MINERAL CONTENTS OF SOYBEAN SEED COATS AND EMBRYOS
DURING DEVELOPMENT

Joseph A. Laszlo

Northern Regional Research Center
Agricultural Research Service
U.S. Department of Agriculture
1815 N. University St.
Peoria, IL 61604

ABSTRACT: Little is known about mineral metabolism in legume seeds during development and maturation. This study examines the distribution of Mg, Ca, Cu, Fe, Zn and Mn between seed coat and embryo in five soybean (Glycine max [L.] Merr.) cultivars during seed development. Levels of Mg and Fe in seed coat and embryo varied with reproductive growth stage, but in no consistent manner across the various cultivars. Seed coat Ca and Zn levels initially decreased, then rose in the final stages, while embryonic levels decreased or remained constant. Cu and Mn contents of seed coats initially increased, then dropped — accompanied by an increase of these minerals in the embryo. These findings suggest that cationic metals are not passively assimilated in conjunction with dry matter accumulation, but rather are subject to ion-specific seed coat unloading, transport, and cotyledonary uptake processes.

1. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.
INTRODUCTION

During the reproductive growth stage of legumes, the developing seed represents the major sink for mineral nutrients. As with all nutrients accumulating in the cotyledons, minerals must pass through the seed coat. However, there are no direct vascular connections between cotyledons and maternal vascular system, which includes the seed coat (25, 37-39). Thus, imported minerals must traverse an apoplastic route from seed coat phloem cells to cotyledonary epidermal cells. The influence that the seed coat exerts in the accumulation of minerals in the developing embryo is unknown.

The mineral composition of soybean seeds at various stages of maturation has been examined recently (4, 11), but in both cases the bean was treated as a whole, with no distinction drawn between the mineral contents of seed coat and embryo. Given the vast morphological differences between these tissues, it would seem unlikely that their mineral contents would be similar. Therefore, a study was undertaken of the cationic metal content of the seed coat and embryo of developing and mature soybean seeds.

MATERIALS AND METHODS

Growth of Soybeans: Five soybean cultivars were investigated: Century, Williams 82, Peking, Sooty, and Wilson. Century and Williams 82 are maturity group III cultivars. Sooty, Peking, and Wilson are group IV cultivars. Williams 82 seed was the generous gift of T. M. Kuo (USDA/ARS, Peoria, IL). The other four cultivars were obtained from R. L. Bernard (U.S. Regional Soybean Laboratory, Urbana, IL). A sixth cultivar, Hardee (maturity group VIII), for which partial results were collected, was obtained from E. E. Hartwig (USDA/ARS, Stoneville, MS).

Plants were grown under greenhouse conditions. Seeds were germinated in 2.6-L pots (3 plants/pot, 3 pots for each cultivar) containing soil (Terra-Lite Soil Mixes and Conditioners, W. R. Grace & Co., Cambridge, MA) enriched with Micromax micronutrients and Osmocote Bedding Plant Food 14-14-14 (Sierra Chemical Co., Milpitas, CA). Plants were watered daily and fertilized as needed with Peters 20-20-20 (W. R. Grace & Co., Fogelsville, PA) containing
Sequestrene 138Fe Iron Chelate (Ciba-Geigy, Greensboro, NC). Natural light was supplemented with incandescent and fluorescent lighting, providing 370 µE • m⁻² • s⁻¹ to the top of the leaf canopy. Initially, the plants were kept on a 15-h light/9-h dark cycle, then switched after 2 months to a 10-h light period to induce flowering. Daylight temperature was maintained at 24–27°C. Nighttime temperature was 20–22°C. Data presented in Figures 1-6 are based on a single crop season (May–August, 1988).

Seed Collection and Processing: Immature and mature soybean seeds were collected and segregated into seven developmental stages (Table 1) based on the reproductive growth stages defined by Fehr and coworkers (5). In order to obtain a more detailed characterization of developmental changes, some stages were further subdivided. The primary determinant in assigning seeds to stages R4–R6.5 was fresh weight. Seeds with weights falling between defined weight intervals were discarded. Pod and seed coat color were the determinants for the final two stages. Table 1 also lists the approximate days after flowering, another commonly employed growth stage classification system, for each stage to facilitate comparison of these results with related studies. Seeds from a common cultivar and growth stage were pooled.

Following categorization of seeds into growth stages, seed coats and embryos (i.e., cotyledons and embryonic axis) were separated. These fractions were placed initially in containers on ice then stored at -20°C. Collected seed coat fractions were freeze dried overnight. Embryos were vacuum dried at 60°C. After drying, sample fractions were ground to a coarse powder and stored under vacuum at room temperature until analyzed.

Mineral Analysis: Samples (100 mg) were wet ashed following the microwave digestion procedure described previously (13). Quantitation of minerals was by ion chromatography (13). One to three replicates of each fraction, depending on available sample mass, were analyzed in duplicate. Insufficient quantities of embryo stages R4 and R5 precluded their analysis. Data in Figures 1-6 are presented as the mean ± one standard deviation of 2–6 determinations. Significant differences in mineral content between maturation stages within a cultivar were detected by analysis of variance.
TABLE I
Classification of Soybean Seed Developmental Stages.

<table>
<thead>
<tr>
<th>Reproductive Stage</th>
<th>Approximate DAF</th>
<th>Fresh Weight (mg)</th>
<th>Stage Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R4</td>
<td>14</td>
<td>≤20</td>
<td>Initiation of seed formation</td>
</tr>
<tr>
<td>R5</td>
<td>21</td>
<td>21-40</td>
<td>Beginning seed fill</td>
</tr>
<tr>
<td>R5.5</td>
<td>28</td>
<td>45-85</td>
<td>Intermediate linear seed fill</td>
</tr>
<tr>
<td>R6</td>
<td>35</td>
<td>90-210</td>
<td>Late linear seed fill</td>
</tr>
<tr>
<td>R6.5</td>
<td>42</td>
<td>≥220</td>
<td>Maximum fresh weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pods green &amp; seed coats green</td>
</tr>
<tr>
<td>R7</td>
<td>63</td>
<td>-</td>
<td>Physiological maturity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pods yellow &amp; seed coats yellow-green</td>
</tr>
<tr>
<td>R8</td>
<td>80</td>
<td>-</td>
<td>Harvest maturity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pods brown &amp; seed coats yellow</td>
</tr>
</tbody>
</table>

a Adapted from scheme devised by Fehr & Caviness (1980).
b Days after flowering.
c Seed coats of Peking, Wilson, and Sooty cultivars have black spots at this stage.
d Seed coats of Peking, Wilson, and Sooty cultivars are black at this stage.

RESULTS

Magnesium: Figure 1 displays the changes in the Mg content of seed coat and embryo fractions during maturation. Although significant (P < 0.05) differences between stages were found in seed coat Mg content, the seed coats did not display a consistent pattern of change in Mg levels across all cultivars. Four of the five cultivars showed a moderate increase in Mg from stages R6 through R8 in the embryo.
FIGURE 1. Magnesium content of seed coat (upper panel) and embryo (lower panel) fractions of developing soybean seeds.
Calcium: Unlike Mg, the Ca content of seed coat and embryo fractions varied in a highly consistent manner during development (Figure 2). In the seed coat, Ca concentrations decreased from R4 to R5.5/R6, then rapidly rose in the final stages in all five cultivars. A sixth cultivar, Hardee, also displayed this pattern (data not shown). Except for Century, embryonic Ca contents were nearly constant after an initial decrease during linear seed fill. Century Ca values continued to decrease ($P < 0.05$) in the embryo through stage R8.

Copper: Except for Sooty, the Cu content of seed coats decreased during linear seed fill (R5-R6), then remained nearly constant (Figure 3). The pattern of a slow increase in Cu in Sooty seed coats was consistently observed over several crop seasons, although the absolute concentrations differed somewhat, indicating that this is a cultivar-specific trait. Another cultivar-dependent trait displayed by the seed coats was their Cu content at harvest maturity (R8). Century (lowest) and Sooty (highest) differed by 4-fold in Cu at R8 in the data shown. In a previous crop, the difference was 14-fold (data not shown).

The Cu content of the embryo fraction of all five cultivars increased slowly (i.e., slightly faster relative to dry matter accumulation) throughout development (Figure 3). The large difference between Century and Sooty in R8 Cu content in the seed coat was not reflected in the embryo.

Iron: The changes in Fe concentration in developing seed coats varied with cultivar (Figure 4). Within each cultivar, Fe content changed significantly ($P < 0.05$) during the course of development.

Embryonic Fe concentrations increased significantly from R5.5 to R6 in all cultivars, but subsequent changes varied depending on cultivar (Figure 4). The Fe content of seed coats exceeded that of the corresponding embryo fraction at all stages for all cultivars.

Zinc: Seed coat Zn content varied during seed development in a pattern consistent across all cultivars (Figure 5), including Hardee (data not shown). Zinc content decreased dramatically from R4 to R6.5, then increased slightly (but significantly) to R8.

Embryonic Zn levels decreased slightly or remained constant during maturation (Figure 5).
FIGURE 2. Calcium content of seed coat (upper panel) and embryo (lower panel) fractions of developing soybean seeds.
FIGURE 3. Copper content of seed coat (upper panel) and embryo (lower panel) fractions of developing soybean seeds. Note that upper and lower panel ordinates are not drawn to same scale.
FIGURE 4. Iron content of seed coat (upper panel) and embryo (lower panel) fractions of developing soybean seeds. Note that upper and lower panel ordinates are not drawn to same scale.
FIGURE 5. Zinc content of seed coat (upper panel) and embryo (lower panel) fractions of developing soybean seeds.
FIGURE 6. Manganese content of seed coat (upper panel) and embryo (lower panel) fractions of developing soybean seeds. Note that upper and lower panel ordinates are not drawn to same scale.
Manganese: The Mn content of seed coats of all cultivars changed in a similar manner (Figure 6), and was observed to do so for two crop seasons. Manganese levels rose through linear seed fill (up to R5.5/R6), then decreased in subsequent stages. There was a 4- to 5-fold difference between the stage with the maximum Mn content and the final (R8) stage Mn content.

Figure 6 demonstrates that the Mn contents of the embryo increased (in four of five cultivars) over the period that seed coat manganese levels were dropping.

DISCUSSION

Two recent studies have reported on the mineral contents of maturing soybean seeds. The work of Iskanker (11) on three soybean varieties suggested that there were no significant differences in macro- and micronutrient content between immature (presumably stage R6), green mature (R7), and dry mature whole seeds (R8). Dornbos and McDonald (4) examined Williams 79 soybean seeds at stages R5, R6, R7, and R8, and found: (i) Fe, Cu and Mn content (on a dry weight basis) varied little or not at all, (ii) Ca and Zn decreased slightly, and (iii) Mg increased slightly. The present work essentially corroborates the Dornbos and McDonald findings for Mg, Ca, Fe and Zn changes in the embryo. Unlike their work, I that Cu and Mn levels increased slightly in embryos during development. However, far more drastic changes in mineral content occurred in seed coats. For some minerals, most notably Mn, the changes in the embryo opposed changes in the seed coat. While the seed coat represents only 6-12% of the dry matter in mature soybean seeds, it represents a more substantial fraction of the whole bean in earlier stages (Table 2). Thus, offsetting changes in seed coat and embryo mineral levels may have masked some changes when the whole bean was examined in the above mentioned studies.

Observation of changes in mineral levels during maturation serve little purpose unless those changes can be ascribed to physiologically significant processes. Assimilates accumulated by the developing embryo arrive solely via the phloem, which terminates within the seed coat parenchyma layer (16, 25, 27, 37). The pathway(s) and energetics of carbon (sucrose) and nitrogen (amino acid) unloading from phloem sieve cells, symplastic and apoplastic transfer through parenchymal cells, and uptake by cotyledonary epidermal cells have been studied in
TABLE 2
Dry Weight of Seed Coat and Embryo During Development.

<table>
<thead>
<tr>
<th>Reproductive Stage</th>
<th>Cultivar</th>
<th>Dry Weight (mg)(a)</th>
<th>Seed Coat Dry Wt (% of Whole Seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Embryo</td>
<td>Seed Coat</td>
</tr>
<tr>
<td>R4</td>
<td>Century</td>
<td>0.64</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>Williams 82</td>
<td>0.43</td>
<td>1.17</td>
</tr>
<tr>
<td>R5</td>
<td>Century</td>
<td>2.30</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>Williams 82</td>
<td>1.95</td>
<td>2.45</td>
</tr>
<tr>
<td>R5.5</td>
<td>Century</td>
<td>4.40</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>Williams 82</td>
<td>6.00</td>
<td>4.75</td>
</tr>
<tr>
<td>R6</td>
<td>Century</td>
<td>22.7</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Williams 82</td>
<td>28.4</td>
<td>8.3</td>
</tr>
<tr>
<td>R6.5</td>
<td>Century</td>
<td>109</td>
<td>16.2</td>
</tr>
<tr>
<td></td>
<td>Williams 82</td>
<td>117</td>
<td>13.9</td>
</tr>
<tr>
<td>R7</td>
<td>Century</td>
<td>193</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>Williams 82</td>
<td>165</td>
<td>14.8</td>
</tr>
<tr>
<td>R8</td>
<td>Century</td>
<td>195</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>Williams 82</td>
<td>189</td>
<td>13.8</td>
</tr>
</tbody>
</table>

\(a\) Mean of ≥ 20 seeds.

legumes (1, 6, 7, 10, 22, 24, 26, 27). Other than the work of Van Bel and Patrick (29, 30) on proton and K fluxes, nothing is known about cation movements in these tissues. The presented data suggest several aspects of how the seed coat may impact mineral import. However, a number of caveats must be mentioned first: (i) although the presumed flux of assimilates is from plant vegetative parts to embryo, ions may also efflux via the seed coat xylem with the excess water returned by the cotyledons (14, 16, 21, 27); (ii) because mineral
concentrations expressed on a dry weight basis cannot be used to draw conclusions about mineral concentrations in hydrated tissues, it is not possible to conclude from these data whether ions are moving with or against a concentration gradient between the seed coat and embryo; (iii) minerals present in the seed coat serve other metabolic purposes than to simply await transport to the cotyledons, thus may not be subject to influences controlling ion fluxes; (iv) factors in the seed can not be considered exclusively with regards to mineral assimilation — other source-sink relationships and phloem ion mobility effects need to be considered (15, 17, 19); and (v) the influence of environmental conditions (i.e., soil, moisture, temperature, etc.) on mineral content of fully matured soybeans are well documented (2, 20, 23). Investigation of the impact of these factors on mineral levels in immature beans was not considered in the present study.

Several trends, with regards to ion movements from seed coat to cotyledons, can be suggested from the observed pattern of changes in mineral levels during seed development. The behavior of the two macronutrients, Mg and Ca, were entirely different (Figures 1 and 2). Mg is highly phloem mobile (15) and only weakly bound by soybean seed coat cell walls (12), thus it responds readily to changes in sink water fluxes and other solute diffusional forces. This may account for the highly fluctuating levels of Mg in both seed coat and embryo. Conversely, Ca is poorly mobile in the phloem and tightly bound to cell walls. The attraction of Ca to cell walls may explain why Ca accumulates in the seed coat in the later stages of maturation (although this requires a further postulate that an alteration occurs in the seed coat parenchyma cell walls to effect this change). Pronounced seed coat Ca accumulation during maturation has been observed in other legumes (9). The behavior of Zn is very much like that of Ca (compare Figures 2 and 5). After an initial decrease, Zn is accumulated in the seed coat, again indicating a loss of transportability. The behavior of Cu and Mn are similar (compare Figures 3 and 6), and distinctly different from the other minerals. The loss of these two minerals from the seed coat roughly coincides with their accumulation in the embryo. Transport of Cu and Mn into the embryo apparently continues well after seed maximum fresh weight has been reached — the point at which Ca and Zn import becomes restricted. The data on Fe levels (Figure 4) offer little insight into Fe transport behavior.
It is clear that the unloading/transport/uptake behavior of minerals is highly individualistic and not readily explained by current concepts of ion transport mechanisms in plants (3, 28, 32-36). That Zn transport appears to be effected differently from Cu and Mn is totally unexpected given their similar chemical properties. Perhaps the forms in which the ions are transported represents the key. There are a plethora of potential ion-binding carrier molecules available (such as small organic acids, phytoferritin, phytochelatin, etc.), but free amino acids are the only metal-chelating solutes actually known to transfer from seed coat to embryo (10, 22). The organic acid constituents of seed coat exudates have not been reported in the literature.

An issue related to partitioning of minerals within the developing seed concerns the source of those minerals. Harper (8) has shown that Ca and Mg uptake by soybean plants remains high during reproductive growth stages, but the level of these macronutrients remains relatively constant in vegetative portions of the plant. This implies that Ca and Mg accumulated in the seed are derived from root-based sites, possibly via a xylem-to-phloem transfer prior to or within the pod (18). Harper (8) found micronutrient levels generally declined in vegetative parts during seed development, implying a redistribution of these minerals to the seed. However, Vasilas (31) reported that changes in macro- and micronutrient levels varied with respect to vegetative part (i.e., stem, leaflet and petiole) and planting date. Thus, drawing firm conclusions as to the source(s) of the minerals accumulated by the seed must await more definitive results.

REFERENCES


