Cucurbita maxima*. Phytochem. 18: 1939-1942.

Graf, E. 1986. Chemistry and applications of phytic acid: An overview. **In** Phytic Acid: Chemistry and Applications. Edited by E. Graf. Pilatus Press, Minneapolis. pp.1-22.

Graf, E., Empson, K.L. and Eaton, J.W. 1987. Phytic acid: a natural antioxidant. J.Biol. Chem. 262: 11647-11650.

Greenwood, J.S. 1989. Phytin synthesis and deposition. **In** Recent Advances in the Development and Germination of Seeds. Edited by R.B. Taylorson. Plenum Press, New York. pp.109-125.

Greenwood, J.S. and Bewley, J.D. 1984. Subcellular distribution of phytin in the endosperm of developing castor bean: a possibility for its synthesis in the cytoplasm prior to deposition within protein bodies. Planta 160: 113-120.

Greenwood, J.S. and Bewley, J.D. 1985. Seed development in *Ricinus communis* cv. Hale (castor bean). III. Pattern of storage protein and phytin accumulation in the endosperm. Can. J. Bot. 63: 2121-2129.

Greenwood, J.S. and Chrispeels, M.J. 1985. Immunocytochemical localization of phaseolin and phytohemagglutinin in the endoplasmic reticulum and Golgi complex of developing bean cotyledon. Planta 164: 295-302.

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.

Guardiola, J.L. and Suttcliffe, 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.

Gutterman, Y. 1992. Influences of daylength and red or far-red light during the storage of ripe *Cucumis prophetarum* fruits, on seed germination in light. J. Arid Environ. 23: 443-449.

Halaweish, F.T. and Tallamy, D.W. 1993. A new cucurbitacin profile for *Cucurbita andreana*: a candidate for cucurbitacin tissue culture. J. Chem. Ecology 19: 1135-1141.

Hanson, J.B. 1984. The functions of calcium in plant nutrition. **In** Advances in Plant Nutrition. Vol.1. Edited by P.B. Tinker and A. Läuchli. Praeger Publishers, New York. 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 Cell Physiol. 21: 219-232.

Hara, I., Wada, K. and Matsubara, H. 1976a. Pumpkin (*Cucurbita* sp.) seed globulin. II. Alterations during germination. Plant 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 Cell Physiol. 17: 799-814.

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.

Hayakawa, T., Suzuki, K., Miura, H., Ohno, T. and Igaue, I. 1990. Myo-inositol polyphosphate intermediates in the dephosphorylation of phytic acid by acid phosphatase with phytase activity from rice. Agric. Biol. Chem. 54: 279-286.

Heiser, C.B. 1979. The Gourd Book. University of Oklahoma Press, Norman.

Hendersen, C.W., Scheerens, J.C. and Berry, J.W. 1986. Antinutritional factors in *Cucurbita* seed meals. J. Agric. Food Chem. 34: 434-436.

Hoagland, D.R. and Arnon, D.I. 1950. The Water-Culture Method for Growing Plants Without Soil. Circular 347. The College of Agriculture, University of California,

Berkeley.

Hocking, P.J. 1980. Redistribution of nutrient elements from cotyledons of two species of annual legumes during germination and seeling groeth. Ann. Bot. 45: 383-396.

Hocking, P.J. and Pate, J.S. 1977. Mobilization of minerals to developing seeds of legumes. Ann. Bot. 41: 51-62.

Hsiao, T.C. and Läuchli, A. 1986. Role of potassium in plant-water relations. **In** Advances in Plant Nutrition. Vol.2. Edited by B. Tinker and A. Läuchli. Praeger Press, New York. pp.281-311.

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.

Jacks, T.J. 1986. Cucurbit seed protein and oil. **In** Plant Proteins: Applications, Biological Effects and Chemistry. Edited by R.L. Ory. Amer. Chem. Soc. Symposium Series No. 312. Amer. Chem. Soc. Washington, D.C.

Jacks, T.J. 1990. Cucurbit seeds: cytological, physiochemical and nutritional characterizations. **In** Biology and Utilization of the Cucurbitaceae. Edited by D.M. Bates, R.W. Robinson and C. Jeffrey. Cornell University Press, Ithaca. pp.356-363.

Jackson, C.K. and Hall, J.L. 1993. A fine structural analysis of auxin induced elongation of cucmber hypocotyls, and the effects of calcium antagonists and ionophores. Ann. Bot. 72: 193-204.

Jeffrey, C. 1990a. An outline classification of the Cucurbitaceae. **In** Biology and Utilization of the Cucurbitaceae. Edited by D.M. Bates, R.W. Robinson and C. Jeffrey. Cornell University Press, Ithaca. pp.449-463.

Jeffrey, C. 1990b. Systematics of the Cucurbitaceae: an overview. In Biology and Utilization of the Cucurbitaceae. Edited by D.M. Bates, R.W. Robinson and C. Jeffrey. Cornell University Press, Ithaca. pp.3-9.

Johnson, L.F. and Tate, M.E. 1969. Structure of "phytic acids". Can. J. Chem. 47: 63-73.

Joshi, D.C., Das, S.K. and Mukherjee, R.K. 1993. Physical properties of pumpkin seeds. J. Agric. Eng. Res. 54: 219-229.

Kikunaga, S., Katoh, Y. and Takahashi, M. 1991. Biochemical changes in phosphorus compounds and in the activity of phytase and α-amylase in the rice (*Oryza sativa*) grain during germination. J. Sci. Food Agric. 56: 335-343.

Kuvaeva, E.B. and Kretovich, V.L. 1978. Phytase of germinating pea seeds. Soviet Plant Physiol. 25: 290-295.

Laboure, A.M., Gagnon, J. and Lescure, A.M. 1993. Purification and characterization of a phytase (*myo*-inositol hexakisphosphate phosphohydrolase) acumulated in maize (*Zea mays*) seedlings during germination. Biochem. J. 295: 413-419.

Lasley, S.E. and Garber, M.P. 1978. Photsynthetic contribution of cotyledons to early development of cucumber. Hort. Sci. 13: 191-193.

Lazos, E.S. 1986. Nutritional, fatty acid and oil characteristics of pumpkin and melon seeds. J. Food. Sci. 51: 1382-1383.

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.

Loewus, F.A. 1990. Structure and occurrence of inositols in plants. **In** Inositol Metabolism in Plants. Edited by D.J. Morre, W.F. Boss and F.A. Loewus. Wiley-Liss Inc., New York. pp.1-11.

Loewus, F.A. and Loewus, M.W. 1983. *myo*-Inositol: Its biosynthesis and metabolism. Ann. Rev. Plant Physiol. 34: 137-161.

Loewus, F.A., Everard, J.D. and Young, K.A. 1990. Inositol metabolism: Precursor role and breakdown. **In** Inositol Metabolism in Plants. Edited by D.J. Morre, W.F. Boss and F.A. Loewus. Wiley-Liss Inc., New York. pp.21-45.

Lott, J.N.A. 1970. Changes in the cotyledons of *Cucurbita maxima* during germination. I. General characteristics. Can. J. Bot. 48: 2227-2231.

Lott, J.N.A. 1973. A scanning selectron 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. Edited by P.K. Stumpf and E.E. Conn. Academic Press, New York. pp.589-623.

Lott, J.N.A. 1984. Accumulation of seed reserves of phosphorus and other minerals. **In** Seed Physiology. Vol.1. Edited by D.R. Murray. Academic Press, Sydney. pp.139-166.

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., Goodchild, D.J. and Craig, S. 1984. Studies of mineral reserves in pea (*Pisum sativum*) cotyledons using low-water-content procedures. Aust. J. Plant Physiol. 11: 459-469.

Lott, J.N.A., Greenwood, J. and Vollmer, C. 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. 61: 984-988.

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., Ockenden, I., Kerr, P., West, M., Leech, T. and Skilnyk, H. 1994. The influence of experimentally induced changes in the (Mg + Ca):K balance on protein bodies formed in developing *Cucurbita* seeds. Can. J. Bot. 72: 364-369.

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. Aust. J. Plant Physiol. 12: 341-353.

Lott, J.N.A., Spitzer, E.,and Vollmer, C. 1979. Calcium distribution in globoid crystals of *Cucurbita* cotyledon protein bodies. Plant Physiol. 63: 847-851.

Lott, J.N.A., West, M.M., Clark, B. and Beecroft, P. Unpublished. Changes in the composition of globoids in castor bean cotyledons and endosperm during early seedling growth with and without complete mineral nutrients. Submitted to Seed Sci. Res.

Maga, J.A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30: 1-9.

Marme, D. 1983. Cacium transport and function. **In** Inorganic Plant Nutrition. Encyclopedia of Plant Physiology. Vol.15B. Edited by A. Läuchli and R.L. Bieleski. Springer Verlag, New York. pp.599-625.

McKersie, B.D. and Senaratna, T. 1983. Membrane structure in germinating seeds. **In** Mobilization of Reserves in Germination. Recent advances in Phytochemistry. Vol.17. Edited by C. Nozzolillo and P.J. Lea. Plenum Press, New York. pp.29-52.

Mengel, K. and Kirkby, E.A. 1982. Principles of Plant Nutrition. Worblaufen-Bern, Switzerland: International Potash Institute. pp.387-508

Monma, M., Toshio, S., Hashizume, K and Saio, K. 1992. Biogenesis of protein bodies in embryonic axes of soybean seeds (*Glycine max.* cv. Enrei). Biosci. Biotech. Biochem. 56: 1036-1040.

Murphy, D.J. 1993. Structure, function and biogenesis of storage lipid bodies and oleosins in plants. Prog. Lipid. Res. 32: 247-280.

Murray, D.R. 1984. Axis-cotyledon relationship during reserve mobilization. **In** Seed Physiology. Vol.2. Edited by D.R. Murray. Academic Press, Toronto. pp.247-280.

Nelson, H.C. 1932. Development of the foliaceous cotyledons of *Cucurbita maxima*. Univ. Iowa Stud. Natur. Hist. 14: 3-27.

Nolan, K.B. and Duffin, P.A. 1987. Effects of phytate on mineral bioavailability. *In vitro* studies on Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} (also Cd^{2+}) solubilities in the presence of phytate. J. Sci. Food Agric. 40: 79-85.

Ockenden, I. 1987. Studies of calcium and other storage minerals in embryos of *Cucurbita maxima*, *Cucurbita andreana* and their recipricol hybrids. Ph.D. Dissertation, McMaster University, Hamilton, Ontario. pp.14-39.

Ockenden, I. and Lott, J.N.A. 1988a. Mineral Storage in *Cucurbita* embryos. I. Calcium storage in relation to embryo size. Can. J. Bot. 66: 1477-1481.

Ockenden, I. and Lott, J.N.A. 1988b. Mineral Storage in *Cucurbita* embryos. II. Calcium storage in reciprocal hybrids of *Cucurbita maxima* and *Cucurbita andreana*. Can. J. Bot. 66: 1482-1485.

Ockenden, I. and Lott, J.N.A. 1988c. Mineral Storage in *Cucurbita* embryos. III. Calcium storage as compared with storage of magnesium, potassium and phosphorus. Can. J. Bot. 66: 1486-1489.

Ockenden, I. and Lott, J.N.A. 1988d. Changes in the distribution of magnesium, potassium, calcium and phosphorus during growth of *Cucurbita* seedlings. J. Exp. Bot. 39: 973-980.

Ockenden, I. and Lott, J.N.A. 1990. Elemental storage in *Cucurbita* embryos: X-ray microanalysis of magnesium, potassium, calcium and phosphorus within globoid crystals. Can. J. Bot. 68: 646-650.

Ogawa, M., Tanaka, K., and Kasai, Z. 1975. Isolation of high phytin containing particles from rice grains using an aqueous polymer. Agric. Biol. Chem. 39: 695-700.

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.

Organ, M.G., Greenwood, J.S. and Bewley, J.D. 1988. Phytin is synthesized in the cotyledons of germinated castor-bean seeds in response to exongenously supplied phosphate. Planta 174: 513-517.

Penner, D. and Ashton, F.M. 1967. Hormonal control of proteinase activity in squash cotyledons. Plant Physiol. 42: 791-796.

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.

Pichl, I. 1976. Seed globulins of various species of the Cucurbitaceae. Phytochem. 15: 717-722.

Pichl, I. 1978. Characterizations of albumins isolated from seeds of *Cucurbita maxima* L. Bichem. Physiol. Pflanzen 172: 61-66.

Pino, E., Martin, L., Guerra, H., Nicolas, G. and Villalobos. 1991. Effect of dihydrozeatin on the mobilization of protein reserves in protein bodies during the germination of chick-pea seeds. J. Plant Physiol. 137: 425-432.

Poovaiah, B.W. and Reddy, A.S.N. 1987. Calcium messenger system in plants. Crit. Rev. Plant Sci. 6: 47-103.

Poovaiah, B.W. and Reddy, A.S.N. 1993. Calcium and signal transduction in plants. Crit. Rev. Plant Sci. 12: 185-211.

Raboy, V. 1990. Bichemistry and genetics of phytic acid synthesis. **In** Inositol Metabolism in Plants. Edited by D.J. Morre, W.F. Boss and F.A. Loewus. Wiley-Liss Inc., New York. pp.55-76.

Raboy, V. and Dickinson, D.B. 1993. Phytic acid levels in seeds of *Glycine max* and *G. soja* as influenced by phosphorus status. Crop Sci. 33: 1300-1305.

Rao, P.U. and Deosthale, Y.G. 1983. Effect of germination and cooking on mineral composition of pulses. J. Food Sci. Tech. 20: 195-197.

Reddy, N.R., Balakrishnan, C.V. and Salunkhe, D.K. 1978. Phytate phosphorus and mineral changes during germination and cooking of black gram (*Phaseolus mungo*) seeds. J. Food Sci. 43: 540-543.

Saski, K and Taylor, I.E.P. 1984. Specific labelling of cell wall polysaccharides with myo-(2-H)-inositol during germination and growth of *Phaseolus vulgaris* L. Plant Cell Physiol. 25: 989-997.

Scott, J.J. and Loewus, F.A. 1986. Phytate metabolism in plants. **In** Phytic Acid: Chemistry and Applications. Edited by E. Graf. Pilatus Press, Minneapolis. pp.23-42.

Siddiqui, K.S., Shah, S.A., Aqra, F.M.A.M., Tabassum, S. and Zaidi, S.A.A. 1993. Equilibrium studies on interactions of rare earth ions with phytic acid. Ind. J. Chem. 32: 421-423.

Simon, E.W. 1984. Early events in germination. **In** Seed Physiology. Vol.2. Edited by D.R. Murray. Academic Press, Toronto. pp.77-115.

Singh, D. and Dathan, A.S.R. 1972. Structure and development of seed coat structure in the Cucurbitaceae. VI. Seeds of *Cucurbita*. Phytomorphology 22: 29-45.

Slack, P.T., Black, M. and Chapman, J.M. 1977. The control of lipid mobilization in *Cucumis* cotyledons. J. Exp. Bot. 28: 569-577.

Sobolev, A.M. and Rodionova, M.A. 1966. Phytin synthesis by aleurone grains in ripening sunflower seeds. Sov. Plant Physiol. 13: 958-961.

Splittstoesser, W.E. 1983. Effects of embryo removal upon reserve protein degradation in *Cucurbita moschata*. J. Seed Technol. 8: 25-30.

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.). Hort. Sci. 17: 402-403.

Stewart, A., Neild, H. and Lott, J.N.A. 1988. An investigation of the mineral content of barley grains and seedlings. Plant Physiol. 86: 93-97.

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.

Tsuyuki, H., Itoh, S. and Yamagata, K. 1985. Lipid and triacylglycerol compositions of total lipids in pumpkin seeds. Nippo Skokuhin Kogyo Gakkaishi 32: 1-15.

Vaughan, J.G. 1970. The Structure and Utilization of Oil Seeds. Chapman and Hall Ltd., London. pp.63-69

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.

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 origins 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. Interscience Publishers. New York. pp. 14-36.

Whiting, A.G. 1938. Development and anatomy of primary structures in the seedling of *Cucurbita maxima*. Bot. Gaz. 99: 497-528.

Wilson, H.D., Doebley, J. and Duvall, M. 1992. Chloroplast DNA diversity among wild and cultivated members of *Cucurbita* (Cucurbitaceae).Theor. Appl. Genet. 84: 859-865.

Xu, P., Price, J., Wise, A. and Aggett, P.J. 1992. Interaction of inositol phosphates with calcium, zinc, and histidine. J. Inorganic Biochem. 47: 119-130.

Yang, S.L. and Walters, T.W. 1992. Ethnobotany and the economic role of the Cucurbitaceae of China. Econ. Bot. 46: 349-367.

APPENDIX A: CALCULATION OF CORRECTION FACTORS

In an EDX analysis spectra, each element may produce multiple peaks, one for each of the possible inter-orbital electron transitions which occur as the ionized atom returns to a stable state. For the elements of interest here, the K_α is the largest peak, and is usually the peak used in the calculation of P/B ratios. The secondary K_β peak, is much smaller, but contains a constant fraction of the total counts in the K_α peak. Knowing the size of the K_α peak, therefore, allows one to calculate the size of the K_β peak. In EDX analysis spectra, the Ca K_α peak is overlapped by the K K_β peak. Because the size of the K K_β peak is constant relative to the size of the K K_α peak, the contribution of the K K_β peak to the Ca K_α peak is also constant relative to the K K_α peak. By calculating the size of the "Ca K_α" peak produced by a potassium salt which does not contain Ca, the contribution of the K K_β peak to the Ca K_α peak can be determined. When expressed relative to the size of the K K_α peak, this is a correction factor which can be applied to any spectra in order to calculate the actual contribution of Ca to its K_α peak. In addition to the K/Ca overlap, in EDX analysis spectra the Fe K_α peak is overlapped by the Mn K_β peak and the Zn K_α peak is overlapped by the Cu K_β peak. Using the same approach, correction factors can be derived for Fe and Zn by the EDX analysis of Mn and Cu salts respectively.

To derive these correction factors, some salts were analyzed in powder form, spread on formvar-carbon coated copper grids, and others were analyzed after being embedded in Spurr's resin and thick sectioned. The salts analyzed to determine the correction factor for each element were:

Ca: - KH_2PO_4 powder

- KH_2PO_4 plastic embedded

- K,Mg phytic acid powder

Fe: - $MnSO_4$ plastic embedded

Zn: - $CuSO_4$ plastic embedded

- results from analyses of all other salts were used as well. Although they were not Cu salts, Cu peaks were produced in each spectra by the Cu grids.

To calculate the correction factor for calcium the following procedure was used. The total counts in K K_α and Ca K_α peaks before and after background subtraction were calculated (the gross and net counts, respectively). The contribution of the K K_β peak to the Ca K_α was calculated relative to the net counts in the K K_α peak by making the calculation:

$$\frac{\text{Ca-b (net counts in Ca } K_\alpha \text{ peak)}}{\text{K-b (net counts in K } K_\alpha \text{ peak)}}$$

This calculation was carried out for each individual spectrum, and the mean value for each salt was calculated. The overall mean correction factor was then calculated from all the K salts analyzed.

This same procedure was followed to calculate the correction factors for Fe and Zn.

Table A1 Correction factors for Ca, Fe and Zn calculated by the analysis of various pure salts.

Salt	N	Ca-b/K-b	Fe-b/Mn-b	Zn-b/Cu-b
KH_2PO_4 - plastic	20	0.0821		0.0219
- powder	20	0.0820		0.0189
Cu-sulphate	20			0.0163
Mn-sulphate	20		0.1160	0.0201
K,Mg phytic acid	20	0.1007		0.0210
mean		0.0883	0.1160	0.0196

Note: N = the number of spectra collected

The correction factors were then used to calculate the actual contribution of K, Mn and Cu to the respective peaks of Ca, Fe and Zn.

For example, the Ca correction factor was applied to the following data:

net K (K-b)	gross Ca	net Ca (Ca-b)	Ca P/B	Corrected Ca P/B
14356	2526	1631	1.82	0.41

Before the correction factor was applied the Ca P/B ratio is 1.82, corrected for the overlap of the K K_β peak the actual Ca P/B was only 0.41.

To calculate the corrected P/B ratio:

1. A corrected Ca value is determined:

Corrected Ca = net Ca - (correction factor X net K)

CCa = 1631 - (0.088 X 14356)

CCa = 368

2. This corrected Ca value is used to calculate the P/B ratio:

Corrected Ca P/B = corrected Ca peak / background

CCa P/B = CCa / (gross Ca - net Ca)

CCA P/B = 368 / (2526 - 1631)

CCa P/B = 0.41

NB: The calculation of the correction factors was a collaborative effort between P. Beecroft and M. West.
Plastic embedded samples were embedded by Dr. T. Wada.

APPENDIX B: MEAN PEAK-TO-BACKGROUND (P/B) AND ELEMENT-TO-PHOSPHORUS (element/P) VALUES FOR GLOBOIDS FROM EACH SEED FROM EACH TREATMENT

C. maxima - Tables B1a - B1m
C. andreana - Tables B2a - B2m

Note: For each table, values in the same column followed by the same letter are not significantly different at $P > 0.05$.

Table B1a Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage I, *C.maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.39 ± 1.13 a	13.10 ± 1.95 bc	1.07 ± 0.18 ijk	3.99 ± 0.81 d	0.32 ± 0.07 l	0.21 ± 0.28 e	0.018 ±0.024 m	0.09 ± 0.13 f	0.007 ±0.010 n	0.26 ± 0.09 g	0.021 ±0.007 p	0.20 ± 0.15 h	0.016 ±0.010 q
2	12.28 ± 1.73 a	12.25 ± 1.76 bc	1.01 ± 0.15 ijk	4.08 ± 0.58 d	0.34 ± 0.48 l	0.16 ± 0.14 e	0.013 ±0.014 m	0.08 ± 0.12 f	0.006 ±0.009 n	0.18 ± 0.10 g	0.014 ±0.008 p	0.27 ± 0.16 h	0.022 ±0.013 q
3	12.31 ± 1.65 a	13.61 ± 2.12 bc	1.11 ± 0.10 ijk	4.67 ± 1.02 d	0.38 ± 0.07 l	0.00 ± 0.15 e	0.001 ±0.013 m	0.11 ± 0.19 f	0.008 ±0.014 n	0.25 ± 0.08 g	0.020 ±0.005 p	0.20 ± 0.13 h	0.016 ±0.009 q
4	11.52 ± 1.44 a	14.04 ± 2.39 bc	1.22 ± 0.15 i	3.99 ± 0.68 d	0.35 ± 0.06 l	-0.09 ± 0.11 e	-0.008 ±0.009 m	0.13 ± 0.14 f	0.012 ±0.014 n	0.36 ± 0.22 g	0.031 ±0.020 p	0.20 ± 0.18 h	0.017 ±0.015 q
5	12.22 ± 0.52 a	14.63 ± 2.58 b	1.19 ± 0.18 ij	4.36 ± 0.61 d	0.36 ± 0.06 l	-0.07 ± 0.24 e	-0.006 ±0.019 m	0.13 ± 0.07 f	0.010 ±0.006 n	0.31 ± 0.16 g	0.025 ±0.012 p	0.30 ± 0.17 h	0.025 ±0.014 q
6	11.90 ± 1.00 a	13.26 ± 2.01 bc	1.22 ± 0.21 ijk	3.96 ± 1.21 d	0.33 ± 0.10 l	-0.04 ± 0.14 e	-0.003 ±0.011 m	0.07 ± 0.12 f	0.006 ±0.010 n	0.26 ± 0.10 g	0.022 ±0.009 p	0.29 ± 0.13 h	0.024 ±0.011 q
7	12.10 ± 1.41 a	11.24 ± 2.48 c	0.93 ± 0.16 k	3.65 ± 0.97 d	0.30 ± 0.08 l	0.08 ± 0.18 e	0.007 ±0.016 m	0.02 ± 0.08 f	0.002 ±0.006 n	0.36 ± 0.17 g	0.030 ±0.016 p	0.14 ± 0.15 h	0.012 ±0.012 q
8	11.73 ± 1.66 a	12.85 ± 1.22 bc	1.13 ± 0.32 ijk	4.12 ± 0.97 d	0.36 ± 0.09 l	0.19 ± 0.37 e	0.015 ±0.030 m	0.08 ± 0.13 f	0.007 ±0.011 n	0.23 ± 0.11 g	0.020 ±0.009 p	0.15 ± 0.16 h	0.015 ±0.017 q
9	12.59 ± 0.44 a	12.05 ± 1.07 bc	0.96 ± 0.10 jk	3.69 ± 0.92 d	0.29 ± 0.07 l	0.22 ± 0.31 e	0.018 ±0.024 m	0.05 ± 0.15 f	0.004 ±0.012 n	0.34 ± 0.24 g	0.027 ±0.020 p	0.16 ± 0.11 h	0.013 ±0.009 q
10	12.79 ± 1.19 a	13.10 ± 1.96 bc	1.02 ± 0.10 ijk	4.36 ± 0.72 d	0.34 ± 0.04 l	0.14 ± 0.26 e	0.012 ±0.024 m	0.12 ± 0.15 f	0.009 ±0.011 n	0.19 ± 0.07 g	0.015 ±0.005 p	0.22 ± 0.19 h	0.017 ±0.014 q
mean	12.18 ± 1.28	13.01 ± 2.14	1.08 ± 0.19	4.09 ± 0.88	0.34 ± 0.07	0.08 ± 0.25	0.007 ±0.021	0.09 ± 0.13	0.007 ±0.011	0.27 ± 0.15	0.023 ±0.013	0.21 ± 0.16	0.018 ±0.013

Table B1b Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Dark/No Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.20 ± 0.70 a b c	10.32 ± 1.07 d e	0.85 ± 0.08 q r	3.71 ± 0.80 i j	0.30 ± 0.06 u	0.04 ± 0.18 k	0.003 ±0.014 v	0.14 ± 0.09 m	0.011 ±0.007 w	0.19 ± 0.09 n	0.016 ±0.007 x	0.20 ± 0.12 p	0.016 ±0.009 y z
2	12.44 ± 1.38 a b c	10.34 ± 1.91 d e	0.83 ± 0.12 q r s	3.98 ± 1.01 i j	0.32 ± 0.06 u	-0.04 ± 0.06 k	-0.003 ±0.005 v	0.03 ± 0.09 m	0.003 ±0.007 w	0.22 ± 0.12 n	0.017 ±0.008 x	0.22 ± 0.16 p	0.018 ±0.012 y z
3	13.75 ± 0.92 c	12.70 ± 1.59 h	0.93 ± 0.14 q	4.67 ± 0.96 j	0.34 ± 0.06 u	-0.06 ± 0.05 k	-0.005 ±0.004 v	0.16 ± 0.11 m	0.012 ±0.008 w	0.27 ± 0.15 n	0.019 ±0.011 x	0.33 ± 0.15 p	0.024 ±0.011 y z
4	12.57 ± 1.19 a c	8.05 ± 1.68 e f g	0.64 ± 0.11 s t	4.44 ± 0.39 i j	0.36 ± 0.03 u	0.20 ± 0.32 k	0.016 ±0.025 v	0.02 ± 0.05 m	0.002 ±0.004 w	0.29 ± 0.08 n	0.023 ±0.006 x	0.31 ± 0.12 p	0.025 ±0.011 y z
5	11.54 ± 0.78 a b	7.67 ± 1.15 f g	0.66 ± 0.08 r s t	4.42 ± 0.64 i j	0.38 ± 0.06 u	0.14 ± 0.42 k	0.014 ±0.040 v	0.06 ± 0.13 m	0.005 ±0.011 w	0.19 ± 0.07 n	0.017 ±0.005 x	0.35 ± 0.15 p	0.030 ±0.012 y
6	11.85 ± 1.05 a b	8.89 ± 1.75 d e f g	0.75 ± 0.15 q r s t	3.36 ± 0.97 i	0.28 ± 0.08 u	-0.01 ± 0.09 k	0.000 ±0.007 v	0.09 ± 0.04 m	0.007 ±0.003 w	0.21 ± 0.07 n	0.017 ±0.005 x	0.21 ± 0.09 p	0.018 ±0.006 y z
7	12.74 ± 1.17 a c	10.52 ± 2.25 d h	0.83 ± 0.22 q r s	4.49 ± 0.81 i j	0.35 ± 0.06 u	0.01 ± 0.14 k	0.001 ±0.010 v	0.10 ± 0.11 m	0.008 ±0.009 w	0.25 ± 0.07 n	0.020 ±0.007 x	0.22 ± 0.12 p	0.017 ±0.009 y z
8	11.80 ± 0.76 a b	9.77 ± 1.63 d e f	0.83 ± 0.14 q r s	3.68 ± 0.80 i j	0.31 ± 0.07 u	-0.00 ± 0.12 k	0.000 ±0.011 v	0.11 ± 0.12 m	0.010 ±0.011 w	0.24 ± 0.09 n	0.020 ±0.008 x	0.30 ± 0.16 p	0.025 ±0.012 y z
9	11.67 ± 1.27 a b	8.39 ± 1.44 d e f g	0.72 ± 0.11 r s t	3.32 ± 1.03 i	0.28 ± 0.08 u	0.12 ± 0.49 k	0.008 ±0.034 v	0.13 ± 0.06 m	0.011 ±0.004 w	0.26 ± 0.12 n	0.022 ±0.009 x	0.17 ± 0.05 p	0.014 ±0.004 z
10	10.94 ± 1.36 b	6.84 ± 1.01 g	0.63 ± 0.11 t	3.62 ± 0.95 i j	0.34 ± 0.13 u	0.03 ± 0.16 k	0.005 ±0.019 v	0.10 ± 0.12 m	0.009 ±0.010 w	0.26 ± 0.07 n	0.024 ±0.005 x	0.27 ± 0.13 p	0.025 ±0.012 y z
mean	12.15 ± 1.27	9.35 ± 2.24	0.77 ± 0.16	3.97 ± 0.95	0.33 ± 0.08	0.04 ± 0.25	0.004 ±0.021	0.09 ± 0.10	0.008 ±0.008	0.24 ± 0.10	0.020 ±0.007	0.26 ± 0.14	0.021 ±0.011

Table B1c Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Dark/With Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.60 ± 0.99 a b	10.89 ± 1.21 c	0.87 ± 0.08 k	4.49 ± 0.69 d e	0.36 ± 0.06 m n	0.26 ± 0.32 f	0.021 ±0.025 p	0.11 ± 0.10 g	0.008 ±0.007 q	0.36 ± 0.12 h	0.028 ±0.009 r	0.27 ±0.16 i	0.021 ±0.013 s t
2	12.41 ± 1.12 a b	11.45 ± 1.49 c	0.93 ± 0.13 k	3.84 ± 0.97 e	0.31 ± 0.07 n	0.13 ± 0.26 f	0.012 ±0.024 p	0.13 ± 0.11 g	0.011 ±0.008 q	0.32 ± 0.11 h	0.025 ±0.007 r	0.25 ± 0.25 i j	0.021 ±0.022 s t
3	13.01 ± 1.38 a b	10.96 ± 2.29 c	0.84 ± 0.12 k	4.25 ± 0.99 d e	0.32 ± 0.05 m n	0.00 ± 0.13 f	0.000 ±0.010 p	0.14 ± 0.15 g	0.011 ±0.011 q	0.29 ± 0.11 h	0.022 ±0.008 r	0.05 ± 0.14 j	0.003 ±0.011 t
4	12.60 ± 1.04 a b	10.00 ± 1.29 c	0.80 ± 0.11 k	4.29 ± 0.85 d e	0.34 ± 0.06 m n	0.29 ± 0.31 f	0.024 ±0.025 p	0.05 ± 0.11 g	0.004 ±0.008 q	0.29 ± 0.20 h	0.024 ±0.017 r	0.23 ± 0.16 i j	0.018 ±0.012 s t
5	12.00 ± 0.95 a b	9.86 ± 2.04 c	0.82 ± 0.17 k	4.76 ± 1.45 d e	0.39 ± 0.11 m n	0.22 ± 0.56 f	0.017 ±0.042 p	0.12 ± 0.14 g	0.010 ±0.012 q	0.37 ± 0.22 h	0.031 ±0.017 r	0.22 ± 0.12 i j	0.018 ±0.011 s t
6	11.78 ± 1.41 a	9.77 ± 2.23 c	0.85 ± 0.22 k	4.43 ± 0.88 d e	0.38 ± 0.08 m n	0.13 ± 0.20 f	0.012 ±0.018 p	0.13 ± 0.10 g	0.011 ±0.009 q	0.36 ± 0.19 h	0.031 ±0.015 r	0.20 ± 0.10 i j	0.018 ±0.009 s t
7	13.40 ± 0.88 b	9.75 ± 2.96 c	0.74 ± 0.24 k	5.51 ± 0.76 d	0.41 ± 0.04 m	0.08 ± 0.30 f	0.006 ±0.022 p	0.13 ± 0.17 g	0.010 ±0.013 q	0.44 ± 0.37 h	0.032 ±0.025 r	0.19 ± 0.12 i j	0.014 ±0.010 s t
8	11.66 ± 0.84 a	9.65 ± 2.62 c	0.82 ± 0.20 k	4.38 ± 0.52 d e	0.38 ± 0.05 m n	0.07 ± 0.12 f	0.006 ±0.010 p	0.07 ± 0.14 g	0.007 ±0.013 q	0.29 ± 0.13 h	0.025 ±0.011 r	0.29 ± 0.12 i	0.025 ±0.011 s
mean	12.43 ± 1.19	10.29 ± 2.11	0.83 ± 0.17	4.49 ± 0.99	0.36 ± 0.07	0.15 ± 0.30	0.012 ±0.024	0.11 ± 0.13	0.009 ±0.010	0.34 ± 0.20	0.027 ±0.015	0.21 ± 0.16	0.017 ±0.014

Table B1d Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Light/No Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	10.66 ± 1.51 b	7.31 ± 2.03 c	0.68 ± 0.15 i	3.08 ± 0.76 d	0.29 ± 0.07 j	0.19 ± 0.14 e	0.019 ±0.013 k	0.03 ± 0.05 f	0.003 ±0.004 m	0.22 ± 0.08 g	0.021 ±0.007 n	0.22 ± 0.09 h	0.020 ±0.006 p
2	12.33 ± 1.15 a b	7.98 ± 1.45 c	0.65 ± 0.10 i	4.01 ± 1.00 d	0.33 ± 0.08 j	0.01 ± 0.13 e	0.001 ±0.012 k	0.07 ± 0.14 f	0.006 ±0.012 m	0.34 ± 0.19 g	0.027 ±0.013 n	0.24 ± 0.17 h	0.019 ±0.014 p
3	10.91 ± 1.31 a b	7.62 ± 1.53 c	0.70 ± 0.14 i	3.20 ± 1.38 d	0.29 ± 0.10 j	0.24 ± 0.25 e	0.024 ±0.024 k	0.02 ± 0.09 f	0.002 ±0.007 m	0.35 ± 0.25 g	0.031 ±0.019 n	0.19 ± 0.09 h	0.017 ±0.007 p
4	12.63 ± 1.40 a	8.90 ± 1.72 c	0.70 ± 0.12 i	3.94 ± 1.03 d	0.31 ± 0.08 j	0.02 ± 0.23 e	0.002 ±0.018 k	0.07 ± 0.17 f	0.006 ±0.014 m	0.21 ± 0.08 g	0.017 ±0.005 n	0.21 ± 0.18 h	0.016 ±0.015 p
5	12.09 ± 1.64 a b	9.50 ± 2.49 c	0.79 ± 0.20 i	3.79 ± 1.38 d	0.31 ± 0.09 j	-0.05 ± 0.05 e	-0.004 ±0.004 k	0.10 ± 0.08 f	0.008 ±0.006 m	0.28 ± 0.11 g	0.023 ±0.007 n	0.27 ± 0.16 h	0.022 ±0.012 p
6	12.11 ± 1.06 a b	8.75 ± 1.50 c	0.72 ± 0.11 i	4.11 ± 1.10 d	0.34 ± 0.08 j	0.35 ± 0.53 e	0.028 ±0.041 k	0.13 ± 0.11 f	0.011 ±0.009 m	0.26 ± 0.07 g	0.022 ±0.005 n	0.23 ± 0.16 h	0.019 ±0.012 p
7	12.49 ± 1.06 a	9.03 ± 1.80 c	0.72 ± 0.09 i	3.91 ± 0.92 d	0.31 ± 0.06 j	0.09 ± 0.21 e	0.007 ±0.016 k	0.11 ± 0.07 f	0.009 ±0.006 m	0.30 ± 0.16 g	0.025 ±0.015 n	0.30 ± 0.08 h	0.024 ±0.007 p
8	11.69 ± 0.91 a b	9.19 ± 1.60 c	0.79 ± 0.14 i	4.00 ± 0.99 d	0.34 ± 0.06 j	0.22 ± 0.50 e	0.017 ±0.038 k	0.09 ± 0.08 f	0.008 ±0.007 m	0.25 ± 0.07 g	0.021 ±0.006 n	0.19 ± 0.11 h	0.017 ±0.010 p
9	11.37 ± 0.88 a b	7.30 ± 1.34 c	0.64 ± 0.11 i	4.11 ± 0.88 d	0.36 ± 0.06 j	-0.01 ± 0.21 e	-0.001 ±0.019 k	0.12 ± 0.08 f	0.010 ±0.007 m	0.20 ± 0.05 g	0.018 ±0.005 n	0.21 ± 0.15 h	0.018 ±0.011 p
10	11.56 ± 0.99 a b	7.73 ± 0.66 c	0.67 ± 0.06 i	3.98 ± 1.06 d	0.34 ± 0.08 j	0.26 ± 0.34 e	0.022 ±0.029 k	0.04 ± 0.05 f	0.004 ±0.004 m	0.26 ± 0.13 g	0.022 ±0.011 n	0.18 ± 0.12 h	0.016 ±0.011 p
mean	11.76 ± 1.32	8.33 ± 1.78	0.71 ± 0.13	3.81 ± 1.08	0.32 ± 0.08	0.13 ± 0.31	0.011 ±0.026	0.08 ± 0.10	0.007 ±0.008	0.27 ± 0.14	0.023 ±0.011	0.22 ± 0.13	0.019 ±0.011

Table B1e Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Light/With Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.08 ± 1.21 a b	10.38 ± 2.62 c d	0.85 ± 0.17 m n	4.50 ± 0.85 e	0.37 ± 0.05 p	0.12 ± 0.19 f	0.010 ±0.016 q	0.09 ± 0.08 h	0.008 ±0.006 s	0.42 ± 0.52 i	0.036 ±0.048 t	0.19 ± 0.12 j	0.016 ±0.011 u
2	13.02 ± 1.10 a b	11.91 ± 1.33 d	0.92 ± 0.13 m	4.47 ± 1.19 e	0.34 ± 0.07 p	0.28 ± 0.61 f	0.020 ±0.046 q	0.13 ± 0.14 h	0.010 ±0.011 s	0.40 ± 0.47 i	0.030 ±0.034 t	0.31 ± 0.18 i	0.024 ±0.013 u
3	11.86 ± 1.25 a b	10.59 ± 1.99 c d	0.89 ± 0.14 m n	3.87 ± 0.87 e	0.32 ± 0.62 p	0.35 ± 0.40 f	0.029 ±0.032 q	0.08 ± 0.10 h	0.007 ±0.008 s	0.24 ± 0.10 i	0.020 ±0.008 t	0.29 ± 0.19 i	0.024 ±0.016 u
4	13.44 ± 1.52 a	9.50 ± 1.67 c d	0.71 ± 0.13 m n	4.56 ± 0.85 e	0.34 ± 0.07 p	1.41 ± 1.35 g	0.104 ±0.097 r	0.02 ± 0.09 h	0.001 ±0.007 s	0.54 ± 0.35 i	0.042 ±0.031 t	0.18 ± 0.11 i	0.013 ±0.008 u
5	12.56 ± 1.66 a b	8.71 ± 2.05 c	0.69 ± 0.15 n	4.46 ± 0.98 e	0.35 ± 0.06 p	0.12 ± 0.23 f	0.010 ±0.020 q	0.03 ± 0.10 h	0.002 ±0.008 s	0.26 ± 0.13 i	0.021 ±0.010 t	0.16 ± 0.10 i	0.013 ±0.009 u
6	12.68 ± 1.10 a b	9.88 ± 1.94 c d	0.78 ± 0.12 m n	4.87 ± 0.91 e	0.38 ± 0.05 p	0.04 ± 0.10 f	0.003 ±0.008 q	0.05 ± 0.07 h	0.004 ±0.006 s	0.36 ± 0.26 i	0.029 ±0.020 t	0.34 ± 0.23 i	0.026 ±0.017 u
7	12.27 ± 0.84 a b	9.60 ± 2.24 c d	0.79 ± 0.19 m n	4.58 ± 0.99 e	0.37 ± 0.08 p	-0.05 ± 0.08 f	-0.004 ±0.007 q	0.13 ± 0.14 h	0.011 ±0.012 s	0.28 ± 0.15 i	0.023 ±0.012 t	0.29 ± 0.14 i	0.023 ±0.011 u
8	11.25 ± 1.29 b	8.56 ± 1.44 c	0.78 ± 0.18 m n	4.17 ± 0.55 e	0.38 ± 0.07 p	0.26 ± 0.34 f	0.023 ±0.029 q	0.11 ± 0.13 h	0.011 ±0.014 s	0.30 ± 0.07 i	0.027 ±0.007 t	0.20 ± 0.10 i	0.018 ±0.009 u
mean	12.40 ± 1.37	9.89 ± 2.12	0.80 ± 0.16	4.44 ± 0.92	0.36 ± 0.06	0.32 ± 0.70	0.024 ±0.051	0.08 ± 0.11	0.007 ±0.010	0.35 ± 0.31	0.028 ±0.025	0.24 ± 0.21	0.020 ±0.013

Table B1f Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Dark/No Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	11.10 ± 2.20 b	3.30 ± 1.84 c d	0.29 ± 0.14 r s	4.26 ± 1.32 g	0.38 ± 0.08 v w	0.02 ± 0.04 j	0.002 ±0.004 x	0.27 ± 0.18 k	0.023 ±0.011 y	0.34 ± 0.22 m	0.029 ±0.016 a	0.41 ± 0.30 p	0.036 ±0.024 c
2	12.13 ± 1.34 a b	2.50 ± 1.01 c	0.21 ± 0.09 r	5.20 ± 0.85 g h i	0.43 ± 0.04 v w	0.01 ± 0.05 j	0.001 ±0.004 x	0.84 ± 0.81 l	0.070 ±0.066 z	0.87 ± 0.73 n	0.074 ±0.069 b	1.15 ± 0.67 q	0.093 ±0.051 d
3	11.14 ± 1.84 b	3.30 ± 0.80 c d	0.30 ± 0.09 r s	4.71 ± 0.51 g h	0.43 ± 0.07 v w	0.07 ± 0.11 j	0.006 ±0.010 x	0.22 ± 0.13 k	0.020 ±0.011 y	0.28 ± 0.09 m	0.025 ±0.007 a	0.65 ± 0.32 p q	0.057 ±0.027 c d
4	12.37 ± 1.54 a b	5.53 ± 0.98 d e f	0.45 ± 0.07 s t u	4.45 ± 1.55 g h	0.37 ± 0.11 v w	0.02 ± 0.05 j	0.001 ±0.004 x	0.30 ± 0.10 k	0.024 ±0.007 y	0.37 ± 0.09 m	0.031 ±0.009 a	0.50 ± 0.21 p	0.042 ±0.020 c
5	11.73 ± 1.06 a b	3.56 ± 1.12 c d	0.31 ± 0.10 r s	4.16 ± 0.70 g	0.36 ± 0.07 w	0.10 ± 0.21 j	0.008 ±0.017 x	0.34 ± 0.16 k	0.029 ±0.014 y	0.54 ± 0.42 m n	0.047 ±0.037 a b	0.66 ± 0.43 p q	0.057 ±0.040 c d
6	13.42 ± 1.70 a	2.93 ± 2.00 c d	0.22 ± 0.13 r	6.20 ± 1.00 i	0.46 ± 0.04 v	-0.02 ± 0.07 j	-0.001 ±0.005 x	0.32 ± 0.20 k	0.023 ±0.013 y	0.51 ± 0.45 m n	0.037 ±0.030 a b	0.37 ± 0.21 p	0.027 ±0.013 c
7	12.22 ± 0.88 a	4.22 ± 1.62 c d e	0.32 ± 0.11 r s t	5.45 ± 1.15 g h i	0.41 ± 0.08 v w	0.02 ± 0.11 j	0.002 ±0.008 x	0.35 ± 0.29 k	0.026 ±0.021 y	0.57 ± 0.28 m n	0.043 ±0.023 a b	0.55 ± 0.33 p	0.042 ±0.026 c
8	13.35 ± 0.37 a	7.82 ± 2.32 f	0.59 ± 0.18 u	5.51 ± 0.94 g h i	0.41 ± 0.07 v w	-0.05 ± 0.05 j	-0.004 ±0.004 x	0.30 ± 0.15 k	0.023 ±0.011 y	0.42 ± 0.13 m n	0.031 ±0.010 a	0.71 ± 0.59 p q	0.053 ±0.044 c d
9	13.55 ± 0.67 a	6.86 ± 2.12 e f	0.51 ± 0.16 t u	6.04 ± 0.56 h i	0.45 ± 0.05 v w	-0.08 ± 0.08 j	-0.006 ±0.006 x	0.17 ± 0.13 k	0.012 ±0.010 y	0.36 ± 0.19 m	0.027 ±0.015 a	0.38 ± 0.36 p	0.029 ±0.028 c
10	13.56 ± 1.12 a	6.42 ± 3.13 e f	0.47 ± 0.21 s t u	6.22 ± 0.56 i	0.46 ± 0.03 v	0.01 ± 0.03 j	0.000 ±0.002 x	0.34 ± 0.17 k	0.025 ±0.012 y	0.53 ± 0.16 m n	0.039 ±0.012 a b	0.68 ± 0.33 p q	0.050 ±0.023 c d
mean	12.56 ± 1.62	4.64 ± 2.49	0.37 ± 0.18	5.24 ± 1.19	0.42 ± 0.07	0.01 ± 0.10	0.001 ±0.008	0.35 ± 0.34	0.027 ±0.027	0.48 ± 0.36	0.038 ±0.031	0.61 ± 0.44	0.049 ±0.035

Table B1g Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Dark/With Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.51 ± 1.39 a	2.22 ± 1.09 b	0.16 ± 0.08 m	6.01 ± 1.05 e	0.45 ± 0.08 p	0.04 ± 0.09 f	0.003 ±0.007 q	0.71 ± 0.35 h	0.053 ±0.025 s	0.86 ± 0.78 i	0.064 ±0.059 t	1.11 ± 0.58 jk	0.081 ±0.039 u
2	13.47 ± 0.88 a	2.37 ± 0.71 b	0.18 ± 0.05 m	5.51 ± 0.81 e	0.41 ± 0.07 p	0.03 ± 0.10 f	0.002 ±0.007 q	0.75 ± 0.17 h	0.056 ±0.014 s	1.17 ± 0.35 i	0.087 ±0.026 t	2.67 ± 0.94 l	0.20 ±0.074 v
3	13.23 ± 1.11 a	2.77 ± 1.46 b	0.21 ± 0.10 m	5.27 ± 0.84 e	0.40 ± 0.05 p	0.04 ± 0.09 f	0.003 ±0.007 q	0.39 ± 0.35 g	0.028 ±0.026 r	1.22 ± 0.93 i	0.094 ±0.075 t	1.18 ± 1.06 k	0.087 ±0.078 u
4	13.51 ± 2.05 a	5.68 ± 2.40 cd	0.43 ± 0.18 no	6.03 ± 1.71 e	0.44 ± 0.09 p	0.00 ± 0.07 f	0.000 ±0.005 q	0.36 ± 0.22 g	0.025 ±0.015 r	0.54 ± 0.47 i	0.038 ±0.030 t	0.64 ± 0.44 jk	0.046 ±0.029 u
5	13.05 ± 1.28 a	3.45 ± 2.93 bc	0.28 ± 0.24 mn	6.02 ± 0.74 e	0.46 ± 0.04 p	0.41 ± 0.98 f	0.032 ±0.079 q	0.16 ± 0.28 g	0.011 ±0.020 r	0.90 ± 1.11 i	0.071 ±0.094 t	0.46 ± 0.38 jk	0.034 ±0.028 u
6	12.47 ± 1.32 a	7.31 ± 2.69 d	0.59 ± 0.23 o	5.15 ± 1.24 e	0.41 ± 0.08 p	0.21 ± 0.66 f	0.017 ±0.052 q	0.22 ± 0.12 g	0.017 ±0.010 r	0.44 ± 0.18 i	0.035 ±0.015 t	0.40 ± 0.17 j	0.032 ±0.014 u
7	12.43 ± 1.18 a	5.64 ± 1.35 cd	0.46 ± 0.13 no	5.80 ± 1.07 e	0.47 ± 0.07 p	0.17 ± 0.21 f	0.013 ±0.016 q	0.35 ± 0.17 g	0.027 ±0.012 r	0.46 ± 0.12 i	0.037 ±0.010 t	0.58 ± 0.22 jk	0.046 ±0.015 u
8	13.24 ± 0.86 a	4.54 ± 2.71 bc	0.34 ± 0.19 mn	5.30 ± 0.89 e	0.40 ± 0.05 p	0.46 ± 0.45 f	0.035 ±0.033 q	0.33 ± 0.18 g	0.025 ±0.012 r	0.49 ± 0.22 i	0.037 ±0.016 t	0.56 ± 0.29 jk	0.042 ±0.022 u
mean	13.11 ± 1.31	4.25 ± 2.64	0.33 ± 0.21	5.64 ± 1.10	0.43 ± 0.07	0.17 ± 0.47	0.013 ±0.037	0.41 ± 0.31	0.030 ±0.023	0.76 ± 0.67	0.058 ±0.053	0.95 ± 0.91	0.071 ±0.067

Table B1h Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Light/No Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.76 ± 1.66 a	2.37 ± 4.57 b d	0.20 ± 0.39 p q r	5.59 ± 1.37 e	0.44 ± 0.07 s t	0.08 ± 0.05 f	0.006 ±0.003 u	0.93 ± 1.48 g	0.066 ±0.106 v	1.41 ± 0.69 i j	0.108 ±0.050 x y	2.50 ± 1.94 m n	0.19 ±0.15 b c
2	12.89 ± 1.63 a	0.52 ± 0.39 d	0.04 ± 0.04 r	4.75 ± 1.61 e	0.36 ± 0.11 s	0.14 ± 0.21 f	0.012 ±0.017 u	0.48 ± 0.32 g	0.038 ±0.024 v	1.56 ± 1.01 j	0.12 ±0.077 y	2.54 ± 1.91 n	0.20 ±0.15 c
3	12.42 ± 1.20 a	5.12 ± 2.36 b c	0.42 ± 0.20 p q	5.32 ± 0.87 e	0.43 ± 0.08 s t	0.28 ± 0.56 f	0.023 ±0.045 u	0.33 ± 0.11 g	0.026 ±0.008 v	0.48 ± 0.42 h	0.038 ±0.032 w	0.78 ± 0.88 k	0.061 ±0.067 a b
4	12.53 ± 1.13 a	3.08 ± 1.84 b c d	0.25 ± 0.15 p q r	5.75 ± 1.02 e	0.46 ± 0.08 s t	0.01 ± 0.10 f	0.001 ±0.008 u	0.49 ± 0.30 g	0.039 ±0.025 v	0.38 ± 0.25 h	0.031 ±0.020 w	1.27 ± 1.60 k m n	0.11 ±0.14 a b c
5	12.90 ± 0.98 a	5.64 ± 2.78 c	0.44 ± 0.22 p r	4.77 ± 1.20 e	0.37 ± 0.08 s t	1.04 ± 3.16 f	0.076 ±0.231 u	0.26 ± 0.16 g	0.020 ±0.012 v	0.35 ± 0.10 h	0.028 ±0.008 w	0.51 ± 0.42 k	0.040 ±0.029 a
6	12.64 ± 0.53 a	2.38 ± 1.22 b d	0.19 ± 0.11 q	4.88 ± 1.13 e	0.39 ± 0.09 s t	0.06 ± 0.11 f	0.005 ±0.009 u	0.53 ± 0.36 g	0.042 ±0.027 v	0.78 ± 0.28 h i	0.062 ±0.022 w x	1.32 ± 0.27 k m n	0.10 ±0.023 a b c
7	12.31 ± 1.24 a	4.06 ± 1.01 b c	0.33 ± 0.08 p q	5.85 ± 0.54 e	0.48 ± 0.07 t	0.73 ± 1.53 f	0.057 ±0.114 u	0.37 ± 0.12 g	0.030 ±0.008 v	0.49 ± 0.18 h	0.041 ±0.018 w	0.47 ± 0.27 k	0.040 ±0.029 a
8	12.69 ± 1.23 a	3.78 ± 0.81 b c	0.30 ± 0.09 p q	5.76 ± 0.77 e	0.45 ± 0.03 s t	0.04 ± 0.07 f	0.004 ±0.006 u	0.46 ± 0.11 g	0.036 ±0.007 v	0.45 ± 0.06 h	0.036 ±0.007 w	0.60 ± 0.26 k	0.047 ±0.020 a
9	13.44 ± 0.93 a	4.01 ± 1.06 b c	0.30 ± 0.07 p q	4.71 ± 0.80 e	0.35 ± 0.07 s	0.08 ± 0.06 f	0.006 ±0.006 u	0.51 ± 0.21 g	0.039 ±0.017 v	0.47 ± 0.14 h	0.035 ±0.010 w	0.77 ± 0.28 k	0.057 ±0.021 a b
10	12.96 ± 0.63 a	4.57 ± 1.80 b c	0.35 ± 0.13 p q	5.30 ± 0.67 e	0.41 ± 0.05 s t	-0.03 ± 0.07 f	-0.002 ±0.006 u	0.29 ± 0.19 g	0.023 ±0.015 v	0.40 ± 0.19 h	0.031 ±0.015 w	0.89 ± 0.56 k m	0.068 ±0.042 a b
mean	12.76 ± 1.14	3.59 ± 2.38	0.28 ± 0.19	5.26 ± 1.08	0.41 ± 0.08	0.25 ± 1.14	0.019 ±0.084	0.45 ± 0.46	0.035 ±0.033	0.65 ± 0.57	0.051 ±0.044	1.12 ± 1.21	0.088 ±0.096

Table B1i Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Light/With Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	pk/pkg	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.88 ± 1.34 a	6.10 ± 3.04 efg	0.44 ± 0.21 vw	6.20 ± 0.97 ij	0.46 ± 0.04 x	0.03 ± 0.10 k	0.002 ±0.007 a	0.31 ± 0.18 mn	0.022 ±0.011 cd	0.50 ± 0.13 pq	0.036 ±0.009 ef	0.95 ± 0.61 st	0.069 ±0.045 gh
2	13.06 ± 1.49 ab	7.94 ± 1.69 de	0.62 ± 0.15 uv	4.74 ± 1.38 h	0.36 ± 0.09 y	0.02 ± 0.08 k	0.001 ±0.006 a	0.14 ± 0.08 n	0.011 ±0.006 d	0.30 ± 0.11 p	0.023 ±0.008 e	0.32 ± 0.14 r	0.025 ±0.012 g
3	14.36 ± 1.19 a	4.68 ± 2.32 fg	0.33 ± 0.17 w	6.59 ± 0.90 j	0.46 ± 0.04 x	0.40 ± 0.55 kl	0.028 ±0.036 ab	0.37 ± 0.15 m	0.026 ±0.011 c	0.68 ± 0.27 q	0.048 ±0.018 f	1.25 ± 0.77 t	0.087 ±0.053 h
4	13.24 ± 0.77 ab	4.21 ± 2.12 g	0.32 ± 0.17 w	5.30 ± 0.95 hi	0.40 ± 0.08 xy	0.49 ± 0.63 kl	0.036 ±0.045 ab	0.20 ± 0.10 mn	0.015 ±0.007 cd	0.43 ± 0.30 p	0.032 ±0.022 ef	0.61 ± 0.77 rs	0.046 ±0.056 gh
5	13.11 ± 0.67 ab	6.91 ± 1.29 def	0.53 ± 0.08 v	5.21 ± 0.94 hi	0.40 ± 0.07 xy	0.66 ± 0.49 kl	0.051 ±0.039 ab	0.24 ± 0.11 mn	0.018 ±0.008 cd	0.34 ± 0.16 p	0.026 ±0.013 e	0.39 ± 0.15 rs	0.030 ±0.012 g
6	11.37 ± 1.55 c	8.32 ± 1.71 de	0.73 ± 0.11 u	4.32 ± 1.03 h	0.38 ± 0.08 xy	-0.03 ± 0.05 k	-0.002 ±0.004 a	0.22 ± 0.13 mn	0.019 ±0.010 cd	0.26 ± 0.10 p	0.023 ±0.008 e	0.50 ± 0.30 rs	0.043 ±0.023 gh
7	13.51 ± 0.90 a	8.10 ± 2.31 de	0.60 ± 0.15 uv	6.26 ± 0.45 ij	0.46 ± 0.03 x	1.04 ± 1.38 l	0.081 ±0.11 b	0.26 ± 0.12 mn	0.019 ±0.008 cd	0.49 ± 0.21 pq	0.036 ±0.015 ef	0.67 ± 0.23 rst	0.050 ±0.018 gh
8	11.99 ± 1.28 bc	9.16 ± 1.19 d	0.78 ± 0.16 u	4.29 ± 1.14 h	0.35 ± 0.06 y	0.02 ± 0.11 k	0.002 ±0.010 a	0.29 ± 0.21 mn	0.023 ±0.016 cd	0.40 ± 0.14 p	0.033 ±0.009 ef	0.65 ± 0.28 rst	0.055 ±0.028 gh
mean	13.07 ± 1.46	6.93 ± 2.58	0.54 ± 0.22	5.36 ± 1.28	0.41 ± 0.07	0.33 ± 0.68	0.025 ±0.053	0.25 ± 0.15	0.019 ±0.011	0.42 ± 0.22	0.032 ±0.015	0.67 ± 0.54	0.051 ±0.039

Table B1j Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage IV, Dark/No Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.27 ± 1.13 a	4.23 ± 4.03 bcd	0.31 ± 0.29 nop	5.68 ± 1.53 f	0.43 ± 0.10 r	0.27 ± 0.33 g	0.020 ±0.024 st	0.51 ± 0.56 hi	0.038 ±0.042 uv	0.92 ± 0.68 j	0.072 ±0.058 w	0.89 ± 1.07 k	0.065 ±0.076 x
2	13.77 ± 0.90 a	2.57 ± 1.66 cde	0.19 ± 0.13 opq	5.92 ± 1.24 f	0.43 ± 0.09 r	0.35 ± 0.45 g	0.027 ±0.034 st	0.58 ± 0.37 hi	0.042 ±0.026 uv	0.42 ± 0.25 j	0.030 ±0.017 w	1.13 ± 1.00 kl	0.082 ±0.073 x
3	13.46 ± 0.96 a	3.50 ± 1.72 bcde	0.26 ± 0.13 nopq	6.10 ± 1.29 f	0.45 ± 0.09 r	0.15 ± 0.18 g	0.011 ±0.013 st	0.72 ± 0.37 h	0.053 ±0.026 u	0.82 ± 0.56 j	0.060 ±0.040 w	2.30 ± 1.27 lm	0.17 ±0.09 y
4	14.03 ± 1.01 a	0.73 ± 0.41 e	0.05 ± 0.03 q	6.56 ± 1.23 f	0.47 ± 0.08 r	1.43 ± 2.90 g	0.10 ±0.20 s	0.51 ± 0.15 hi	0.036 ±0.009 uv	0.41 ± 0.28 j	0.029 ±0.020 w	1.04 ± 0.85 k	0.074 ±0.058 x
5	13.66 ± 0.76 a	2.25 ± 2.01 de	0.16 ± 0.14 pq	6.29 ± 0.91 f	0.46 ± 0.06 r	0.01 ± 0.10 g	0.000 ±0.007 t	0.82 ± 0.52 h	0.060 ±0.037 u	1.06 ± 0.52 j	0.078 ±0.039 w	2.32 ± 1.09 m	0.17 ±0.08 y
6	12.95 ± 1.77 a	6.45 ± 2.90 bc	0.49 ± 0.20 n	5.29 ± 0.79 f	0.41 ± 0.05 r	0.61 ± 0.09 g	0.048 ±0.010 st	0.34 ± 0.16 hi	0.026 ±0.009 uv	0.29 ± 0.13 j	0.022 ±0.009 w	0.29 ± 0.22 k	0.022 ±0.016 x
7	14.21 ± 1.72 a	5.21 ± 2.11 bcd	0.38 ± 0.16 nop	5.64 ± 1.30 f	0.40 ± 0.09 r	0.84 ± 0.30 g	0.060 ±0.021 st	0.47 ± 0.30 hi	0.032 ±0.020 uv	0.74 ± 0.76 j	0.055 ±0.063 w	0.44 ± 0.26 k	0.032 ±0.019 x
8	13.08 ± 1.43 a	5.88 ± 2.32 b	0.45 ± 0.17 n	5.24 ± 1.19 f	0.40 ± 0.08 r	0.88 ± 0.32 g	0.067 ±0.023 st	0.39 ± 0.23 hi	0.030 ±0.017 uv	0.68 ± 1.01 j	0.052 ±0.073 w	0.64 ± 0.62 k	0.051 ±0.050 x
9	13.41 ± 1.24 a	4.54 ± 2.31 bcd	0.34 ± 0.16 nop	6.67 ± 1.03 f	0.42 ± 0.05 r	0.64 ± 0.26 g	0.048 ±0.020 st	0.35 ± 0.16 hi	0.026 ±0.011 uv	0.37 ± 0.25 j	0.026 ±0.016 w	0.44 ± 0.55 k	0.032 ±0.038 x
10	12.48 ± 1.52 a	5.09 ± 2.12 bcd	0.42 ± 0.18 no	5.85 ± 0.72 f	0.47 ± 0.05 r	0.61 ± 0.20 g	0.050 ±0.017 st	0.16 ± 0.09 i	0.013 ±0.008 v	0.28 ± 0.13 j	0.022 ±0.008 w	0.37 ± 0.18 k	0.029 ±0.012 x
mean	13.43 ± 1.32	4.04 ± 2.80	0.31 ± 0.21	5.82 ± 1.16	0.43 ± 0.08	0.58 ± 0.99	0.043 ±0.069	0.49 ± 0.36	0.036 ±0.026	0.60 ± 0.58	0.045 ±0.044	0.99 ± 1.05	0.073 ±0.078

Table B1k Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage IV, Dark/With Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	pk/bk	Zn/P
1	12.54 ± 1.81 a	1.80 ± 1.05 c	0.15 ± 0.11 p	5.61 ± 0.88 d	0.45 ± 0.05 q	0.15 ± 0.14 e	0.012 ±0.012 r	0.50 ± 0.37 ghi	0.038 ±0.025 t	0.40 ± 0.32 j	0.031 ±0.022 v	1.02 ± 1.33 n	0.08 ± 0.09 y
2	13.10 ± 1.27 ab	3.40 ± 1.29 c	0.27 ± 0.11 p	5.26 ± 1.23 d	0.40 ± 0.09 q	0.00 ± 0.07 e	-0.000 ±0.005 r	0.95 ± 0.59 g	0.075 ±0.047 u	1.33 ± 0.72 m	0.10 ±0.056 x	2.59 ± 1.56 o	0.21 ± 0.13 z
3	12.66 ± 1.19 ab	2.90 ± 1.40 c	0.23 ± 0.12 p	5.11 ± 1.80 d	0.40 ± 0.13 q	0.24 ± 0.27 ef	0.019 ±0.022 rs	0.52 ± 0.26 ghi	0.042 ±0.023 tu	0.77 ± 0.49 jklm	0.060 ±0.037 vwx	1.74 ± 0.89 no	0.14 ± 0.06 yz
4	12.70 ± 0.97 ab	3.38 ± 0.88 c	0.27 ± 0.08 p	5.38 ± 0.92 d	0.42 ± 0.06 q	0.35 ± 1.06 ef	0.025 ±0.074 rs	0.91 ± 0.51 gh	0.074 ±0.043 u	0.65 ± 0.30 jkl	0.052 ±0.025 vw	1.63 ± 1.01 no	0.13 ± 0.08 yz
5	13.49 ± 1.28 ab	3.52 ± 1.72 c	0.26 ± 0.13 p	5.93 ± 0.66 d	0.44 ± 0.07 q	-0.03 ± 0.05 e	-0.002 ±0.004 r	0.41 ± 0.14 i	0.030 ±0.011 t	0.46 ± 0.18 jk	0.035 ±0.014 vw	0.84 ± 0.60 n	0.06 ± 0.05 y
6	14.08 ± 0.94 ab	2.50 ± 1.53 c	0.18 ± 0.11 p	6.43 ± 0.64 d	0.46 ± 0.04 q	0.89 ± 0.76 f	0.062 ±0.052 s	0.62 ± 0.27 ghi	0.044 ±0.020 tu	0.99 ± 0.57 klm	0.071 ±0.042 vwx	1.28 ± 0.42 n	0.09 ± 0.03 y
7	12.51 ± 1.54 a	3.23 ± 1.80 c	0.26 ± 0.13 p	5.45 ± 1.33 d	0.43 ± 0.07 q	0.47 ± 0.54 ef	0.039 ±0.043 rs	0.46 ± 0.22 hi	0.036 ±0.015 t	0.55 ± 0.26 jkl	0.044 ±0.019 vw	0.85 ± 0.80 n	0.07 ± 0.06 y
8	14.22 ± 0.75 b	2.40 ± 0.69 c	0.17 ± 0.05 p	6.10 ± 0.72 d	0.43 ± 0.05 q	0.07 ± 0.48 e	0.006 ±0.037 r	0.64 ± 0.22 ghi	0.045 ±0.017 tu	1.07 ± 0.41 lm	0.076 ±0.031 wx	1.76 ± 0.70 no	0.12 ± 0.05 yz
mean	13.16 ± 1.37	2.89 ± 1.41	0.22 ± 0.11	5.66 ± 1.13	0.43 ± 0.07	0.27 ± 0.59	0.020 ±0.042	0.63 ± 0.39	0.048 ±0.031	0.78 ± 0.52	0.059 ±0.039	1.46 ± 1.09	0.11 ± 0.08

Values in the same column followed by the same letter are not significantly different at $P > 0.05$.

Table B1m Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage IV, Light/No Nutrients *C. maxima* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.25 ± 1.03 a	1.17 ± 0.80 b	0.09 ± 0.07 q	5.50 ± 0.76 de	0.42 ± 0.05 tu	0.00 ± 0.04 f	0.000 ±0.003 v	0.86 ± 0.33 j	0.065 ±0.026 xy	1.38 ± 0.71 m	0.11 ± 0.06 a	2.35 ± 1.20 nop	0.18 ± 0.09 cd
2	13.16 ± 1.43 a	0.88 ± 0.48 b	0.07 ± 0.04 q	5.48 ± 1.20 de	0.42 ± 0.09 tu	0.24 ± 0.33 fg	0.018 ±0.026 vw	1.57 ± 0.60 jk	0.12 ±0.05 yz	1.48 ± 0.43 m	0.11 ± 0.03 a	4.64 ± 1.07 p	0.36 ± 0.09 e
3	14.03 ± 1.24 a	0.98 ± 0.86 b	0.07 ± 0.06 q	6.36 ± 1.09 d	0.45 ± 0.06 t	0.54 ± 0.35 fg	0.040 ±0.026 vw	2.40 ± 1.11 k	0.17 ±0.08 z	1.38 ± 0.44 m	0.10 ± 0.03 a	3.27 ± 1.62 nop	0.23 ± 0.12 cde
4	14.57 ± 1.36 a	2.44 ± 1.00 bc	0.17 ± 0.07 qr	5.79 ± 1.35 de	0.40 ± 0.10 tu	0.67 ± 0.25 fgh	0.046 ±0.016 vw	0.61 ± 0.34 j	0.041 ±0.020 x	0.65 ± 0.68 m	0.04 ± 0.05 a	1.03 ± 1.92 n	0.07 ± 0.13 c
5	13.48 ± 1.54 a	3.47 ± 1.39 c	0.26 ± 0.11 r	4.28 ± 1.52 e	0.32 ± 0.10 u	1.28 ± 0.46 h	0.097 ±0.038 s	0.65 ± 0.38 j	0.046 ±0.024 x	1.53 ± 0.97 m	0.12 ± 0.08 a	2.06 ± 1.42 no	0.15 ± 0.10 cd
6	14.09 ± 1.62 a	1.73 ± 2.20 b	0.14 ± 0.19 qr	6.59 ± 1.26 d	0.47 ± 0.06 t	0.75 ± 0.87 gh	0.054 ±0.061 ws	1.32 ± 1.05 j	0.088 ±0.066 xy	1.53 ± 1.20 m	0.12 ± 0.12 a	4.16 ± 2.52 op	0.30 ± 0.19 de
mean	13.78 ± 1.44	1.76 ± 1.58	0.13 ± 0.13	5.74 ± 1.40	0.42 ± 0.09	0.59 ± 0.63	0.043 ±0.046	1.25 ± 0.94	0.090 ±0.067	1.35 ± 0.85	0.10 ± 0.08	3.04 ± 2.11	0.22 ± 0.16

Table B2a Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage I, *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	14.06 ± 1.11 a	10.75 ± 2.49 de	0.77 ± 0.18 mn	5.22 ± 1.08 g	0.37 ± 0.07 pq	0.98 ± 0.79 h	0.07 ± 0.05 r	-0.05 ± 0.09 i	-0.004 ±0.006 s	0.28 ± 0.13 j	0.020 ±0.009 t	0.19 ± 0.12 k	0.014 ±0.008 u
2	13.25 ± 1.08 abc	11.17 ± 3.13 d	0.85 ± 0.23 mn	4.33 ± 1.23 g	0.33 ± 0.09 q	1.29 ± 0.97 h	0.10 ± 0.07 r	0.02 ± 0.12 i	0.001 ±0.008 s	0.36 ± 0.20 j	0.027 ±0.014 t	0.31 ± 0.27 k	0.023 ±0.019 u
3	12.53 ± 1.23 abc	11.99 ± 1.42 d	0.96 ± 0.13 m	4.90 ± 0.92 g	0.39 ± 0.06 pq	0.95 ± 0.69 h	0.08 ± 0.06 r	-0.05 ± 0.09 i	-0.004 ±0.007 s	0.33 ± 0.11 j	0.026 ±0.009 t	0.19 ± 0.10 k	0.015 ±0.009 u
4	13.98 ± 1.21 ab	11.67 ± 1.98 d	0.83 ± 0.09 mn	5.47 ± 1.22 g	0.39 ± 0.09 pq	1.37 ± 1.24 h	0.10 ± 0.10 r	-0.01 ± 0.10 i	-0.006 ±0.007 s	0.40 ± 0.18 j	0.029 ±0.013 t	0.27 ± 0.21 k	0.020 ±0.016 u
5	13.00 ± 0.79 abc	10.84 ± 2.34 de	0.84 ± 0.18 mn	4.87 ± 0.83 g	0.38 ± 0.07 pq	0.76 ± 0.60 h	0.06 ± 0.04 r	0.04 ± 0.15 i	0.003 ±0.012 s	0.33 ± 0.21 j	0.026 ±0.016 t	0.29 ± 0.11 k	0.022 ±0.009 u
6	12.38 ± 1.02 bc	7.97 ± 1.30 ef	0.65 ± 0.13 n	5.25 ± 1.13 g	0.42 ± 0.08 pq	1.05 ± 0.59 h	0.09 ± 0.05 r	0.08 ± 0.20 i	0.006 ±0.015 s	0.24 ± 0.11 j	0.019 ±0.008 t	0.23 ± 0.28 k	0.018 ±0.020 u
7	12.93 ± 1.40 abc	6.91 ± 2.19 f	0.54 ± 0.16 o	5.05 ± 0.96 g	0.39 ± 0.07 pq	1.70 ± 0.35 h	0.13 ± 0.03 r	0.04 ± 0.10 i	0.003 ±0.008 s	0.30 ± 0.22 j	0.023 ±0.018 t	0.25 ± 0.14 k	0.020 ±0.012 u
8	11.98 ± 1.32 c	9.84 ± 2.14 def	0.82 ± 0.16 mno	5.54 ± 1.15 g	0.46 ± 0.08 p	0.97 ± 0.49 h	0.08 ± 0.04 r	-0.03 ± 0.09 i	-0.002 ±0.007 s	0.29 ± 0.18 j	0.024 ±0.013 t	0.23 ± 0.12 k	0.020 ±0.010 u
mean	13.01 ± 1.30	10.14 ± 2.70	0.78 ± 0.20	5.08 ± 1.09	0.39 ± 0.08	1.13 ± 0.78	0.09 ± 0.06	0.00 ± 0.13	0.002 ±0.010	0.31 ± 0.17	0.024 ±0.013	0.24 ± 0.18	0.019 ±0.013

Table B2b Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Dark/No Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	9.97 ± 2.02 b	4.32 ± 2.95 c e	0.46 ± 0.35 m n	4.41 ± 1.08 f	0.44 ± 0.07 p	1.44 ± 1.33 h	0.14 ± 0.12 q	0.05 ± 0.13 i	0.006 ±0.015 r	0.47 ± 0.51 j	0.047 ±0.047 t	0.23 ± 0.22 k	0.022 ±0.019 u
2	12.28 ± 1.38 a b	4.68 ± 1.90 c	0.38 ± 0.16 m	5.00 ± 1.22 f	0.42 ± 0.13 p	1.20 ± 0.86 h	0.09 ± 0.06 q	0.02 ± 0.13 i	0.001 ±0.011 r	0.18 ± 0.09 j	0.015 ±0.007 s	0.30 ± 0.43 k	0.024 ±0.034 u
3	12.72 ± 1.32 a	5.66 ± 1.83 c d	0.44 ± 0.12 m n	5.98 ± 0.89 f g	0.47 ± 0.07 p	0.82 ± 0.68 h	0.07 ± 0.06 q	0.06 ± 0.22 i	0.004 ±0.017 r	0.47 ± 0.29 j	0.037 ±0.024 s t	0.31 ± 0.14 k	0.024 ±0.009 u
4	13.95 ± 1.29 a	6.11 ± 2.60 c d	0.44 ± 0.19 m n	5.28 ± 1.44 f	0.38 ± 0.11 p	1.78 ± 1.36 h	0.13 ± 0.10 q	0.00 ± 0.11 i	0.000 ±0.008 r	0.30 ± 0.14 j	0.021 ±0.010 s t	0.14 ± 0.15 k	0.010 ±0.012 u
5	12.60 ± 1.80 a	8.45 ± 1.66 d	0.68 ± 0.13 n	5.66 ± 1.40 f g	0.45 ± 0.07 p	0.61 ± 0.74 h	0.05 ± 0.06 q	-0.03 ± 0.05 i	-0.003 ±0.004 r	0.47 ± 0.26 j	0.037 ±0.021 s t	0.38 ± 0.48 k	0.029 ±0.034 u
6	13.08 ± 1.15 a	5.78 ± 2.22 c d	0.44 ± 0.17 m n	5.68 ± 1.26 f g	0.43 ± 0.08 p	1.43 ± 1.71 h	0.12 ± 0.15 q	-0.01 ± 0.07 i	-0.001 ±0.005 r	0.25 ± 0.16 j	0.019 ±0.011 s t	0.26 ± 0.22 k	0.019 ±0.017 u
7	14.14 ± 1.06 a	5.43 ± 1.78 c	0.39 ± 0.13 m	5.91 ± 0.92 f g	0.42 ± 0.06 p	2.33 ± 1.30 h	0.16 ± 0.09 q	-0.02 ± 0.11 i	-0.001 ±0.008 r	0.23 ± 0.10 j	0.017 ±0.007 s	0.34 ± 0.27 k	0.024 ±0.019 u
8	13.71 ± 1.68 a	1.64 ± 1.16 e	0.13 ± 0.10 o	6.91 ± 1.03 g	0.51 ± 0.05 p	1.48 ± 1.06 h	0.11 ± 0.07 q	-0.03 ± 0.15 i	-0.002 ±0.011 r	0.25 ± 0.12 j	0.018 ±0.009 s t	0.49 ± 0.31 k	0.035 ±0.019 u
mean	12.80 ± 1.90	5.26 ± 2.68	0.42 ± 0.22	5.60 ± 1.32	0.44 ± 0.09	1.39 ± 1.23	0.11 ± 0.10	0.00 ± 0.13	0.001 ±0.011	0.33 ± 0.26	0.026 ±0.023	0.30 ± 0.30	0.023 ±0.022

Table B2c Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Dark/With Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.84 ± 0.71 a	7.10 ± 1.20 b	0.55 ± 0.09 m	5.58 ± 0.62 e f	0.44 ± 0.05 q r	1.14 ± 1.24 g h	0.09 ± 0.09 s	-0.01 ± 0.06 i	-0.001 ±0.005 t	0.43 ± 0.22 j	0.034 ±0.018 u	0.28 ± 0.18 k	0.022 ±0.015 v
2	11.98 ± 0.48 a	5.17 ± 1.32 b c	0.43 ± 0.11 m n o	4.54 ± 0.94 f	0.38 ± 0.08 r	1.73 ± 0.67 g h	0.14 ± 0.05 s	0.00 ± 0.10 i	0.000 ±0.008 t	0.30 ± 0.08 j	0.025 ±0.007 u	0.28 ± 0.12 k	0.023 ±0.010 v
3	12.47 ± 1.54 a	6.82 ± 2.46 b	0.55 ± 0.21 m	6.23 ± 0.96 e	0.50 ± 0.06 q	0.73 ± 0.43 h	0.06 ± 0.04 s	0.00 ± 0.03 i	0.000 ±0.003 t	0.31 ± 0.13 j	0.025 ±0.009 u	0.19 ± 0.16 k	0.016 ±0.014 v
4	12.78 ± 1.10 a	3.00 ± 2.07 c d	0.24 ± 0.16 o p	5.42 ± 1.05 e f	0.43 ± 0.08 q r	1.74 ± 1.20 g h	0.14 ± 0.09 s	0.08 ± 0.09 i	0.006 ±0.007 t	0.36 ± 0.09 j	0.028 ±0.006 u	0.28 ± 0.14 k	0.022 ±0.010 v
5	12.84 ± 1.33 a	5.10 ± 2.49 b c	0.40 ± 0.20 m n o	5.58 ± 1.00 e f	0.43 ± 0.06 q r	2.15 ± 1.18 g h	0.17 ± 0.09 s	-0.05 ± 0.07 i	0.000 ±0.005 t	0.32 ± 0.17 j	0.025 ±0.013 u	0.18 ± 0.07 k	0.014 ±0.006 v
6	13.23 ± 0.99 a	6.93 ± 2.06 b	0.53 ± 0.16 m n	6.32 ± 0.83 e	0.48 ± 0.04 q	1.50 ± 1.46 g h	0.11 ± 0.10 s	0.00 ± 0.11 i	0.000 ±0.008 t	0.39 ± 0.25 j	0.029 ±0.018 u	0.21 ± 0.12 k	0.016 ±0.009 v
7	13.29 ± 1.27 a	2.13 ± 2.16 d	0.17 ± 0.19 p	6.47 ± 0.62 e	0.49 ± 0.03 q	2.36 ± 1.29 g	0.18 ± 0.10 s	-0.01 ± 0.04 i	-0.001 ±0.003 t	0.38 ± 0.14 j	0.029 ±0.011 u	0.21 ± 0.14 k	0.016 ±0.013 v
8	11.69 ± 1.23 a	3.69 ± 1.06 c d	0.32 ± 0.09 n o p	5.43 ± 1.26 e f	0.46 ± 0.10 q r	1.45 ± 1.07 g h	0.13 ± 0.10 s	0.13 ± 0.16 i	0.012 ±0.014 t	0.26 ± 0.10 j	0.023 ±0.011 u	0.16 ± 0.08 k	0.014 ±0.007 v
mean	12.64 ± 1.22	4.99 ± 2.57	0.40 ± 0.20	5.70 ± 1.07	0.45 ± 0.07	1.60 ± 1.17	0.13 ± 0.09	0.02 ± 0.10	0.001 ±0.009	0.34 ± 0.16	0.027 ±0.012	0.22 ± 0.13	0.018 ±0.011

Table B2d Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Light/No Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.92 ± 1.1 ab	5.55 ± 2.85 e	0.43 ± 0.21 tu	6.19 ± 0.78 gh	0.48 ± 0.06 w	1.26 ± 0.71 ik	0.10 ± 0.06 x	-0.04 ± 0.05 m	-0.003 ±0.004 z	0.16 ± 0.07 n	0.012 ±0.005 a	0.25 ± 0.11 qr	0.019 ±0.009 c
2	12.07 ± 1.61 abc	2.74 ± 1.65 d	0.24 ± 0.15 st	5.65 ± 1.23 gh	0.46 ± 0.06 w	1.10 ± 0.83 ik	0.09 ± 0.07 x	0.04 ± 0.11 m	0.003 ±0.009 z	0.20 ± 0.11 no	0.017 ±0.009 a	0.30 ± 0.18 qr	0.026 ±0.017 cd
3	12.84 ± 1.04 ab	2.52 ± 1.87 d	0.20 ± 0.15 s	5.44 ± 1.90 g	0.42 ± 0.15 w	2.73 ± 1.46 j	0.21 ± 0.11 y	0.00 ± 0.07 m	0.000 ±0.005 z	0.30 ± 0.10 nop	0.024 ±0.009 ab	0.29 ± 0.17 qr	0.023 ±0.014 c
4	14.16 ± 1.12 b	3.06 ± 1.08 de	0.22 ± 0.08 st	7.20 ± 0.95 h	0.51 ± 0.06 w	1.86 ± 1.02 ij	0.13 ± 0.07 xy	0.10 ± 0.14 m	0.007 ±0.009 z	0.40 ± 0.24 p	0.028 ±0.016 ab	0.43 ± 0.26 qr	0.030 ±0.017 cd
5	12.29 ± 1.91 ab	9.28 ± 2.50 f	0.75 ± 0.13 v	5.79 ± 0.86 gh	0.47 ± 0.06 w	0.97 ± 0.71 ik	0.08 ± 0.05 x	0.00 ± 0.12 m	0.001 ±0.010 z	0.18 ± 0.09 n	0.015 ±0.008 a	0.21 ± 0.14 q	0.017 ±0.011 c
6	10.07 ± 2.05 c	5.50 ± 1.97 e	0.56 ± 0.23 uv	5.20 ± 1.23 g	0.52 ± 0.10 w	0.51 ± 0.44 k	0.06 ± 0.05 x	0.05 ± 0.09 m	0.004 ±0.008 z	0.18 ± 0.08 n	0.019 ±0.010 ab	0.53 ± 0.42 r	0.048 ±0.028 d
7	10.84 ± 1.40 ac	5.12 ± 1.32 de	0.48 ± 0.16 u	5.48 ± 1.31 gh	0.50 ± 0.09 w	1.64 ± 0.63 ijk	0.15 ± 0.06 xy	0.03 ± 0.08 m	0.004 ±0.009 z	0.22 ± 0.12 nop	0.021 ±0.011 ab	0.30 ± 0.14 qr	0.027 ±0.013 cd
8	11.40 ± 1.42 ac	2.68 ± 1.78 d	0.24 ± 0.14 st	4.74 ± 1.29 g	0.42 ± 0.10 w	1.76 ± 0.91 ij	0.15 ± 0.07 xy	0.04 ± 0.07 m	0.004 ±0.007 z	0.38 ± 0.20 op	0.034 ±0.019 b	0.18 ± 0.07 q	0.015 ±0.006 c
mean	12.07 ± 1.88	4.56 ± 2.87	0.39 ± 0.24	5.71 ± 1.37	0.47 ± 0.09	1.48 ± 1.06	0.12 ± 0.08	0.03 ± 0.10	0.003 ±0.008	0.25 ± 0.16	0.021 ±0.013	0.31 ± 0.23	0.026 ±0.018

Table B2e Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage II, Light/With Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	11.45 ± 1.70 b	1.37 ± 1.14 c	0.12 ± 0.10 r	4.99 ±0.67 g	0.44 ± 0.07 v	1.14 ± 0.70 jk	0.10 ± 0.06 wx	0.03 ± 0.12 m	0.003 ±0.010 y	0.19 ± 0.10 n	0.016 ±0.007 z	0.47 ± 0.24 o	0.042 ±0.024 b
2	11.97 ± 1.18 ab	5.46 ± 1.73 de	0.45 ± 0.12 stu	5.01 ± 0.56 g	0.42 ± 0.05 v	0.48 ± 0.75 j	0.04 ± 0.06 w	0.06 ± 0.06 m	0.004 ±0.005 y	0.25 ± 0.13 n	0.020 ±0.010 z	0.21 ± 0.11 pq	0.017 ±0.009 a
3	11.94 ± 1.16 ab	3.19 ± 2.01 cd	0.28 ± 0.20 st	5.59 ± 0.53 ghi	0.47 ± 0.03 v	1.16 ± 0.95 jk	0.09 ± 0.07 wx	0.03 ± 0.07 m	0.002 ±0.007 y	0.18 ± 0.07 n	0.015 ±0.007 z	0.10 ± 0.10 q	0.008 ±0.009 a
4	13.49 ± 0.91 a	8.92 ± 2.23 f	0.66 ± 0.15 u	5.66 ± 1.13 ghi	0.42 ± 0.08 v	0.75 ± 0.46 jk	0.06 ± 0.04 wx	-0.01 ± 0.04 m	-0.006 ±0.003 y	0.24 ± 0.08 n	0.018 ±0.006 z	0.18 ± 0.07 pq	0.013 ±0.006 a
5	12.80 ± 1.64 ab	6.87 ± 2.81 ef	0.53 ± 0.19 tu	5.12 ± 0.89 gh	0.40 ± 0.05 v	0.48 ± 0.50 j	0.04 ± 0.03 w	0.09 ± 0.11 m	0.001 ±0.009 y	0.30 ± 0.09 n	0.024 ±0.007 z	0.27 ± 0.11 opq	0.021 ±0.007 a
6	13.33 ± 0.58 a	7.93 ± 2.15 ef	0.59 ± 0.15 tu	6.10 ± 0.84 hi	0.46 ± 0.05 v	1.09 ± 0.87 jk	0.08 ± 0.07 wx	0.01 ± 0.05 m	0.000 ±0.003 y	0.30 ± 0.13 n	0.023 ±0.011 z	0.32 ± 0.11 op	0.024 ±0.008 a
7	13.55 ± 1.06 a	5.43 ± 2.28 de	0.40 ± 0.17 st	6.37 ± 0.72 i	0.47 ± 0.04 v	1.36 ± 1.30 jk	0.11 ± 0.11 wx	-0.02 ± 0.07 m	-0.002 ±0.006 y	0.39 ± 0.35 m	0.029 ±0.029 z	0.33 ± 0.19 op	0.025 ±0.016 ab
8	13.18 ± 0.75 a	7.48 ± 1.85 ef	0.57 ± 0.15 tu	5.87 ± 0.57 ghi	0.45 ± 0.04 v	1.78 ± 0.91 k	0.14 ± 0.07 x	-0.05 ± 0.09 m	-0.004 ±0.007 y	0.30 ± 0.09 m	0.023 ±0.007 z	0.29 ± 0.14 opq	0.023 ±0.011 a
mean	12.71 ± 1.36	5.83 ± 3.10	0.45 ± 0.23	5.59 ± 0.88	0.44 ± 0.06	1.03 ± 0.91	0.08 ± 0.07	0.02 ± 0.09	0.001 ±0.007	0.27 ± 0.16	0.021 ±0.013	0.27 ± 0.17	0.022 ±0.015

Table B2f Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Dark/No Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	12.24 ± 1.88 a	1.75 ± 1.18 b	0.14 ± 0.09 mn	6.84 ± 1.36 d	0.56 ± 0.10 o	3.24 ± 1.12 g	0.27 ± 0.09 r	0.03 ± 0.11 h	0.003 ±0.010 s	0.26 ± 0.22 ij	0.023 ±0.021 tu	0.46 ± 0.23 k	0.039 ±0.020 vw
2	13.34 ± 1.30 a	2.96 ± 1.94 bc	0.23 ± 0.15 mn	7.24 ± 1.20 d	0.54 ± 0.08 o	0.69 ± 0.70 e	0.05 ± 0.05 p	-0.01 ± 0.06 h	-0.001 ±0.005 s	0.63 ± 0.37 ij	0.048 ±0.029 tu	0.51 ± 0.22 k	0.039 ±0.019 vw
3	12.88 ± 2.08 a	2.21 ± 1.14 bc	0.18 ± 0.10 mn	7.14 ± 0.79 d	0.57 ± 0.12 o	1.24 ± 1.31 ef	0.09 ± 0.09 pq	0.03 ± 0.20 h	0.003 ±0.014 s	0.47 ± 0.27 ij	0.035 ±0.018 tu	0.54 ± 0.31 kl	0.042 ±0.022 vw
4	13.42 ± 1.37 a	0.73 ± 0.65 b	0.06 ± 0.05 n	7.55 ± 0.96 d	0.56 ± 0.05 o	2.06 ± 1.50 efg	0.15 ± 0.10 pq	0.01 ± 0.08 h	0.000 ±0.007 s	0.23 ± 0.23 j	0.017 ±0.018 u	0.27 ± 0.15 k	0.021 ±0.013 v
5	13.45 ± 1.41 a	4.30 ± 2.33 c	0.32 ± 0.16 m	6.52 ± 0.86 d	0.49 ± 0.07 o	1.27 ± 0.97 ef	0.09 ± 0.07 pq	0.02 ± 0.18 h	0.002 ±0.013 s	0.69 ± 0.39 i	0.052 ±0.030 tu	1.24 ± 1.29 l	0.088 ±0.088 w
6	13.58 ± 1.48 a	2.86 ± 1.65 bc	0.21 ± 0.12 mn	6.92 ± 0.84 d	0.51 ± 0.06 o	2.54 ± 1.02 fg	0.19 ± 0.07 qr	-0.01 ± 0.13 h	-0.001 ±0.009 s	0.42 ± 0.20 ij	0.030 ±0.012 tu	0.28 ± 0.25 k	0.020 ±0.016 v
7	13.71 ± 0.94 a	2.09 ± 1.82 bc	0.15 ± 0.13 mn	7.31 ± 0.73 d	0.53 ± 0.05 o	2.65 ± 1.17 fg	0.19 ± 0.08 qr	0.13 ± 0.16 h	0.009 ±0.012 s	0.54 ± 0.27 ij	0.039 ±0.018 tu	0.70 ± 0.41 kl	0.051 ±0.029 vw
8	13.12 ± 0.99 a	3.14 ± 2.40 bc	0.24 ± 0.19 mn	7.11 ± 1.29 d	0.54 ± 0.08 o	1.68 ± 0.14 efg	0.13 ± 0.08 pq	0.05 ± 0.13 h	0.004 ±0.010 s	0.67 ± 0.49 ij	0.053 ±0.045 t	0.59 ± 0.20 kl	0.045 ±0.014 vw
mean	13.23 ± 1.48	2.50 ± 1.94	0.19 ± 0.14	7.08 ± 1.03	0.54 ± 0.08	1.92 ± 1.34	0.14 ± 0.10	0.03 ± 0.14	0.002 ±0.010	0.49 ± 0.35	0.037 ±0.028	0.57 ± 0.57	0.043 ±0.040

Table B2g Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Dark/With Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.22 ± 1.05 a	1.67 ± 1.69 b	0.13 ± 0.14 j	6.30 ± 1.05 c	0.48 ± 0.07 k	0.66 ± 0.63 d	0.05 ± 0.05 m	0.00 ± 0.07 g	0.000 ±0.005 p	0.42 ± 0.18 h	0.032 ±0.013 q	0.53 ± 0.33 i	0.041 ±0.025 r
2	11.94 ± 1.00 a	1.52 ± 0.96 b	0.13 ± 0.09 j	6.34 ± 0.73 c	0.53 ± 0.05 k	1.56 ± 1.56 de	0.14 ± 0.14 mn	0.04 ± 0.09 g	0.003 ±0.007 p	0.36 ± 0.16 h	0.030 ±0.015 q	0.46 ± 0.22 i	0.038 ±0.017 r
3	11.69 ± 1.00 a	2.45 ± 1.34 b	0.21 ± 0.13 j	5.63 ± 1.13 c	0.48 ± 0.07 k	2.33 ± 1.15 def	0.20 ± 0.10 mn	0.12 ± 0.10 g	0.010 ±0.008 p	0.41 ± 0.18 h	0.036 ±0.018 q	0.37 ± 0.15 i	0.031 ±0.013 r
4	12.75 ± 1.30 a	1.50 ± 1.24 b	0.12 ± 0.10 j	6.43 ± 0.50 c	0.51 ± 0.05 k	2.12 ± 1.21 def	0.16 ± 0.08 mn	0.06 ± 0.07 g	0.005 ±0.006 p	0.63 ± 0.38 h	0.050 ±0.031 q	0.42 ± 0.12 i	0.033 ±0.010 r
5	12.90 ± 1.83 a	1.82 ± 1.39 b	0.15 ± 0.12 j	6.69 ± 1.01 c	0.52 ± 0.05 k	1.55 ± 1.03 de	0.12 ± 0.07 mn	0.02 ± 0.09 g	0.001 ±0.006 p	0.48 ± 0.33 h	0.039 ±0.031 q	0.38 ± 0.19 i	0.030 ±0.017 r
6	12.64 ± 1.09 a	2.01 ± 1.34 b	0.16 ± 0.11 j	6.95 ± 1.04 c	0.55 ± 0.06 k	2.64 ± 1.62 def	0.21 ± 0.12 mn	0.08 ± 0.14 g	0.006 ±0.010 p	0.40 ± 0.15 h	0.032 ±0.013 q	0.41 ± 0.23 i	0.032 ±0.018 r
7	12.98 ± 0.98 a	2.78 ± 1.35 b	0.22 ± 0.11 j	6.31 ± 0.79 c	0.49 ± 0.06 k	3.68 ± 1.18 f	0.29 ± 0.10 n	0.03 ± 0.14 g	0.002 ±0.011 p	0.48 ± 0.21 h	0.037 ±0.016 q	0.29 ± 0.16 i	0.023 ±0.013 r
8	13.20 ± 0.89 a	2.92 ± 1.93 b	0.22 ± 0.15 j	6.47 ± 0.48 c	0.49 ± 0.05 k	3.27 ± 2.72 ef	0.26 ± 0.23 n	0.04 ± 0.09 g	0.003 ±0.007 p	0.33 ± 0.26 h	0.025 ±0.019 q	0.38 ± 0.16 i	0.029 ±0.012 r
mean	12.66 ± 1.25	2.08 ± 1.46	0.17 ± 0.12	6.39 ± 0.91	0.51 ± 0.06	2.23 ± 1.71	0.18 ± 0.14	0.05 ± 0.10	0.004 ±0.008	0.44 ± 0.25	0.035 ±0.021	0.41 ± 0.21	0.032 ±0.016

Table B2h Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Light/No Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.79 ± 0.71 a	0.77 ± 1.09 de	0.06 ± 0.08 op	7.50 ± 0.73 f	0.54 ± 0.05 q	2.44 ± 0.88 gh	0.18 ± 0.06 r	0.14 ± 0.33 i	0.011 ±0.025 s	0.41 ± 0.14 j	0.030 ±0.009 t	0.31 ± 0.09 k	0.023 ±0.007 u
2	13.97 ± 1.19 a	1.01 ± 0.76 cde	0.07 ± 0.04 nop	7.19 ± 1.17 f	0.52 ± 0.09 q	2.07 ± 1.22 gh	0.15 ± 0.09 r	0.06 ± 0.12 i	0.004 ±0.009 s	0.44 ± 0.24 j	0.031 ±0.016 t	0.42 ± 0.18 k	0.030 ±0.014 u
3	14.52 ± 1.26 a	1.87 ± 1.14 bcd	0.13 ± 0.08 mno	7.48 ± 0.67 f	0.52 ± 0.05 q	1.49 ± 0.59 h	0.10 ± 0.04 r	0.04 ± 0.15 i	0.003 ±0.010 s	0.32 ± 0.23 j	0.022 ±0.014 t	0.31 ± 0.18 k	0.022 ±0.013 u
4	14.10 ± 1.28 a	0.32 ± 0.14 e	0.02 ± 0.01 p	7.82 ± 0.80 f	0.56 ± 0.05 q	4.30 ± 1.54 g	0.31 ± 0.12 r	0.08 ± 0.13 i	0.006 ±0.009 s	0.43 ± 0.15 j	0.031 ±0.012 t	0.35 ± 0.17 k	0.025 ±0.011 u
5	13.76 ± 1.32 a	2.39 ± 1.18 bc	0.17 ± 0.08 mn	7.07 ± 1.04 f	0.51 ± 0.06 q	2.79 ± 2.85 gh	0.22 ± 0.26 r	0.01 ± 0.10 i	0.000 ±0.008 s	0.29 ± 0.17 j	0.021 ±0.011 t	0.38 ± 0.19 k	0.028 ±0.014 u
6	13.85 ± 1.82 a	2.50 ± 1.24 b	0.18 ± 0.09 m	7.48 ± 1.34 f	0.54 ± 0.05 q	2.94 ± 0.95 gh	0.21 ± 0.06 r	0.11 ± 0.11 i	0.008 ±0.008 s	0.31 ± 0.16 j	0.022 ±0.012 t	0.31 ± 0.12 k	0.023 ±0.009 u
7	113.93 ± 1.16 a	1.06 ± 1.16 cde	0.08 ± 0.08 mnop	7.40 ± 0.82 f	0.53 ± 0.05 q	2.25 ± 1.68 gh	0.16 ± 0.12 r	0.17 ± 0.14 i	0.012 ±0.010 s	0.35 ± 0.17 j	0.026 ±0.013 t	1.09 ± 1.65 k	0.080 ±0.012 u
8	13.68 ± 1.06 a	1.32 ± 0.96 bcde	0.10 ± 0.07 mnop	6.91 ± 0.95 f	0.51 ± 0.06 q	3.08 ± 2.72 gh	0.23 ± 0.20 r	0.03 ± 0.20 i	0.002 ±0.015 s	0.67 ± 0.66 j	0.048 ±0.044 t	0.72 ± 0.64 k	0.053 ±0.049 u
mean	13.95 ± 1.23	1.40 ± 1.22	0.10 ± 0.09	7.36 ± 0.96	0.53 ± 0.06	2.67 ± 1.84	0.19 ± 0.14	0.08 ± 0.17	0.006 ±0.013	0.40 ± 0.30	0.029 ±0.020	0.49 ± 0.67	0.035 ±0.050

Table B2i Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage III, Light/With Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.00 ± 1.21 a	6.29 ± 2.72 c	0.48 ± 0.20 n	6.26 ± 0.95 cd	0.48 ± 0.05 o	0.49 ± 0.40 e	0.04 ± 0.03 q	0.04 ± 0.09 gh	0.003 ±0.007 t	0.36 ± 0.07 ij	0.027 ±0.006 u	0.32 ± 0.15 k	0.025 ±0.014 v
2	12.64 ± 0.98 a	1.24 ± 1.26 b	0.10 ± 0.11 m	6.53 ± 0.95 cd	0.52 ± 0.07 op	3.56 ± 2.37 fg	0.29 ± 0.19 rs	0.00 ± 0.09 h	0.000 ±0.007 t	0.57 ± 0.76 ij	0.044 ±0.054 u	0.41 ± 0.20 k	0.032 ±0.015 v
3	12.71 ± 1.29 a	2.06 ± 1.85 b	0.16 ± 0.15 m	5.79 ± 0.75 c	0.46 ± 0.05 o	1.96 ± 1.11 ef	0.15 ± 0.08 qr	0.03 ± 0.06 gh	0.002 ±0.005 t	0.27 ± 0.13 j	0.022 ±0.009 u	0.34 ± 0.09 k	0.027 ±0.006 v
4	12.70 ± 1.04 a	1.50 ± 1.34 b	0.12 ± 0.11 m	7.20 ± 0.88 d	0.57 ± 0.08 p	2.62 ± 1.84 ef	0.21 ± 0.14 qr	0.12 ± 0.08 g	0.009 ±0.006 t	0.31 ± 0.09 ij	0.025 ±0.007 u	0.41 ± 0.22 k	0.033 ±0.017 v
5	13.63 ± 1.17 a	1.78 ± 1.39 b	0.14 ± 0.11 m	6.78 ± 0.78 cd	0.50 ± 0.05 op	1.54 ± 0.90 ef	0.11 ± 0.06 qr	0.02 ± 0.06 gh	0.002 ±0.005 t	0.42 ± 0.27 ij	0.030 ±0.018 u	0.27 ± 0.24 k	0.020 ±0.019 v
6	12.91 ± 1.51 a	1.27 ± 1.10 b	0.10 ± 0.09 m	6.08 ± 1.21 cd	0.47 ± 0.07 o	5.79 ± 2.36 g	0.46 ± 0.19 s	0.07 ± 0.09 gh	0.005 ±0.006 t	0.71 ± 0.58 ij	0.056 ±0.044 u	0.48 ± 0.40 k	0.039 ±0.035 v
7	13.67 ± 0.88 a	0.51 ± 0.49 b	0.04 ± 0.03 m	6.81 ± 0.95 cd	0.50 ± 0.07 op	6.09 ± 1.69 g	0.45 ± 0.13 s	0.04 ± 0.11 gh	0.003 ±0.008 t	0.82 ± 0.38 i	0.060 ±0.027 u	0.51 ± 0.35 k	0.038 ±0.029 v
8	11.93 ± 1.50 a	1.76 ± 0.81 b	0.15 ± 0.08 m	6.18 ± 0.88 cd	0.52 ± 0.05 op	3.22 ± 2.65 f	0.26 ± 0.19 rs	0.11 ± 0.06 gh	0.009 ±0.005 t	0.48 ± 0.12 ij	0.040 ±0.008 u	0.22 ± 0.09 k	0.018 ±0.007 v
mean	12.90 ± 1.28	2.05 ± 2.21	0.16 ± 0.17	6.45 ± 0.99	0.50 ± 0.07	3.16 ± 2.54	0.25 ± 0.20	0.05 ± 0.09	0.004 ±0.007	0.49 ± 0.41	0.038 ±0.030	0.37 ± 0.25	0.029 ±0.020

Table B2j Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage IV, Dark/No Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.59 ± 1.16 a	1.02 ± 0.61 cd	0.08 ± 0.05 m	6.82 ± 1.07 e	0.50 ± 0.07 o	4.08 ± 3.33 fg	0.31 ± 0.26 pq	0.05 ± 0.10 i	0.004 ±0.008 s	0.63 ± 0.57 j	0.047 ±0.045 t	0.40 ± 0.17 k	0.029 ±0.012 u
2	12.47 ± 1.25 ab	0.90 ± 0.85 cd	0.07 ± 0.07 m	6.68 ± 0.73 e	0.54 ± 0.05 o	3.20 ± 1.99 fgh	0.26 ± 0.17 pqr	0.07 ± 0.11 i	0.006 ±0.009 s	0.45 ± 0.25 j	0.036 ±0.020 t	0.43 ± 0.22 k	0.034 ±0.016 u
3	13.61 ± 0.61 a	1.51 ± 1.24 cd	0.11 ± 0.09 mn	6.70 ± 1.08 e	0.49 ± 0.07 o	4.08 ± 1.86 fg	0.30 ± 0.14 pqr	0.06 ± 0.11 i	0.004 ±0.008 s	0.32 ± 0.15 j	0.024 ±0.012 t	0.46 ± 0.27 k	0.034 ±0.019 u
4	13.53 ± 1.00 a	0.81 ± 0.62 d	0.06 ± 0.05 m	6.88 ± 1.20 e	0.51 ± 0.07 o	4.62 ± 2.66 f	0.35 ± 0.22 p	0.08 ± 0.12 i	0.006 ±0.009 s	0.50 ± 0.57 j	0.038 ±0.047 t	0.44 ± 0.22 k	0.032 ±0.016 u
5	12.63 ± 2.01 ab	1.35 ± 0.74 cd	0.12 ± 0.07 mn	6.47 ± 1.26 e	0.51 ± 0.06 o	1.25 ± 0.60 gh	0.10 ± 0.06 qr	0.17 ± 0.41 i	0.011 ±0.029 s	0.29 ± 0.26 j	0.023 ±0.018 t	0.58 ± 0.39 k	0.044 ±0.025 u
6	12.68 ± 1.27 ab	1.54 ± 1.10 cd	0.12 ± 0.09 mn	6.40 ± 1.05 e	0.50 ± 0.05 o	2.98 ± 2.40 fgh	0.23 ± 0.19 pqr	0.01 ± 0.08 i	0.001 ±0.006 s	0.51 ± 0.31 j	0.040 ±0.023 t	0.80 ± 0.71 k	0.062 ±0.052 u
7	11.76 ± 1.28 b	2.33 ± 1.57 c	0.20 ± 0.13 n	5.50 ± 0.80 e	0.47 ± 0.07 o	0.78 ± 0.98 h	0.07 ± 0.09 r	0.11 ± 0.12 i	0.010 ±0.010 s	0.34 ± 0.24 j	0.030 ±0.021 t	0.55 ± 0.31 k	0.046 ±0.026 u
8	14.03 ± 0.82 a	1.77 ± 1.26 cd	0.13 ± 0.09 mn	6.85 ± 1.15 e	0.49 ± 0.08 o	4.26 ± 1.13 f	0.30 ± 0.08 pqr	-0.01 ± 0.03 i	-0.001 ±0.002 s	0.38 ± 0.08 j	0.027 ±0.006 t	0.44 ± 0.14 k	0.032 ±0.010 u
mean	13.04 ± 1.38	1.40 ± 1.11	0.11 ± 0.09	6.54 ± 1.10	0.50 ± 0.07	3.16 ± 2.38	0.24 ± 0.18	0.07 ± 0.17	0.005 ±0.013	0.43 ± 0.35	0.033 ±0.027	0.51 ± 0.36	0.039 ±0.026

Table B2k Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage IV, Dark/With Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.32 ± 0.67 a b	1.45 ± 1.40 c	0.11 ± 0.11 m	7.53 ± 0.70 d e	0.56 ± 0.03 n	4.43 ± 1.55 g	0.34 ± 0.13 o p	0.14 ± 0.11 h i	0.011 ±0.008 q r	0.46 ± 0.10 j	0.034 ±0.008 s	0.54 ± 0.28 k	0.040 ±0.020 t
2	12.90 ± 1.12 a	1.12 ± 1.09 c	0.09 ± 0.09 m	6.91 ± 0.68 d e f	0.54 ± 0.05 n	6.39 ± 1.37 g	0.50 ± 0.10 o	0.21 ± 0.12 h i	0.016 ±0.009 q r	0.39 ± 0.20 j	0.030 ±0.016 s	0.74 ± 0.38 k	0.059 ±0.033 t
3	12.83 ± 0.95 a	1.63 ± 1.29 c	0.13 ± 0.10 m	6.54 ± 0.65 f	0.51 ± 0.05 n	4.37 ± 2.50 g	0.34 ± 0.19 o p	0.16 ± 0.11 h i	0.012 ±0.008 q r	0.44 ± 0.18 j	0.034 ±0.014 s	0.47 ± 0.36 k	0.036 ±0.024 t
4	12.94 ± 1.26 a	1.18 ± 0.85 c	0.09 ± 0.07 m	6.81 ± 0.72 d e f	0.53 ± 0.06 n	4.97 ± 3.11 g	0.39 ± 0.26 o p	0.07 ± 0.14 i	0.005 ±0.011 r	0.50 ± 0.28 j	0.039 ±0.020 s	0.86 ± 0.33 k	0.065 ±0.020 t
5	12.97 ± 1.67 a b	1.05 ± 1.32 c	0.08 ± 0.10 m	6.96 ± 0.78 d e f	0.54 ± 0.06 n	3.61 ± 2.05 g	0.29 ± 0.19 o p	0.13 ± 0.13 h i	0.010 ±0.009 q r	0.43 ± 0.23 j	0.033 ±0.017 s	0.75 ± 0.42 k	0.057 ±0.028 t
6	13.24 ± 1.00 a b	1.72 ± 1.59 c	0.13 ± 0.12 m	6.68 ± 0.53 f	0.51 ± 0.04 n	5.24 ± 1.14 g	0.40 ± 0.11 o p	0.24 ± 0.21 h i	0.018 ±0.017 q r	0.45 ± 0.16 j	0.035 ±0.013 s	0.41 ± 0.18 k	0.032 ±0.015 t
7	14.42 ± 0.52 b	0.83 ± 0.78 c	0.06 ± 0.05 m	7.68 ± 0.66 d	0.53 ± 0.05 n	4.34 ± 2.54 g	0.30 ± 0.18 o p	0.33 ± 0.23 h	0.023 ±0.016 q	0.41 ± 0.12 j	0.029 ±0.008 s	1.09 ± 0.70 k	0.076 ±0.049 t
8	13.91 ± 0.68 a b	1.43 ± 1.14 c	0.10 ± 0.08 m	7.34 ± 0.76 d e f	0.53 ± 0.05 n	3.26 ± 1.67 g	0.24 ± 0.13 p	0.14 ± 0.11 h i	0.010 ±0.007 q r	0.38 ± 0.21 j	0.028 ±0.016 s	0.98 ± 0.98 k	0.069 ±0.066 t
mean	13.31 ± 1.13	1.30 ± 1.19	0.10 ± 0.09	7.06 ± 0.76	0.53 ± 0.05	4.58 ± 2.20	0.35 ± 0.18	0.18 ± 0.16	0.013 ±0.012	0.43 ± 0.19	0.033 ±0.014	0.73 ± 0.54	0.054 ±0.038

Table B2m Mean (±SD) P/B and element/P ratios derived from the EDX analysis of globoids from Stage IV, Light/No Nutrients *C. andreana* cotyledons.

seed	P	K		Mg		Ca		Mn		Fe		Zn	
	P/B	P/B	K/P	P/B	Mg/P	P/B	Ca/P	P/B	Mn/P	P/B	Fe/P	P/B	Zn/P
1	13.51 ± 1.29 a	0.38 ± 0.20 b	0.029 ±0.016 p	7.20 ± 1.19 cde	0.54 ± 0.10 qr	1.92 ± 0.92 j	0.14 ± 0.07 w	0.09 ± 0.15 k	0.007 ±0.010 x	0.51 ± 0.21 m	0.039 ±0.017 y	0.87 ± 0.34 no	0.064 ±0.027 z
2	13.07 ± 0.84 a	0.58 ± 0.44 b	0.044 ±0.032 p	6.05 ± 1.33 de	0.46 ± 0.10 r	4.86 ± 3.39 hij	0.37 ± 0.25 uvw	0.03 ± 0.12 k	0.003 ±0.009 x	0.74 ± 0.78 m	0.056 ±0.060 y	1.05 ± 1.03 n	0.080 ±0.077 z
3	13.39 ± 1.36 a	0.60 ± 0.62 b	0.047 ±0.050 p	7.37 ± 1.12 cd	0.55 ± 0.06 qr	4.54 ± 1.28 ij	0.34 ± 0.22 vw	0.03 ± 0.17 k	0.002 ±0.012 x	0.70 ± 0.56 m	0.052 ±0.042 y	0.57 ± 0.58 no	0.043 ±0.045 z
4	13.19 ± 1.04 a	0.54 ± 0.50 b	0.043 ±0.043 p	6.01 ± 0.84 de	0.46 ± 0.07 r	10.72 ± 3.03 f	0.80 ± 0.18 s	0.18 ± 0.15 k	0.013 ±0.011 x	0.43 ± 0.18 m	0.032 ±0.014 y	0.59 ± 0.65 no	0.47 ±0.56 z
5	14.20 ± 1.09 a	0.26 ± 0.21 b	0.019 ±0.016 p	8.06 ± 1.06 c	0.57 ± 0.08 q	6.27 ± 2.22 ghi	0.44 ± 0.14 uv	-0.02 ± 0.13 k	-0.001 ±0.009 x	0.36 ± 0.17 m	0.025 ±0.012 y	0.29 ± 0.15 o	0.20 ±0.10 z
6	13.18 ± 1.44 a	0.39 ± 0.26 b	0.032 ±0.025 p	5.88 ± 0.91 e	0.45 ± 0.04 r	9.35 ± 2.77 fg	0.71 ± 0.20 st	0.04 ± 0.09 k	0.003 ±0.008 x	0.37 ± 0.15 m	0.028 ±0.011 y	0.34 ± 0.17 no	0.26 ±0.12 z
7	13.89 ± 0.90 a	0.22 ± 0.16 b	0.016 ±0.010 p	7.32 ± 0.88 cd	0.53 ± 0.04 qr	8.33 ± 2.63 fgh	0.60 ± 0.19 stu	0.20 ± 0.17 k	0.015 ±0.013 x	0.49 ± 0.22 m	0.036 ±0.018 y	0.37 ± 0.19 no	0.26 ±0.12 z
8	13.33 ± 1.74 a	0.16 ± 0.14 b	0.013 ±0.012 p	7.83 ± 0.70 c	0.60 ± 0.08 q	6.25 ± 2.27 ghi	0.47 ± 0.15 tuv	0.12 ± 0.13 k	0.010 ±0.013 x	0.37 ± 0.13 m	0.028 ±0.009 y	0.33 ± 0.15 o	0.27 ±0.17 z
mean	13.47 ± 1.24	0.39 ± 0.38	0.030 ±0.031	6.96 ± 1.27	0.52 ± 0.09	6.53 ± 3.65	0.48 ± 0.27	0.08 ± 0.15	0.007 ±0.012	0.49 ± 0.38	0.037 ±0.029	0.55 ± 0.55	0.042 ±0.043

APPENDIX C: RESULTS OF STATISTICAL ANALYSES

The following tables summarize the findings of various statistical analyses carried out on segments of the P/B data. Mutiple analyses were carried out in an attempt to determine the effects of light and mineral nutrient conditions on the changes occuring in the globoid composition over time. Three-way ANOVA's were carried out on various portions of the P/B data to examine the influence of individual factors under limited conditions. The analyses tested for the influence of the two identified factors, as well as for seed-to-seed variation. The effect of each factor individually, as well as in combination with each other factor, were considered. A single analysis of all the data was not possible due to the absence of globoids from the Stage IV light/with nutrients samples. Analyses were carried out on all possible subsets of P/B data.

Table #	Information Gathered
C1	The effect of light and mineral nutrients at Stage II.
C2	The effect of light and mineral nutrients at Stage III.
C3	The effect of stage and mineral nutrients in dark grown seedlings (Stages I, II, and IV).
C4	The effect of stage and mineral nutrients in light grown seedlings (Stages I, II and III).
C5	The effect of stage and light in seedlings grown without mineral nutrients (Stages I, II, III, and IV).
C6	The effect of stage and light in seedlings grown with added mineral nutrients (Stages I, II, and III).

General notes on the tables:

- Variability between seeds from the same treatment was also considered in each analysis. In most treatments, there was significant seed-to-seed variation for each element. These differences are displayed in Appendix B.
- SE = the factor or interaction had a significant effect on the mean P/B ratio for that element at $P \leq 0.05$.
- NA indicates that the mean P/B ratios were too low to allow for accurate analysis of any effects.
- _ * _ designates the interaction between two factors such as stage and light conditions (S * LC).

Table C1a The effect of light and mineral nutrient conditions on the composition of globoids from Stage II *C. maxima* cotyledons.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Light Conditions (LC)		SE		NA			
Mineral Nutrient Conditions (MN)		SE	SE	NA		SE	
LC * MN				NA			

Note: This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from Stage II.

Table C1b The effect of light and mineral nutrient conditions on the composition of globoids from Stage II *C.andreana* cotyledons.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Light Conditions (LC)					NA	SE	
Mineral Nutrient Conditions (MN)					NA		
LC * MN					NA		

Note: This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from Stage II.

Table C2a The effect of light and mineral nutrient conditions on the composition of globoids from Stage III *C. maxima* cotyledons.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Light Conditions (LC)				NA			
Mineral Nutrient Conditions (MN)	SE	SE		NA			
LC * MN		SE		NA	SE	SE	SE

Note: This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from Stage III.

Table C2b The effect of light and mineral nutrient conditions on the composition of globoids from Stage III *C.andreana* cotyledons.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Light Conditions (LC)	SE				NA		
Mineral Nutrient Conditions (MN)	SE		SE		NA		
LC * MN					NA		

Note: This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from Stage III.

Table C3a The effect of stage of growth and mineral nutrient conditions on the composition of globoids from the cotyledons of etiolated *C. maxima* seeds and seedlings.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	NA	SE	SE	SE
Mineral Nutrient Conditions (MN)				NA		SE	
S * MN				NA			

Note: This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from dark grown seedlings (Stages I, II, III, and IV).

Table C3b The effect of stage of growth and mineral nutrient conditions on the composition of globoids from the cotyledons of etiolated *C. andreana* seeds and seedlings.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)		SE	SE	SE	NA	SE	SE
Mineral Nutrient Conditions (MN)				SE	NA		
S * MN			SE		NA		SE

Note: This table represents the results of a multifactor analysis-of-variance, carried out on all P/B data from dark grown seedlings (Stages I, II, III, and IV).

Table C4a The effect of stage of growth and mineral nutrient conditions on the composition of globoids from the cotyledons of light grown *C. maxima* seeds and seedlings.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	NA	SE	SE	SE
Mineral Nutrient Conditions (MN)		SE		NA	SE		
S * MN		SE		NA	SE	SE	

Note: This table represents the results of a multifactor analysis-of-variance, carried out on P/B data from light grown seedlings at Stages I, II, and III.

Table C4b The effect of stage of growth and mineral nutrient conditions on the composition of globoids from the cotyledons of light grown *C. andreana* seeds and seedlings.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	SE	NA	SE	SE
Mineral Nutrient Conditions (MN)			SE		NA		
S * MN	SE		SE		NA		

Note: This table represents the results of a multifactor analysis-of-variance, carried out on P/B data from light grown seedlings at Stages I, II, and III.

Table C5a The effect of stage of growth and light conditions on the composition of globoids from the cotyledons of *C. maxima* seeds and seedlings grown without added mineral nutrients.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	NA	SE	SE	SE
Light Conditions (LC)				NA			
S * LC				NA			SE

Note: This table represents the results of a multifactor analysis-of-variance, carried out on P/B data from seedlings grown with deionized water (Stages I, II, III and IV).

Table C5b The effect of stage of growth and light conditions on the composition of globoids from the cotyledons of *C. andreana* seeds and seedlings grown without added mineral nutrients.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	SE	NA	SE	SE
Light Conditions (LC)				SE	NA		
S * LC				SE	NA		

Note: This table represents the results of a multifactor analysis-of-variance, carried out on P/B data from seedlings grown with deionized water (Stages I, II, III and IV).

Table C6a The effect of stage of growth and light conditions on the composition of globoids from the cotyledons of *C. maxima* seeds and seedlings grown with added mineral nutrients.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)	SE	SE	SE	NA	SE	SE	SE
Light Conditions (LC)				NA	SE	SE	
S * LC		SE		NA		SE	

Note: This table represents the results of a multifactor analysis-of-variance, carried out on P/B data from seedlings grown with Hoagland's Solution (Stages I, II,and III).

Table C6b The effect of stage of growth and light conditions on the composition of globoids from the cotyledons of *C. andreana* seeds and seedlings grown with added mineral nutrients.

Factor / Interaction	P	K	Mg	Ca	Mn	Fe	Zn
Stage (S)		SE	SE	SE	NA	SE	SE
Light Conditions (LC)					NA		
S * LC					NA		

Note: This table represents the results of a multifactor analysis-of-variance, carried out on P/B data from seedlings grown with Hoagland's Solution (Stages I, II, and III).