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MICROSTRUCTURE OF WINGED BEANS

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Abstract

Microstructures of seven plant introductions of winged beans (*Psophocarpus tetragonolobus*) produced in Okinawa, Japan were investigated. In cotyledonary cells of winged beans, protein bodies plus numerous lipid bodies were distributed in a cytoplasmic network. Starch granules were often found in some introductions but rarely in others. All seven introductions had very thick cell walls. The high protein, fat and hemicellulose contents of winged beans are consistent with the numerous protein bodies, lipid bodies and thick cell walls in the mature cotyledonary cells. The cell walls contained a number of depressions or cavities 1 to 2 μm deep which frequently occurred opposite complementary pits in adjacent cells (presumably pit-pairs). Plasmodesmata traverse the cell walls in the pit-pairs. In order to determine changes during development, cultivar UPS-32 cultivated at Fukuoka-city was used. In cotyledonary cells at 30 days after flowering, cell walls which had pit-pairs with plasmodesmata, developing amyloplasts with starch granules, vacuoles with dense flocculent materials, tubular rough endoplasmic reticulum, mitochondria etc., were observed but no protein bodies or lipid bodies were apparent. Protein bodies and lipid bodies were, however, found at 45 days after flowering. Cotyledonary cells at 45 days contained many starch granules but mature seeds contained few, if any.

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Introduction

Winged beans (*Psophocarpus tetragonolobus*) are indigenous to Papua New Guinea and Southeast Asia but are attracting attention elsewhere as a potential food resource because of their high protein and fat contents. In their tropical and subtropical regions of origin, they are cultivated in domestic gardens and are consumed in immature stages as a vegetable for table use. Not only the seeds but also the pods, leaves, flowers, stems and tubers of winged beans are edible. Research on food use of mature seeds is still under investigation and a few trials to make foods have been reported (1, 4, 8). It is known that the hard structure of the beans is unfavourable for food processing (7) but there are few reports on the ultrastructure of this potentially important legume (10).

Materials and Methods

In the first experiment, we examined mature seeds of seven plant introductions (PIs) which were introduced and cultivated by the Okinawa Branch, Tropical Agriculture Research Center. The Center designated the PIs 001, 002 and so on in the order in which they were introduced. The beans used were of the 1978 crop. Their characteristics are listed in Table 1.

Table 1:
Characteristics of winged beans introduced and cultivated by the Okinawa Branch, Tropical Agriculture Research Center

Plant introduction (PI)	Country of origin	Cultivar (strain)	Beginning of flowering	Average time of maturation
001	Indonesia	local	Oct. 30	middle of Feb.
002	Indonesia	No.902	Nov. 17	beg. of Mar.
003	Indonesia	No.909	Nov. 9	beg. of Mar.
004	Indonesia	No.1126a	Oct. 15	middle of Jan.
007	Nigeria	TPT-2	Oct.29	middle of Feb.
012	Papua New Guinea	UPS-122	Oct. 26	end of Dec.
013	Okinawa	local	Nov. 1	beg. of Feb.

Data from Takada (9)

In the second experiment, cv. UPS-32 introduced from Papua New Guinea and cultivated in 1979 at

Fukuoka-city, Japan, was used to determine changes during development. Samples at 30, 45 and 58 days after flowering (DAF) were studied. Seed was sown in the greenhouse on July 15 and began to flower on October 14. Maturation was assumed at the time of complete browning and drying of the pods.

Soybeans used for comparative analysis of carbohydrate fractions were of the IOM type (grown in Indiana, Ohio and Michigan) imported from the United States.

Preparation of microscopic specimens

Small pieces of cotyledonary tissue were cut out with a razor blade, fixed with 5% glutaraldehyde solution and then with 1% osmium tetroxide solution (both in phosphate buffer containing 5% sucrose, pH 6.7), dehydrated with a graded acetone series (40 to 100%) and embedded in Epon resin.

For the light microscope (LM) the block prepared as described above was sliced to about 1 μm thickness with an LKB Ultratome and affixed on a glass slide. Protein was stained with 0.5% solution of Coomassie Brilliant Blue in 7% acetic acid and 50% methanol and then decolorized with 7% acetic acid and 50% methanol. Lipids were stained with a saturated solution of Sudan Black B in 50% ethanol and polysaccharides were detected with Schiff's reagent after oxidation with 0.5% periodic acid solution.

For the transmission electron microscope (TEM, JEM 100-B or JEM EX-1200), the same block used for LM was ultrathin-sliced and stained with saturated uranyl acetate solution and then saturated lead acetate solution in ethanol which was filtered just before staining.

Analysis of chemical components

PI 003 (Table 1) was used for chemical analysis. Moisture content was measured by drying at 105°C for 4 hours. Crude protein was determined by the Kjeldahl method (N x 6.25) and crude fat was measured by Soxhlet extraction with ethyl ether for 16 hours, drying in vacuum at 50°C and weighing. Ash was determined by heating in a muffle furnace at 550°C overnight. Crude fiber was measured after hydrolysis with 25% HCl and then with 25% NaOH, each for 3 hours.

The fractionation of polysaccharides was as follows (6): The beans were ground and extracted with n-hexane. The defatted powder was extracted with cold water and centrifuged. The residue from the centrifugation was extracted with boiling water and again centrifuged. The residue was dried and weighed (A). A was treated with sodium hypochlorite solution (100 μl of acetic acid and 750 mg of sodium chlorate were added to 50 ml of water at 75°C), filtered and washed through a glass filter, dried and weighed (B). B was treated with 10% NaOH solution for 18 to 24 hours with stirring, centrifuged, washed, dried and weighed (C). C was ashed at 900°C and weighed (D). Lignin was calculated as A - B, hemicellulose as B - C and cellulose as C - D. To compare with the values for winged beans, polysaccharides in soybeans were fractionated by the same method.

Results

Figure 1 shows LM-images of sections of mature seeds of seven PIs stained with Coomassie Brilliant Blue. The cotyledonary cells of all 7 PIs contained many protein bodies. On the other hand, the cell sections of PI 003 contained 2 to 4 protein bodies that were 6 to 8 μm in diameter plus many protein bodies that were only 2 to 3 μm in diameter; the protein bodies were nearly circular in shape. In PIs 001, 004, 007 and 013 numerous protein bodies, which were 3 to 4 μm in diameter, filled each cell and their shapes were slightly distorted. In PIs 002 and 012, the number and shape of protein bodies were rather similar to PI 003.

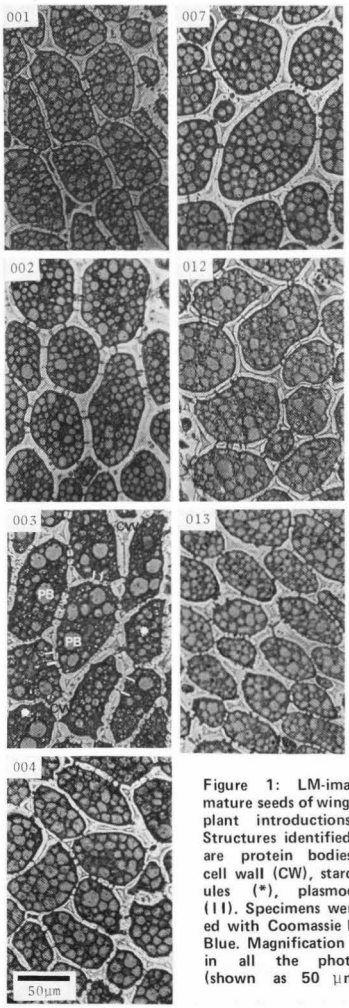

Figure 1: LM-images of mature seeds of winged bean plant introductions (PIs). Structures identified in 003 are protein bodies (PB), cell wall (CW), starch granules (*), plasmodesmata (↓↓). Specimens were stained with Coomassie Brilliant Blue. Magnification is same in all the photographs (shown as 50 μm bar.)

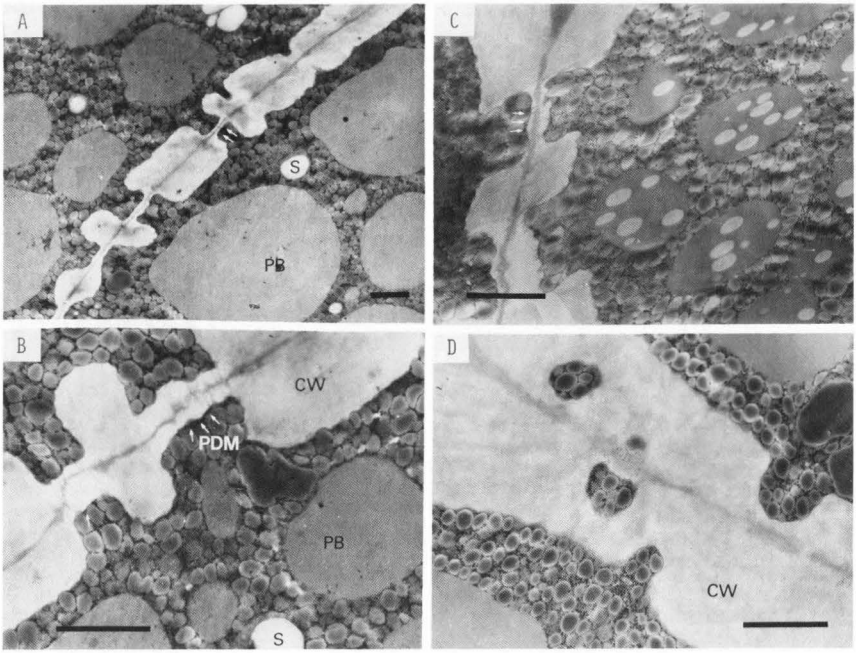

Figure 2: TEM-images of winged bean PI 003 (A,B) and PI 007 (C,D). Micrographs show protein bodies (PB), cell walls (CW), starch granules (S), lipid bodies (LB) and plasmodesmata (PDM, white arrows). Photograph C shows electron translucent inclusions in protein bodies. Bar in each photograph represents 2 µm.

Sudan Black B stained the cytoplasmic network and periodic acid-Schiff reagent stained the cell walls and starch granules. In PI 003 each cell section contained 10 to 20 starch granules which were 1 to 2 µm in diameter, whereas the other PIs contained hardly any starch granules. All PIs had cell walls 1 to 10 µm thick which included many depressions or cavities.

Figure 2 shows TEM-images of PIs 003 and 007 which were previously observed to be different under LM. The cytoplasmic portion stained with Sudan Black B contained numerous lipid bodies which were 0.4 to 0.7 µm in diameter. Protein bodies generally had no inclusions but in some cells they contained ovoid structures of low electron density (Fig. 2-C). There appeared to be a cell-to-cell variation in the distribution of these inclusions. The cell walls contained a number of depressions or cavities 1 to 2 µm deep which frequently occurred opposite complementary pits in adjacent cells (Figs. 2-A and 2-B). These paired depressions or cavities presumably are pit-pairs (3). The pit-pairs were filled with cytoplasm and the cell walls in these regions were less than 1 µm thick. Plasmodesmata traverse the cell walls in the pit-pairs (Figs. 2-A, 2-B and 2-C). Figure 2-D is an example of pits that do not oppose each other in the walls of two neighboring cells.

Table 2 summarizes the chemical composition of PI 003 and the content of polysaccharides fractionated from it. The high contents of crude protein and fat support the abundant occurrence of protein bodies and lipid bodies, respectively. The high content of

Table 2; Chemical composition of winged bean seeds (PI 003) and polysaccharide data for soybeans[a]

	Winged beans (%)	Soybeans (%)
moisture	10.43	
crude protein	34.89	
crude fat	17.70	
ash	3.86	
crude fiber	7.65	
total carbohydrate	27.42	
lignin	3.2	1.3
hemicellulose	25.3	8.2
cellulose	9.4	7.5

a: all values on dry basis except moisture

polysaccharides in winged beans corresponds to their thick cell wall structure but hemicellulose was extraordinarily dominant, when compared with that in soybeans.

LM-images of cotyledonary cells of winged beans of cv. UPS-32 cultivated at Fukuoka-city harvested at 30, 45, and 58 DAF are shown in Figure 3. At 30 DAF, cells were still less than 50 μm in diameter; they contained several vacuoles and small starch granules but no protein bodies. Nuclei stained with periodic acid-Schiff reagent were often observed and some were seen undergoing cell division. At 45 DAF cell size and thickness of cell walls had developed to the same extent as those at 58 DAF (mature seed), being about 100 μm in diameter and 1 to 10 μm thick, respectively. Pit-pairs were clearly recognized even under the LM. The cotyledonary cells at 45 DAF contained numerous starch granules and protein bodies of different sizes (1 to 30 μm). At 58 DAF starch granules were hardly to be found and protein bodies of nearly uniform size (5 to 10 μm) filled the cells.

Figures 4 and 5 show TEM-images of cotyledonary cells of winged beans harvested at 30 and 45 DAF, respectively. In Fig. 4-A are shown cell walls which have pit-pairs with plasmodesmata, amyloplasts with starch granules and several vacuoles. Dense flocculent materials were dispersed in most of the vacuoles but appeared coagulated in some cells as shown in Fig. 4-D. In Fig. 4-B (enlarged portion of Fig. 4A) the cytoplasm contained numerous ribosomes, tubular rough endoplasmic reticulum, dictyosomes (arrows) and dense bodies (*). Numerous mitochondria (arrows) were found (Fig. 4-C). The developing amyloplast in Fig. 4-E exhibited thylacoids (arrows). In the 45 DAF sample (Fig. 5-A), protein bodies and lipid bodies were already observed but many starch granules still remained. Plasmodesmata were observed through the center of a pit-pair as shown in Fig. 5-B.

Discussion

As far as the seven introductions of mature winged beans used were concerned, the microstructures of their cotyledonary cells were rather similar. Using the same samples, Yanagi et al. (11) found that the proteins of winged beans consisted of fractions of about 3S and 6 to 7S (main storage protein) by ultracentrifugal analysis and that no significant differences between introductions existed except for differences in the 3S fraction. The numerous protein bodies and lipid bodies in the mature cotyledonary cells are consistent with the high protein and fat contents of the seeds. The protein content of winged beans was comparable to that of soybeans but hemicellulose of winged beans was much higher than for soybeans as shown in Table 2. The plasmodesmata were characteristically found between pit-pairs which had pores connecting adjacent cells. They were observed in mature seeds but were also found at 30 and 45 DAF in cv. UPS-32 used to determine changes during development. It is also noteworthy that starch granules increased at 45 DAF and decreased again at 58 DAF, compared to the changes in starch content of ripening soybeans. In the latter period of maturation protein bodies appeared to become more uniform in size and starch granules rapidly decreased. The slight difference in microstructure of PI 003 might be connected with variations in maturation.

The maturity of winged beans seems to vary with cultivation conditions. As reported by Data and Pratt (2), winged beans of PI 007 which were sown in September and flowered from early December to the end of the following March reached maximum pod size at 20 to 22 DAF, maximum pod fresh weight at about 25 DAF, maximum seed size at about 30 DAF and maximum seed fresh weight at about 45 DAF. Pods, pedicels and seeds were completely dried after 60 DAF. On the other

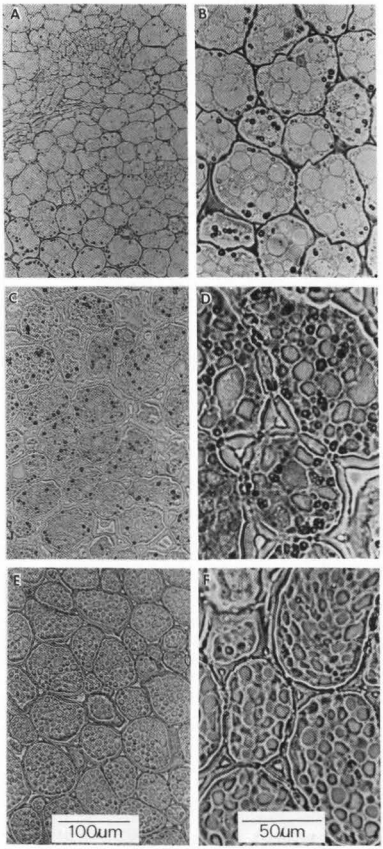

Figure 3: LM-images of cv. UPS-32 harvested at 30 (A, B), 45 (C,D), and 58 (E,F) DAF. The photographs were taken after staining with periodic acid-Schiff reagent.

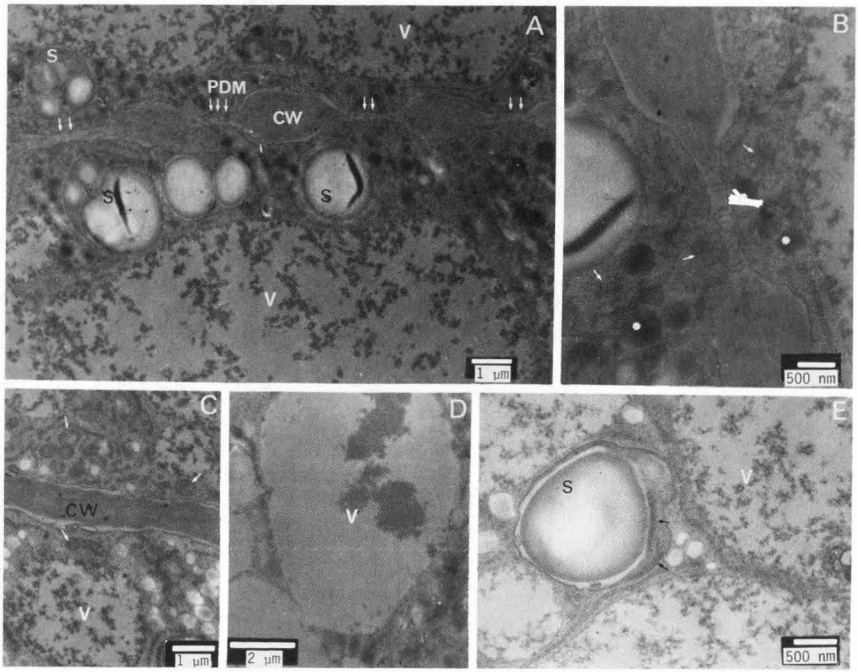

Figure 4: TEM-images of cv. UPS-32 harvested at 30 DAF. A shows pit-pairs with plasmodesmata (PDM, white arrows), cell wall (CW), starch granules (S) and vacuoles (V). B is a higher magnification of a part of A, showing a pit-pair with PDM in the center, dense bodies (*), dictyosomes (white arrows), starch granules and vacuoles. C shows vacuoles and a number of mitochondria (white arrows) and electron translucent bodies. D shows vacuoles in which dense flocculent materials are coagulated, being different from other vacuoles (A, B, C and E). E shows a developing amyloplast in which a starch granule and thylacoids (black arrows) are observed. All micrographs show tubular rough endoplasmic reticulum surrounding vacuoles.

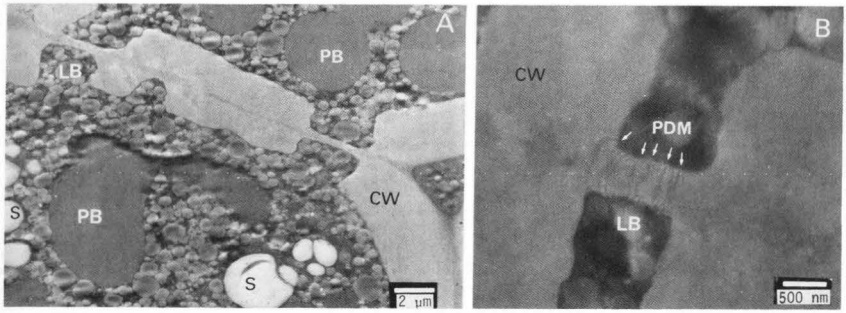

Figure 5: TEM-images of cv. UPS-32 harvested at 45 DAF. A shows pit-pairs of cell wall (CW), protein bodies (PB) and starch (S). Cytoplasm is filled with lipid bodies (LB). B shows a pit-pair with plasmodesmata (PDM).

hand, Kadam et al. (5) collected the pods of 40, 50, 60 and 70 day old plants and completely matured pods of 80 to 85 day old plants. Takada (9) reported that the cultivars in Okinawa that were sown on May 11 flowered from early October to November (120 to 150 days) and matured at 70 to 90 DAF. However, the ones sown on August 17 flowered from early December to the following March (60 to 90 days) and matured at 60 to 100 DAF. Data and Pratt (2) emphasized that winged beans (probably green pods for table use) must be harvested no later than 20 DAF for fresh use because after 20 DAF fiber development increases and tissues become too tough. Kadam et al. (5) showed that the cooking time of the seeds increased gradually until plants were 70 days old and rapidly increased at maturity (80 to 85 days). Cv. UPS-32 which was cultivated at Fukuoka-city in a temperate zone and under the extremely long day for these beans was sown on July 15, flowered from October 14 (about 90 days) and matured at 58 DAF. These seeds at 45 DAF had tough cell walls.

TEM-images of cv. UPS-32 at 30 DAF (Fig. 4) show a morphological change of cellular constituents which may be involved in the formation of protein bodies, lipid bodies and starch granules in cotyledonary cells. However, we do not refer to this question and a systematic fine structural investigation of these constituents during development will be reported in our progressing work.

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Discussion with Reviewers

C. Bair: Would you care to speculate on the nature of the low electron dense inclusions you observed in the protein bodies of winged beans? (Fig. 2C). In my investigations of soybeans I frequently found inclusions similar to those reported here. The inclusions not only varied in size and distribution, but also in electron density. There appeared to be a cell-to-cell (or tissue-to-tissue) variation in the distribution of these inclusions. Most of the inclusions were electron translucent, but some contained electron dense or scattered electron dense materials. X-ray microprobe analysis of these electron dense inclusions revealed a high level of phosphorus, and was believed to be the storage site of phytate.

Authors: In these experiments, we found the low electron dense inclusions but not electron dense ones in the protein bodies. But Saio et al. (1977) have observed electron dense inclusions in the protein bodies of sunflower and they were rich in phosphorus as determined by X-ray microprobe analysis. We agree with your opinion that they may be storage sites for phytate.

C. Bair: How do you explain the difference in the electron density of the lipid bodies shown in Figs. 2 and 5? Those in Fig. 2 are electron dense while those observed in Fig. 5 are electron translucent.

Authors: We have also observed differences in the degree of electron density of lipid bodies. Whenever we used lead nitrate in alkaline pH for electron staining, lipid bodies became electron translucent. Lead acetate used in these experiments made lipid bodies of soybean very electron dense, while those of winged bean were partly translucent (we did the two experiments the same way and at the same time). Saio et al. (1980). have also found that electron density decreased after storage of the seeds, using soybeans. We are not sure that it was caused by differences of quality of lipids.

C. Bair: Fig. 4B shows a considerable amount of electron dense materials scattered throughout the cytoplasm. Upon closer examination, they appear to be in close association with or originating from the rough endoplasmic reticulum. Could this be spherosomal or lipid body development? Numerous other researchers working with various tissues have reported that spherosomes originate from enlarged fragments of the endoplasmic reticulum.

Authors: We are now investigating the fine structural changes of winged beans during development in more detail, using UPS-45 or -99 harvested every 5 days. Consequently, we do not have any comments to make about spherosome development at this time.

C. A. Newell: Since winged beans are quite similar in seed composition to the soybean, how does the microstructure of developing cotyledons in the winged bean compare with those of soybeans? And how does the winged bean compare with other legume seeds in which such studies have been done?

Authors: We have not yet examined the microstructure of developing soybean cotyledons but plan to do so in the future and are also interested in comparative studies on other legumes. In developing seeds of *Vicia faba*, Harris (1979) and Adler and Müntz (1983) reported on development of endoplasmic reticulum or protein bodies. And Baumgartner et al. (1980) described localization of reserve protein in endoplasmic reticulum of *Phaseolus vulgaris*. Bergfeld et al. (1980) also reported on formation of protein bodies in *Sinapis alba L*. Concerning soybeans, Bils and Howell (1963) worked on developing soybean cotyledons and recently Thorne (1981) reported on the morphology and ultrastructure of soybean seed tissues and N. Kaizuma (Univ. of Iwate, Japan) presented papers at the annual meetings of the Japanese Association of Breeding in 1981, 1982 and 1983 on genetical studies on protein body development in soybean cotyledon cells (Kaizuma and Kasai 1981; Sato et al. 1982; Kaizuma and Sato 1983; Kamatsuda et al. 1983).

C. A. Newell: The authors have pointed out the thickness of the cell walls in winged bean cotyledons. How do these compare with other legume seeds? If winged bean cotyledonary cells do have much thicker walls than hitherto found for legumes generally, is there any particular adaptive significance to this characteristic?

Authors: In the regions lacking pit-pairs, the cell walls of winged beans are 5 to 10 times thicker than those found in soybeans and several Phaseolus species that we have examined. Some varieties of lupine (*Lupinus mutabilis*) seeds, however, also have very thick cell walls (unpublished data). We are not aware of any particular adaptive significance for thick cell walls in winged beans.

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