Plant Physiol. (1982) 69, 829–833
0032-0889/82/69/0829/05/$00.50/0

Mineral Reserves in Castor Beans: The Dry Seed[1]

Received for publication July 28, 1981 and in revised form November 20, 1981

JOHN N. A. LOTT, JOHN S. GREENWOOD, AND CATHERINE M. VOLLMER
Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1 (J. N. A. L., C. M. V.); and Department of Biology, University of Calgary, Alberta Canada T2N 0B1 (J. S. G.)

ABSTRACT

Elemental composition and distribution of the mineral reserves in the endosperm and embryo tissues of *Ricinus communis* cultivars Hale and Zanzibarensis were investigated. Energy dispersive x-ray analysis was used to determine the elemental composition of the globoid crystals, while atomic absorption spectrometry allowed quantification of the elements, particularly Ca, in various seed regions. No major differences were found between the two cultivars with regard to the elemental distribution in globoid crystals. While the majority of globoid crystals contained P, K, and Mg, the occasional one also contained Ca. In extremely rare instances, Fe was detected in globoid crystals. Ca-containing globoid crystals were more common in provascular cell protein bodies in the stem and radicle. Polarized light microscopy, micro-incineration, and acid solubility tests demonstrated the presence of calcium oxalate crystals in the innermost testa which adheres to the endosperm and is often mistakenly identified as endosperm. Atomic absorption spectrometry revealed that most of the calcium present in castor bean seeds is localized in the testa. On a per-seed-region basis, the much larger endosperm contains more Ca than does the embryo. However, on a unit-weight basis, the radicle-plus-stem regions contain considerably more Ca than does the cotyledon or endosperm, an observation that is consistent with the observed distribution pattern for Ca-containing globoid crystals.

Plant physiologists have used the seeds of castor bean, *Ricinus communis*, for a great variety of studies. One advantage that castor bean seeds have for experimental work is the presence of a fairly large mass of living endosperm. The endosperm is generally considered to be a comparatively homogeneous tissue, since it never develops vascular tissue. This paper reports the results of studies of the mineral stores in endosperm and embryo regions of castor bean seeds.

Much of the mineral reserves found in seeds are contained inside the proteins bodies, generally in electron-dense inclusions called globoid crystals (4, 7, 12, 13, 15, 18, 20). The mineral reserves occur mainly as phytin, a salt of inositol hexaphosphoric acid (1, 3, 13, 21, 22). The most commonly occurring cations in the phytin-rich globoid crystals seem to be Mg and K, but a range of other cations—including Ba, Ca, Fe, Mn, and Na—have been found in certain cases (2, 4, 8, 9, 12, 15, 22).

The protein bodies of castor bean endosperm have been studied previously (18, 23, 26) and are known to contain prominent globoid crystals. Some chemical studies of globoid crystals isolated from castor bean seeds have also been made (16, 19).

The studies reported here were undertaken for several reasons which are outlined below. Since castor bean seeds are so extensively used in a variety of studies, a clear understanding of the types and exact locations of mineral reserves in the seeds may well be of interest to those studying events during seed formation or germination. Also, we wished to check on the composition and distribution of mineral reserves stored in castor bean seeds, since there was uncertainty from earlier work. Initial studies of Sobolev (16) indicated that the phytin reserves of castor bean seeds were a Mg and Ca salt of phytic acid. Subsequent studies of globoid crystals isolated from whole seeds indicated that phytin was predominantly a K and Mg salt of phytic acid with small amounts of Ca (19). In these later studies, it was not determined whether the Ca was uniformly distributed or not, a feature worthy of investigation, since in some other seeds Ca-containing globoid crystals are concentrated in specific cell types or embryo regions (9, 11, 13). As part of ongoing studies of the way mineral reserves are partitioned in seeds, we wished to study a dicot seed, such as castor bean, in which the endosperm is very large in relation to the embryo. Also in castor bean, the large and very thin cotyledons, which act as haustorial organs during germination, have a much more extensive provascular system than do previously investigated albuminous systems such as those of tomato, rice, and wheat.

In the studies reported here, two different, but complementary, approaches were used. One approach involved quantitative determinations of elemental composition, especially Ca, in various seed regions. The second approach used EDX[2] analysis to study the elemental composition of globoid crystals. With EDX analysis, one can spot-analyze selected cell regions with very good sensitivity. Since one can obtain a simultaneous analysis of all elements with atomic number 11 or heavier, all the elements of interest in globoid crystal studies (P, K, Mg, Ca, Fe, and Mn) can be measured. With EDX analysis, possible tissue-to-tissue, cell-to-cell, and protein body-to-protein body variations could be studied.

MATERIALS AND METHODS

Seeds. Seeds of *Ricinus communis* L., from the relatively large-seeded castor bean variety Zanzibarensis, were obtained from Tregunno Seeds, Hamilton, Canada. These seeds of variety Zanzibarensis, which were 15- to 20-mm long in the long axis, had been obtained by Tregunno's from a European seed producer. Seeds of the smaller-seeded castor bean variety Hale, which have a seed length of 10 to 12 mm, were produced by plants grown in growth chambers at the University of Calgary, Calgary, Alberta. Parent plants were grown in a sand-peat mixture and were routinely watered with half-strength Hoagland mineral nutrient solution throughout vegetative and reproductive growth. Testa markings and color of seeds of the Hale variety were relatively uniform, whereas testa color and markings of seeds of the Zanzibarensis variety were quite variable.

Fixation and Embedding. Testas were removed from dry seeds, and small pieces of endosperm, cotyledon, radicle, and stem

[1] Supported by the Natural Science and Engineering Research Council of Canada.

[2] Abbreviation: EDX, energy dispersive x-ray.

regions were fixed according to the procedure of Lott (6). Samples were fixed on ice for 1.5 h in 5% glutaraldehyde in distilled H_2O (pH adjusted to 7.1), dehydrated in a graded ethanol series, further dehydrated in propylene oxide, and embedded in Spurr's epoxy resin. Sections 150 to 170 nm in thickness were made with a diamond knife and mounted on carbon-Formvar-coated grids.

Freeze-Drying. To check for possible extraction of elements during fixation and dehydration, freeze-dried samples were used. Center endosperm tissue, cotyledon tissue from which the largest veins would develop, and cotyledon tissue lacking very prominent provascular regions were dissected from dry Zanzibarensis castor beans and were low-temperature freeze-dried, according to the method of Lott and Vollmer (12). Twelve seeds with various seed coat colors and markings were studied. Radicle and stem samples were also studied for three seeds of Zanzibarensis. Tissue taken from the cotyledon (tip, middle, base), root-shoot, and endosperm (tip, middle, base) regions of dry seeds of variety Hale were also low-temperature freeze-dried.

After initial studies, the endosperm of both varieties was investigated further using freeze-dried powders. Central endosperm tissue was divided into three portions—an inner region taken from endosperm nearest the cotyledons, a middle portion, and an outer portion nearest the testa. Freeze-dried powders were then dusted onto carbon-coated Formvar-covered grids.

Energy Dispersive X-ray Analysis. EDX analysis was carried out with a Philips EM 300 transmission electron microscope connected to an EDAX model 606 x-ray spectrometer, a model 707A detector, and an EDIT data improvement system (EDAX International Inc., Prairie View, IL). The accelerating voltage used for all analyses was 80 kv. The analysis time for each sample was 60 s. Globoid crystals were identified by their natural electron density.

Determinations of P, K, Mg, and Ca Levels. A 1.0-g sample of embryo tissue (cotyledons and root-shoot axes) and a 10-g sample of endosperm tissue were dissected from dry seeds of variety Zanzibarensis, ball-milled in liquid N_2, and sent to the Australian Mineral Development Laboratory, Frewville, South Australia, for determinations of P, K, Mg, and Ca levels. Atomic absorption spectrometry was used for Ca, K, and Mg determinations, and P was determined by molybdate-blue spectrophotometry.

Atomic Absorption Spectrometry Measurement of Calcium. Dry castor bean seeds of variety Hale were dissected into the following regions: radicle and stem; cotyledons; innermost testa (thin papery layer adhering to the endosperm surface); caruncle; outer testa minus caruncle; and endosperm minus innermost testa. Weights of each region were recorded. The dissected tissue samples were digested in concentrated HNO_3 using low heat until the solution became clear and no tissue fragments remained. Samples were allowed to cool to room temperature, and then 1 to 2 ml of 70% $HClO_4$ were added. Glass-distilled H_2O was used to dilute the samples to a final concentration of 5% HNO_3. Ca analysis was performed using the spectral line for Ca at 211 nm (visible) on a Perkin-Elmer Model 603 atomic absorption spectrophotometer equipped with a hollow cathode Ca lamp. Operating conditions included the following: a lamp current of 18 mamp; slot setting of 4 (0.7 nm); an air-acetylene flame; and a 4-inch burner slot. A 0.25 μl/l standard gave an absorption of 0.012 units. Samples were aspirated, and the concentration of Ca per sample was determined from a standard calibration curve for Ca.

Crystal Identification. The location of birefringent crystals was determined through study of fresh sections with polarized light. Identification of inner testa crystals as Ca oxalate was made by acid solubility studies and microincineration. Disappearance of crystals in 12 M HCl, 1 M HCl, and dilute HNO_3—but retention in 17 M and 2 M acetic acid—gives a good indication that crystals are oxalate. Crystals in fresh sections were also studied with a microincineration procedure modified from Johnson (5). Tissue samples containing birefringent crystals that are insoluble in 2 M acetic acid were heated to 600°C in a furnace. If crystals are Ca oxalate, they will be converted to Ca oxide during heating and, thus, become soluble in 2.0 M acetic acid.

RESULTS

EDX Analysis.

Endosperm. The great majority of endosperm globoid crystals that were examined contained P, K, and Mg in both Zanzibarensis (Figs. 1 and 2) and Hale (Fig. 4) cultivars. In very rare instances, in both cultivars, globoid crystals containing mainly P and K (Fig. 3) or globoid crystals containing P, K, Mg, and some Ca (Fig. 5) were found. In general, globoid crystals in either freeze-dried powders or fixed and sectioned tissue were similar in elemental composition, except that traces of S were more commonly present in globoid crystals in freeze-dried powders, and the K levels were often higher in freeze-dried powders (*cf.* Fig. 1 with Figs. 2 and 3). No differences in elemental content between outer, mid-, and inner endosperm were found.

Cotyledons. Almost all globoid crystals found in cotyledons contained P, K, and Mg, regardless of location in mesophyll cells (Fig. 6), cells of large provascular regions (Fig. 7), of cells or small provascular regions (Fig. 8). In freeze-dried powders, some traces of sulfur were commonly found, along with the P, K, and Mg (Fig. 9). On rare occasions, traces of Ca, or even of Fe (Fig. 6), were detected, in addition to the more commonly occurring elements P, K, and Mg.

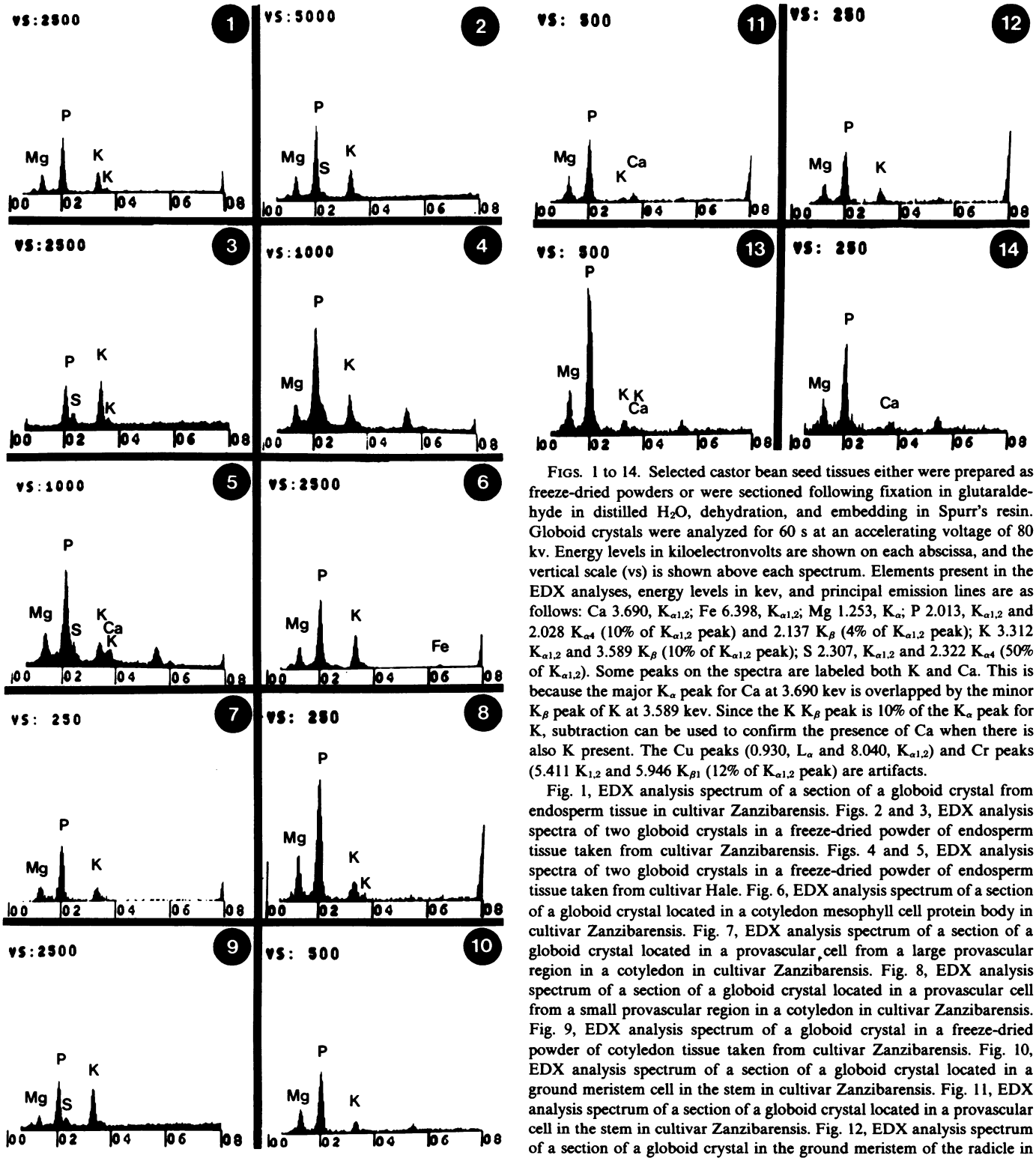
Stem. Globoid crystals located in ground meristem cells of the stem contained P, K, and Mg (Fig. 10), while the small globoid crystals from the provascular regions contained P, Mg, Ca, and only traces of K (Fig. 11). Globoid crystals in freeze-dried powders contained P, K, Mg, and sometimes S. The globoid crystals analyzed in freeze-dried powders in all probability came from ground meristem cells, since globoid crystals derived from the ground meristem cells are both larger and much more numerous than are globoid crystals from the provascular region.

Radicle. Globoid crystals in ground meristem cells from fixed and sectioned radicles contained P, K, and Mg (Fig. 12). Those of the provascular cells contained P, K, Mg, and traces of Ca (Fig. 13) or P, Mg, Ca, and little or no K (Fig. 14). Globoid crystals in freeze-dried powders generally contained P, K, Mg, and sometimes some S. Globoid crystals in freeze-dried powders likely were derived from ground meristem cells rather than from provascular cells, since ground meristem cells contain larger and more numerous globoid crystals.

Elemental Analysis. When very few of the globoid crystals examined with EDX analysis contained Ca, a quantitative investigation of elemental values was begun. An initial study using bulk samples revealed that Ca levels in both endosperm and embryo tissues were indeed much lower than were P, K, and Mg levels (Table I).

After initial studies reported in Table I, methods were devised that permitted determinations of Ca levels in different regions within one seed. Thus, some indication of seed-to-seed variability could be obtained, as could the way in which Ca is apportioned within different regions of a given seed.

It is evident that the overwhelming majority of the Ca found in a castor bean seed is in the testa. Several different testa regions were separated so as to permit a more rigorous examination of Ca compartmentalization. Since it is important for an understanding of the results, a brief description of the various testa fractions is presented here. When a castor bean seed is split open, the hard outer part of the testa readily separates from the innermost part of the testa. The innermost testa is a thin, white, papery layer that adheres to the surface of the endosperm. Thus, the seed region generally called endosperm actually is the endosperm plus a covering of some cells of the testa. Druse (cluster) crystals, thought



FIGS. 1 to 14. Selected castor bean seed tissues either were prepared as freeze-dried powders or were sectioned following fixation in glutaraldehyde in distilled H_2O, dehydration, and embedding in Spurr's resin. Globoid crystals were analyzed for 60 s at an accelerating voltage of 80 kv. Energy levels in kiloelectronvolts are shown on each abscissa, and the vertical scale (vs) is shown above each spectrum. Elements present in the EDX analyses, energy levels in kev, and principal emission lines are as follows: Ca 3.690, $K_{\alpha 1,2}$; Fe 6.398, $K_{\alpha 1,2}$; Mg 1.253, K_{α}; P 2.013, $K_{\alpha 1,2}$ and 2.028 $K_{\alpha 4}$ (10% of $K_{\alpha 1,2}$ peak) and 2.137 K_{β} (4% of $K_{\alpha 1,2}$ peak); K 3.312 $K_{\alpha 1,2}$ and 3.589 K_{β} (10% of $K_{\alpha 1,2}$ peak); S 2.307, $K_{\alpha 1,2}$ and 2.322 $K_{\alpha 4}$ (50% of $K_{\alpha 1,2}$). Some peaks on the spectra are labeled both K and Ca. This is because the major K_{α} peak for Ca at 3.690 kev is overlapped by the minor K_{β} peak of K at 3.589 kev. Since the K K_{β} peak is 10% of the K_{α} peak for K, subtraction can be used to confirm the presence of Ca when there is also K present. The Cu peaks (0.930, L_{α} and 8.040, $K_{\alpha 1,2}$) and Cr peaks (5.411 $K_{1,2}$ and 5.946 $K_{\beta 1}$ (12% of $K_{\alpha 1,2}$ peak) are artifacts.

Fig. 1, EDX analysis spectrum of a section of a globoid crystal from endosperm tissue in cultivar Zanzibarensis. Figs. 2 and 3, EDX analysis spectra of two globoid crystals in a freeze-dried powder of endosperm tissue taken from cultivar Zanzibarensis. Figs. 4 and 5, EDX analysis spectra of two globoid crystals in a freeze-dried powder of endosperm tissue taken from cultivar Hale. Fig. 6, EDX analysis spectrum of a section of a globoid crystal located in a cotyledon mesophyll cell protein body in cultivar Zanzibarensis. Fig. 7, EDX analysis spectrum of a section of a globoid crystal located in a provascular cell from a large provascular region in a cotyledon in cultivar Zanzibarensis. Fig. 8, EDX analysis spectrum of a section of a globoid crystal located in a provascular cell from a small provascular region in a cotyledon in cultivar Zanzibarensis. Fig. 9, EDX analysis spectrum of a globoid crystal in a freeze-dried powder of cotyledon tissue taken from cultivar Zanzibarensis. Fig. 10, EDX analysis spectrum of a section of a globoid crystal located in a ground meristem cell in the stem in cultivar Zanzibarensis. Fig. 11, EDX analysis spectrum of a section of a globoid crystal located in a provascular cell in the stem in cultivar Zanzibarensis. Fig. 12, EDX analysis spectrum of a section of a globoid crystal in the ground meristem of the radicle in cultivar Zanzibarensis. Figs. 13 and 14, EDX analysis spectra of sections of globoid crystals in the provascular cells of the radicle in cultivar Zanzibarensis.

Table I. *Contents of P, Ca, Mg, and K in Endosperm and Embryo Tissue of R. communis cv. Zanzibarensis*

	Mineral Content			
	P	K	Mg	Ca
	μg/mg air-dry tissue			
Endosperm[a]	7.9	4.8	4.1	0.27
Entire embryos	5.7	4.2	3.1	0.24

[a] Contains some of the thin innermost layer of the seed coat.

Table II. *Atomic Absorption Spectroscopy Measurements of Ca per Seed Region in R. communis cv. Hale Seeds*

	Seed 1	Seed 2	Seed 3	Mean
	μg Ca per seed region			
Radicle and stem	4	5	2	3.7
Cotyledons	5	3	4	4.0
Endosperm[a]	22	15	11	16
Innermost testa[a]	40	49	81	56.7
Outer testa[b]	550	700	1,060	770
Caruncle	5	8	7	6.7

[a] The white layer, representing the innermost portion of the testa (24), was removed from the seeds. The endosperm sample, thus, is slightly smaller than actual size, and the innermost testa sample undoubtedly contains a small amount of endosperm.

[b] Excludes caruncle.

Table III. *Atomic Absorption Spectroscopy Measurements of Ca per Unit Weight in R. communis cv. Hale Seed Region*

	Calcium Content			
	Seed 1	Seed 2	Seed 3	Mean
	μg/mg sample			
Radicle and stem	3.6	3.0	2.0	2.87
Cotyledons	1.0	0.9	0.4	0.76
Endosperm[a]	0.1	0.2	0.1	0.13
Innermost testa[a]	1.0	3.0	3.0	2.33
Outermost testa[b]	9.0	10.0	15.0	11.33
Caruncle	5.5	2.0	7.0	4.83

[a] The white layer, representing the innermost portion of the testa (24), was removed from the seeds. The endosperm sample, thus, is slightly smaller than actual size, and the innermost testa sample undoubtedly contains a small amount of endosperm.

[b] Excludes caruncle.

to be Ca oxalate, are present both in the portion of the testa that remains on the endosperm and in the inner part of the bulk of the testa (24). We found that the thin, white, paper-like region of testa that remained attached to the endosperm surface contained numerous birefringent crystals when viewed under polarized light. These crystals—which were soluble in 12 M HCl, 1 M HCl, and dilute HNO_3 but were insoluble in 17 M and 2 M acetic acid—are likely Ca oxalate on the basis of this acid solubility pattern. Microincineration studies provided further proof that these innermost testa crystals were Ca oxalate, since they were insoluble in acetic acid before heating at 600°C but were soluble after heating. In order to obtain an accurate estimation of Ca levels in the endosperm, it was necessary to scrape the surface of the endosperm free of the innermost testa.

The very high Ca levels in the testa are consistent with the existence of numerous Ca-rich crystals in the testa. In our initial studies (Table I) and, we suspect, in most biochemical studies, the Ca-rich innermost testa would routinely be considered as part of the endosperm.

On a per-seed-region basis, the much larger endosperm has more total Ca than does the radicle plus stem or the cotyledon fractions (Table II). However, when these regions are compared on a unit-weight basis (Table III), it is evident that the radicle plus stem regions contain considerably more Ca than do the cotyledon or endosperm regions. This observation is consistent with the EDX analysis results, showing at least some Ca in provascular globoid crystals in the radicle-stem regions.

DISCUSSION

One of the limitations of studies of bulk samples, such as those of Suvorov and Sobolev (19), is the inability to distinguish between a uniform low level of a compound throughout the sample and a higher level in only a portion of the sample. Thus, Suvorov and Sobolev (19), on the basis of quantitative measurements of elements present in isolated globoid crystals, concluded that phytin in castor beans is a K and Mg salt with traces of Ca.

Most globoid crystals that were examined with EDX analysis contained P, K, and Mg, which is consistent with their being rich in a K and Mg salt of phytic acid. Some globoid crystals were found that also contained detectable quantities of Ca. These Ca-containing globoid crystals were especially common in provascular cell protein bodies in the stem and radicle regions. This uneven distribution of the Ca-containing globoid crystals correlates well with the Ca content of the tissues on a unit-weight basis, as given in Table III.

The finding that Ca-containing globoid crystals are especially common in the provascular regions of the stem and radicle of castor bean provides an additional example of nonrandom ion content of globoid crystals in seeds. In *Cucurbita maxima* cotyledons, Ca-containing globoid crystals are often found in protodermal cells, provascular cells, and cells next to the provascular regions, yet are absent from most of the mesophyll cells (11). In wheat grains, there is a distinct distribution pattern for Mn-containing globoid crystals. Mn-containing globoid crystals are found in the base and mid-regions of the stele of the radicle (10). In tomato embryos, globoid crystals containing traces of Fe or Fe plus Mn occur only in certain types of cells or in certain positions (17). Protodermal cell globoid crystals usually contain traces of Fe and Mn. Throughout the embryo, the provascular cell globoid crystals frequently contain some Fe, while globoid crystals in the first layer of ground meristem around the provascular regions always contain some Fe.

In this manuscript, the thin papery layer that adheres to the endosperm surface is called the innermost testa based upon the diagrams of Vaughan (24). However, readers should be aware that the exact origin of this region is not entirely clear, since Scott (14) refers to this region as adherent nucellus.

Much of the Ca present in a castor bean seed comes from the testa. Birefringent crystals, which we demonstrated to be Ca oxalate, are present in some parts of the testa and presumably are responsible for most of the Ca content of the innermost testa. Some areas of the testa, for example the caruncle, are generally free of crystals. When mineral content of endosperm tissues are being studied, great care must be taken to avoid contamination of endosperm samples by the innermost testa. The thin innermost testa, which adheres to the endosperm, contains about 4 times the Ca of the much larger endosperm mass.

Given the observation that an external supply of Ca is often required by seedlings before most other elements, it is interesting to speculate as to whether or not any of the Ca oxalate from the testa is available for use by the seedling plant. While this was not studied in detail here, we suspect that it is not available. The innermost testa layer still contains birefringent crystals at the time when the testa plus remaining endosperm becomes detached from the cotyledons of the castor bean seedling. Recent evidence indicates that Ca from Ca oxalate crystals in endosperm of celery and carrot mericarps in not mobilized to the embryo to any major

extent (J. N. A. Lott, E. Spitzer, C. M. Vollmer, unpublished). Similarly, Ca oxalate crystals in cotyledons of several *Eucalyptus* species are not degraded during germination (M. White, J. N. A. Lott, unpublished).

In the studies reported here, two cultivars differing considerably in size and weight were used. There were no major differences between the cultivars with regard to the distribution of elements in globoid crystals, as studied with EDX analysis. These castor bean findings differ from studies of a series of *Cucurbita* species with different seed sizes, in which a difference in the Ca distribution pattern was observed (11, 12). However, *Cucurbita* is an exalbuminous seed, while *Ricinus* is an albuminous seed. Seeds of the smaller of the two castor bean cultivars studied here are still large by comparison with seeds of the smaller-sized *Cucurbita* species.

A comparison of the data in Tables I and II indicates that the smaller-seeded cultivar Hale has more Ca per unit weight of embryo tissue than does cultivar Zanzibarensis. Conversely, the larger-seeded cultivar Zanzibarensis has more Ca per unit weight of endosperm tissue than does Hale. When only postfertilization tissues (endosperm and embryo) of cultivar Hale are considered, approximately one-third of the total Ca is in the embryo and two-thirds is within the endosperm.

Even though the studies reported here measured K, Mg, and Ca content of embryos and endosperm tissue while Suvorov and Sobolev (19) measured K, Mg, and Ca content of both isolated protein bodies and isolated globoids, there is a good correlation between the data. In all cases, the K content is somewhat higher than is the Mg content, and the Ca content is much less than either the Mg or the K content. Using data from this paper and Suvorov and Sobolev (19), the following Ca contents as a percentage of the total K, Mg, and Ca content can be calculated: isolated globoids, 4.25%; isolated aleurone grains, 4.40%; endosperm tissue, 2.94%; and embryo tissue, 3.18%. These differences, while small, could be accounted for in a number of ways. Different cultivars were used; the growth conditions were likely somewhat different; the isolates might have been contaminated by Ca oxalate crystals; the isolation procedure may have concentrated Ca-containing protein bodies, or there is more K and Mg in the nonprotein body parts of these tissues than there is Ca.

While considering the Ca reserves of castor bean, it is of interest to note that the Ca content of embryo and endosperm tissue is low in this species. When the Ca content of castor bean embryo and endosperm tissue is compared with the Ca content of various seed regions from other genera that are listed in Table IV in Weber and Neumann (25), it is evident that castor bean ranks in the lowest 20%.

Acknowledgment—Technical assistance for portions of this work was provided by Mr. M. White.

LITERATURE CITED

1. ASHTON WM, PC WILLIAMS 1958 The phosphorus compounds of oats. 1. The content of phytate phosphorus. J Sci Food Agric 9: 505–511
2. BUTTROSE MS 1978 Manganese and iron in globoid crystals of protein bodies from *Avena* and *Casuarina*. Aust J Plant Physiol 5: 631–639
3. ERGLE DR, G GUINN 1959 Phosphorus compounds of cotton embryos and their changes during germination. Plant Physiol 34: 476–481
4. HOFSTEN AV 1973 X-ray analysis of microelements in seeds of *Crambe abyssinica*. Physiol Plant 29: 76–81
5. JOHNSON FB 1956 A method for demonstrating calcium oxalate in tissue sections. J. Histochem Cytochem 4: 404–405
6. LOTT JNA 1975 Protein body composition in *Curcurbita maxima* cotyledons as determined by energy dispersive x-ray analysis. Plant Physiol 55: 913–916
7. LOTT JNA, MS BUTTROSE 1978 Globoids in protein bodies of legume seed cotyledons. Aust J Plant Physiol 5: 89–111
8. LOTT JNA, MS BUTTROSE 1978 Thin sectioning, freeze-fracturing, energy dispersive x-ray analysis and chemical analysis in the study of inclusions in seed protein bodies: almond, Brazil nut, and quandong. Can J Bot 56: 2050–2061
9. LOTT JNA, JS GREENWOOD, CM VOLLMER, MS BUTTROSE 1978 Energy dispersive x-ray analysis of P, K, Mg, and Ca in globoid crystals in protein bodies from different regions of *Cucurbita maxima* embryos. Plant Physiol 61: 984–988
10. LOTT JNA, E SPITZER 1980 X-ray analysis studies of elements stored in protein body globoid crystals of *Triticum* grains. Plant Physiol 66: 494–499
11. LOTT JNA, E SPITZER, CM VOLLMER 1979 Calcium distribution in globoid crystals of *Cucurbita* cotyledon protein bodies. Plant Physiol 63: 847–851
12. LOTT JNA, CM VOLLMER 1979 Composition of globoid crystals from embryo protein bodies in five species of *Cucurbita*. Plant Physiol 63: 307–311
13. LUI NST, AM ALTSCHUL 1967 Isolation of globoids from cottonseed aleurone grain. Arch Biochem Biophys 121: 678–684
14. SCOTT FM 1941 Distribution of calcium oxalate crystals in *Ricinus communis* is relation to tissue differentiation and presence of other ergastric substances. Bot Gaz 103: 225–246
15. SHARMA CB, JW DIECKERT 1975 Isolation and partial characterization of globoids from aleurone grains of *Arachis hypogaea* seed. Physiol Plant 33: 1–7
16. SOBOLEV AM 1966 On the state of phytin in the aleurone grains of mature and germinating seeds. Fiziol Rast (Engl Transl) Plant Physiol 13: 177–183
17. SPITZER E, JNA LOTT 1980 Thin-section, freeze-fracture, and energy dispersive X-ray analysis studies of the protein bodies of tomato seeds. Can J Bot 58: 699–711
18. SUVOROV VI, NP BUZULUKOVA, AM SOBOLEV, IN SVESHNIKOVA 1970 Structure and chemical composition of globoids from aleurone grains of castor seeds. Fiziol Rast (Engl Transl) Plant Physiol 18: 1020–1027
19. SUVOROV VI, AM SOBOLEV 1972 Cationic composition of aleurone grains of seeds. Fiziol Rast (Engl Transl) Plant Physiol 19: 486–489
20. TANAKA K, M OGAWA, Z KASAI 1977 The rice scutellum. II. A comparison of scutellar and aleurone electron-dense particles by transmission electron microscopy including energy-dispersive x-ray analysis. Cereal Chem 54: 684–689
21. TANAKA K, T YOSHIDA, K ASADA, Z KASAI 1973 Subcellular particles isolated from aleurone layer of rice seeds. Arch Biochem Biophys 155: 136–143
22. TANAKA K, T YOSHIDA, Z KASAI 1974 Distribution of mineral elements in the outer layer of rice and wheat grains, using electron microprobe x-ray analysis. Soil Sci Plant Nutr 20: 87–91
23. TULLY RE, H BEEVERS 1976 Protein bodies of castor bean endosperm: Isolation, fractionation, and characterization of protein components. Plant Physiol 58: 710–716
24. VAUGHAN JG 1970 The Structure and Utilization of Oil Seeds. Chapman and Hall, London, pp 74–77
25. WEBER E, D NEUMANN 1980 Protein bodies, storage organelles in plant seeds. Biochem Physiol Pflanz 175: 279–306
26. YOULE RJ, ACH HUANG 1976 Protein bodies from the endosperm of castor bean: subfractionation, protein components, lectins and changes during germination. Plant Physiol 58: 703–709