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Fruit and seed development in mung beans (*Phaseolus aureus* Roxb.)

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SUMMARY

A study of fruit set at different nodes was made in mung beans, *Phaseolus aureus* Roxb., under field conditions. Flowering commenced on the fourth node from the base and the percentage fruit set showed a gradual decrease from the fifth node upwards. Yield analysis was carried out for each of the fruiting nodes. When the leaf and inflorescences at a node are taken as a functional unit it is seen that there was a decrease in the ratio of leaf area to fruit and seed weights from the base of the plant upwards indicating that at the upper nodes particularly, some other plant parts also contribute to the photosynthate pool of the developing seeds.

A quantitative study of the dry matter, proteins and starch in the fruit wall and seeds of fruits at different stages of development was made. It showed that the rapid increase in dry matter, proteins and starch in the seeds at the later stages of development is compensated, in part, by a decrease of these components in the fruit wall. Histochemical studies of the fruit wall further supported these observations. This indicated the contribution of substrates by the fruit wall to the developing seeds.

INTRODUCTION

Mung bean is an important grain legume in several parts of Asia and is becoming increasingly used in many other countries due to its nutritional value (Sinha, 1977). The yield is poor and this discourages wide cultivation of it. The plant normally produces a large number of flowers but most of them abscise (Sinha, 1974). Only limited studies have been carried out on its growth and development (Rachie & Roberts, 1974). The present study is intended to fill this gap.

MATERIALS AND METHODS

Phaseolus aureus Roxb. cv. Hybrid 45 crop was raised in the field attached to the Water Technology Centre, IARI, during the *kharif* season of 1974. Seeds were sown in 5 × 5 m plots in the first week of July after inoculation with an effective strain of *Rhizobium* in peat obtained from the Microbiology Division, IARI. Fertilizer at the rate of 20 kg N, 60 kg P and 40 kg K/ha was given at the time of sowing. The inter-row distance was 25 cm and the plants within a row were thinned to 10 cm apart 2 weeks after emergence. Being a monsoon crop, no irrigation was given.

Flowers were tagged at anthesis so that the age of fruits could be determined. Fruits were harvested 2, 4, 6, 8, 10, 12 and 14 days after anthesis (DAA). Each sample consisted of three replicates, and a replicate had 10 fruits. The lengths and circumferences were noted and diameters computed. The fruits were then split into pod covers and seeds and their fresh weights determined. They were dried to constant weight at 85 °C and the dry weights determined.

For determination of respiratory rates fruits of different ages were split into pod covers and seeds. They were chopped into 2–3 mm pieces (only at stages beyond 6 DAA for seeds) and placed in 0·2 M tris HCl buffer, pH 6·8, in Warburg flasks, and the respiration rate determined at 33 °C using Warburg's Constant Volume Respirometer (Umbreit, Burris & Stauffer, 1964). Three replicates were taken for each stage and material pooled from five fruits formed a replicate.

Pod covers and seeds of similar replicates were

fixed in 80 % ethanol for sugar and starch determinations and in 10 % trichloro-acetic acid (TCA) for amino nitrogen (from soluble fraction) and protein nitrogen (from TCA insoluble fraction) determinations. The insoluble starch was hydrolysed using perchloric acid (McCready *et al.* 1950) and sugar contents determined (Dubios *et al.* 1956) using glucose standards. Amino nitrogen was determined using Rosen's (1957) method (leucine standard) and protein nitrogen with Folin's ciocalteau reagent (Lowry *et al.* 1951) using bovine serum albumin as standard.

Rates of [^{14}C]valine incorporation were determined for fruit walls and seeds using 100 mg chopped samples in triplicates. Samples were incubated in 0·2 M tris HCl buffer, pH 6·8, and [^{14}C]valine (obtained from Bhabba Atomic Research Centre, Bombay, India, specific activity 25 μCi/mM) at 30 °C for 30 min. Samples were then washed well in a continuous stream of distilled water and the proteins precipitated with 10 % TCA. The samples were ground and extracted with 80 % alcohol three times and the ethanol insoluble fraction dispersed in formic acid. Aliquots from TCA insoluble fractions were dried in vials and the scintillation mixture [4 g 2,5-diphenyloxazole (PPO) and 100 mg 1,4-bis 2-(5-phenyloxazolyl) (POPOP)/l toluene] added. Counts were obtained using a Packard Scintillating Spectrometer. The [^{14}C]valine incorporation rate was expressed as counts per minute per hour.

For histological studies fruit walls of different ages were fixed in formalin-acetic acid-ethanol (FAA) and then preserved in 70 % ethanol (Johansen, 1940). Materials were passed through ethanol-xylol series and embedded in paraffin. Serial transverse and longitudinal sections were prepared with a rotary microtome at 8 μm thickness. The preparations were stained with periodic-acid Schiff's (Jensen, 1962) or mercuric-bromophenol blue reagent (Mazia, Brewer & Alfert, 1953) or safranin-fast green (Jensen, 1962). Following routine dehydration procedures sections were mounted in DPX.

Collodion peels were prepared from the upper epidermis of fruit wall.

Fifteen unbranched plants were sampled in the month of September when the crop was mature and the number of flower buds formed per inflorescence was counted from the number of abscission scars on the inflorescence axis. Earlier counts of actual flower buds and flowers were made for some samples to check for error in the method of estimation of flowers and fruits by counting scars. The fruits at the different nodes of the plant were also counted.

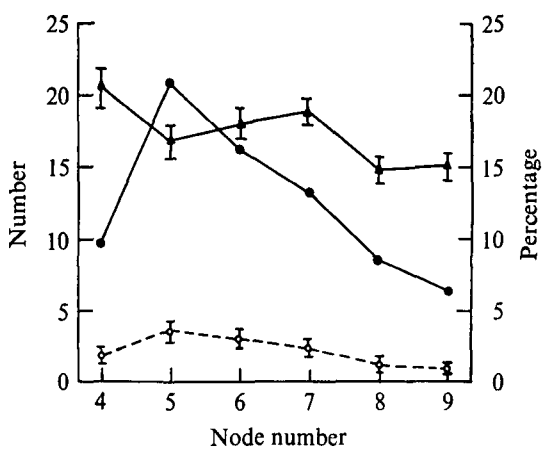
The plants were then grouped into three lots of five each and the leaf areas at different nodes of the plants were determined using an automatic area meter (AAM-7, Hayashi Denkoh). The numbering of nodes was from the oldest node upwards. The dry weights of the leaves, fruits and seeds at the different nodes were determined after drying to constant weight.

RESULTS

At maturity the mung bean plants were about 40 cm in height and had 8–9 nodes. The plants were usually unbranched. Flowering was first noted 30 days after sowing. Inflorescences were borne in the axils of leaves from the fourth node upwards, and the stem also terminated in an inflorescence.

The maximum number of flower buds per inflorescence occurred at node 4 whereas the maximum number of fruits was at node 5 (Text-fig. 1). The percentage fruit set was also a maximum at node 5.

The leaf area and leaf weight at the different nodes increased from the base upwards, reaching a maximum at node 5 and thereafter declined (Table 1). The relationships between leaf area and fruit and seed weights at different nodes of the plant are also shown in Table 1. The fruit and seed weights were also higher at node 5 than at the other fruiting nodes of the plant. The correlation coefficients for leaf area and fruit weights and leaf area and seed weights were highly significant (r = 0·93 for fruit weight and leaf area and r = 0·85 for seed weight and leaf area). However, the ratio of leaf area to fruit and seed weights decreased from the base upwards indicating that a smaller leaf area was capable of producing unit fruit and seed at the upper nodes.

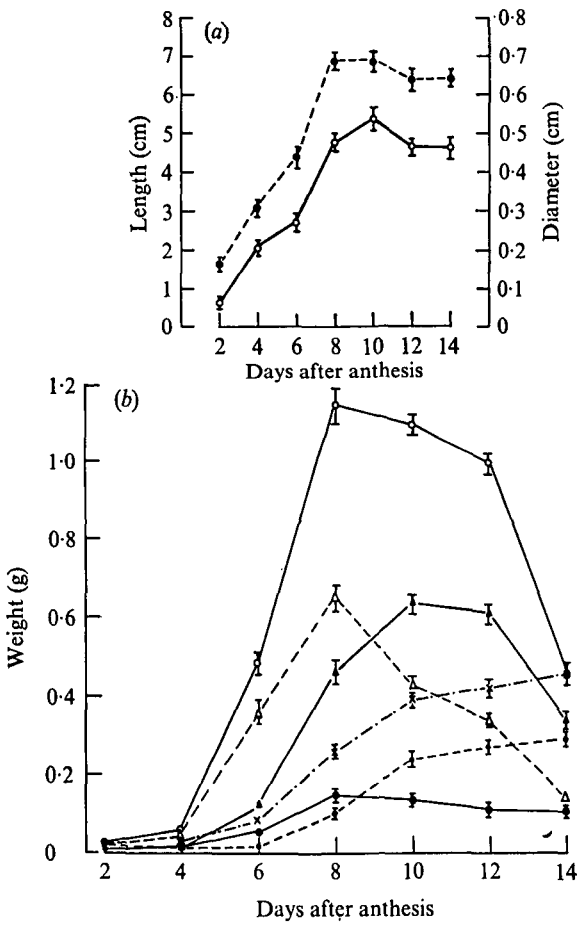


Text-fig. 1. Number of flower buds, fruits and percentage of fruit set at the different flowering nodes of the plant. ▲, Number of flowerbuds; ○ number of fruits; ●, percentage fruit set. I, S.E.

Table 1. *Changes in the leaf area and weight, fruit and seed weight, and ratio of leaf area to fruit and seed weights at the different nodes of the mung plant*

Node number	Leaf area (cm^2)	Leaf weight (g)	Fruit weight (g)	Seed weight (g)	Leaf area/ fruit weight (cm^2/g)	Leaf area/ seed weight (cm^2/g)
1	31·2 ± 1·3	0·13 ± 0·003	—	—	—	—
2	62·4 ± 2·6	0·21 ± 0·004	—	—	—	—
3	68·4 ± 2·6	0·32 ± 0·005	—	—	—	—
4	91·9 ± 3·1	0·45 ± 0·005	1·402 ± 0·013	0·9432 ± 0·011	80·5	97·4
5	107·0 ± 3·6	0·51 ± 0·006	2·13 ± 0·015	1·4 ± 0·015	50·2	76·4
6	78·3 ± 2·9	0·39 ± 0·006	1·84 ± 0·016	1·23 ± 0·011	42·3	63·6
7	30·8 ± 1·8	0·14 ± 0·004	0·98 ± 0·011	0·73 ± 0·009	31·3	42·2
8	4·7 ± 0·3	0·05 ± 0·001	0·48 ± 0·005	0·43 ± 0·005	0·97	1·09
9	—	—	0·186 ± 0·001	0·1457 ± 0·001	—	—

Correlation between leaf area and fruit weight ($r = 0·93$) and leaf area and seed weight ($r = 0·85$) for the nodes 4–8 were found to be significant ($P < 0·01$).

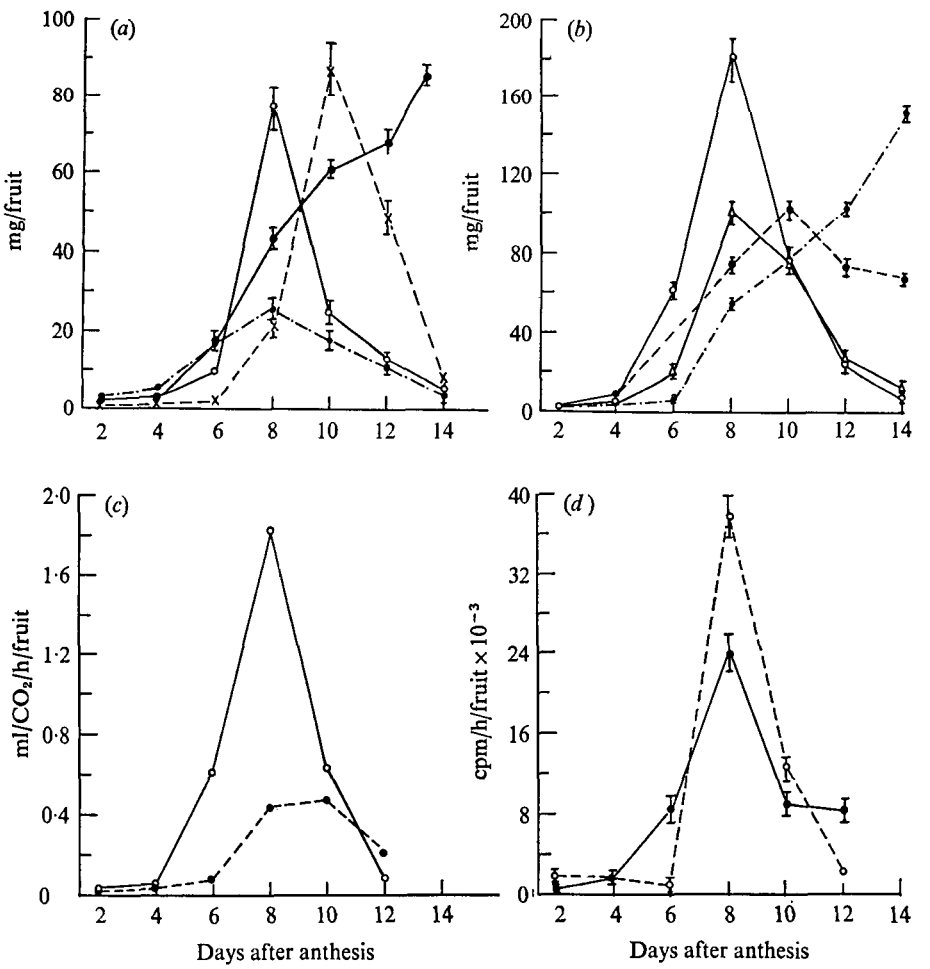


Text-fig. 2. (*a*) Change in the length and diameter of the fruit at different days after anthesis, ●, Length; ○, diameter. (*b*) Changes in fresh and dry weights of the fruit, fruit wall and seeds/fruit with age. ○, Fresh weight fruit; △, fresh weight fruit wall; ▲, fresh weight seeds; ×, dry weight of fruit; ●, dry weight of fruit wall, ——, dry weight seeds. I, S.E.

Changes in the fruit length and diameter at different DAA were studied (Text-fig. 2(*a*)). At 8 and 10 DAA the fruit length and diameter respectively reached their maximum value. Text-figure 2(*b*) shows the changes in fresh and dry weights of fruit, fruit wall and seeds of the fruit at different ages. The fresh weight of the fruit and fruit wall was maximum 8 days after anthesis and thereafter declined. The dry weight of the fruit and seeds showed continued increase whereas that of the fruit wall showed a gradual decrease from 8 DAA onwards. Therefore increases in the weights of the fruit beyond day 8 were due to the growth of the seeds.

Anatomical studies of the fruit wall at 2 DAA revealed three zones (Plate 1A). The innermost zone had large cells with hyaline contents. The outer zone consisted of cells with chloroplasts. The vascular supply was restricted to this zone. The middle zone of small cells had dense cytoplasm. The cells of the inner zone started disintegrating at 6 DAA (Plate 1B). The cells of the outer zone had chloroplast until 10–12 DAA. During this period the walls of the cells of the middle zone became very thick (Plate 1C) and thereafter were completely sclerified. The outer zone became partly disintegrated and the inner completely so by maturity. The epidermal peels of the fruit wall revealed presence of stomata.

Histochemical tests for starch showed its pre-



Text-fig 3. (*a*) Changes in amino-nitrogen and protein nitrogen in the fruit wall and seeds (per fruit) with age. ×, Amino-nitrogen in seeds; ○, amino-nitrogen in fruit wall; ●, protein in seeds; —·— protein in fruit wall. (*b*) Changes in total sugars and starch in the fruit wall and seeds (per fruit). ○, Fruit wall sugar; △, seeds sugar; ●, fruit wall starch, —·—, seeds starch. (*c*) Rate of respiration expressed as ml CO_2 released/h/fruit in the fruit wall and in seeds. ○, Fruit wall; ●, seeds. (*d*) Changes in amount of [^{14}C]valine incorporation into the TCA insoluble fraction of the fruit wall and seeds (per fruit). ○, Fruit wall; ●, seeds.

sence in all three zones of the fruit wall until 6 DAA but later it was confined to the cells of the outer zone (Plate 2). Beyond 8 DAA there was depletion even in the outer zone. The nature of accumulation and depletion of proteins was similar.

Quantitative charges in some constituents of fruit wall and seed were also examined at regular intervals. Beyond 8 DAA the soluble amino-nitrogen was more in the developing seeds than in the fruit wall. It increased considerably between 6 and 10 DAA in seeds and 6 and 8 DAA in fruit wall (Text-fig. 3(*a*)). The protein content of the seeds showed a continued increase with age whereas in the fruit wall there was a decrease after 8 DAA.

Total sugar content of the fruit increased until 8 DAA at which stage it was 290 mg and thereafter declined to 27·4 mg by 14 DAA, by which time the seeds were dehydrated and mature (Text-fig. 3(*b*)). The starch content in the 6-day-old seeds was only 9·4 mg per fruit but it increased considerably afterwards (Text-fig. 3(*b*)). Between 8 and 14 DAA the fruit wall showed a decrease in the total of sugars and starch, whereas during the same period these constituents increased in the seeds. The respiration rate of the fruit wall increased up to 8 DAA and then decreased later considerably (Text-fig. 3(*c*)). In the seeds there was a gradual increase until 10 DAA and thereafter it decreased.

The metabolic activity of the fruit wall and seeds were assessed by feeding [^{14}C]valine and studying the rate of its incorporation into the insoluble fraction (Text-fig. 3(*d*)). Both fruit wall and seeds showed a decline in incorporation 8 DAA but the decrease in the rate of incorporation in seeds was not as much as in the fruit wall. The latter had indicated very little incorporation of [^{14}C]valine.

DISCUSSION

The present study was undertaken with a view to understanding the pattern of fruit and seed development so that it could provide evidence on crop yield. In mung bean cv. Hybrid 45 the fruit setting varied at different nodes, the highest percentage being at node 5. The percentage fruit set and of seed set could be correlated with the leaf area at each node. As leaf area decreased from node 5 upwards so did the percentage of fruit set and the seed weight. Similar results have been obtained in broad beans, *Vicia faba* (Ishag, 1973; Tamaki, Asanuma & Naka, 1973). The leaf area present for 1 g dry weight of fruits and seeds at node 4 was 80·5 cm^2 and 97·4 cm^2 respectively. This ratio decreased to 0·97 and 1·09 at node 8, for fruit and seed respectively. This is suggestive of either (*a*) the upper leaves were more efficient in photosynthesis or (*b*) the fruits of the upper nodes were better exposed to intercept light and hence could have better photosynthetic capacity. It appears that both these possibilities exist.

The correlation coefficient between leaf area and fruit weight was 0·93 and between leaf area and seed weight 0·85. Thus the leaf area at different nodes containing inflorescences is important. There was a steep decrease in leaf area from node 5 upward in this cultivar. It could be related to the time of flowering, emergence of inflorescences and opening of flowers. A study of the flowering behaviour in relation to the vegetative growth of the plant can be important in assessing the factors affecting productivity of the plant.

It was clear from this study that the fruit wall could be photosynthetically active since it was chlorophyllous and also had stomata. The importance of fruit wall in seed development has been shown earlier (Crookston, O'Toole & Ozbun, 1974; Khanna & Sinha, 1976; Sinha & Sane, 1976). However, in mung beans the disintegration of parenchymatous tissue starts by 8 DAA, when the seeds are just entering the active period of their growth. This might restrict the contribution of the fruit wall to the seeds in this species.

In the present study it was observed that the maximum fruit set on any node did not exceed 25% of the flower buds formed. There could have been several reasons for this, as discussed by Sinha (1974, 1977). It is not possible to suggest that only the availability of photosynthates limited the percentage of fruit set and seed production. However, an analysis of the various factors is necessary for improving the properties of fruit set and consequently the plant yield.

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EXPLANATION OF PLATES

PLATE 1

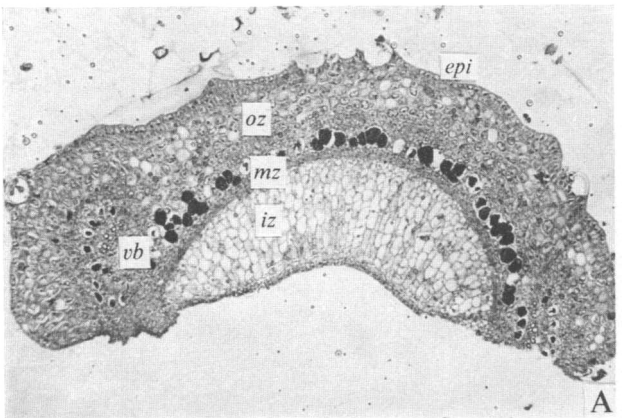
Cross-sections of the fruit wall at different days after anthesis. (A) 2 days after anthesis, ×70. (B) 6 days after anthesis, ×140. (C) 14 days after anthesis, ×450.

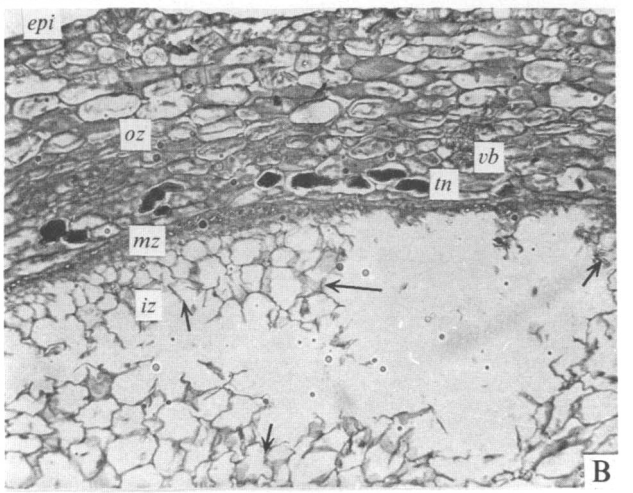
Note the zonation, and the disintegration (indicated by arrows). *Epi*, Epidermis; *iz*, inner zone; *mz*, middle zone; *oz*, outer zone; *scl*, sclerified middle zone; *tn*, tannin.

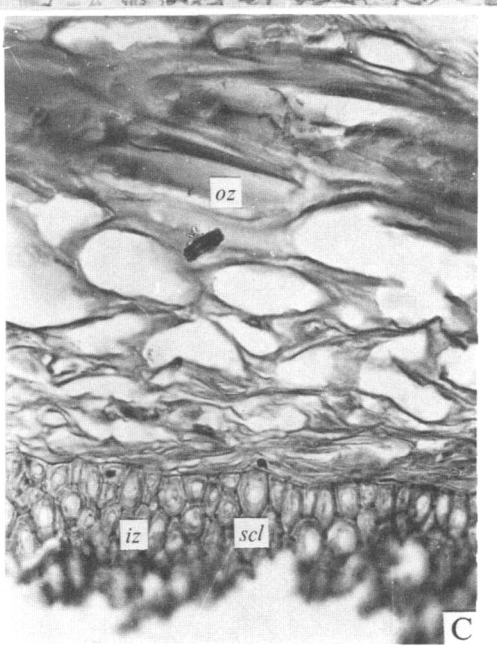
PLATE 2.

Cross-sections of fruit wall stained with PAS-reagent. (A) 4 days after anthesis, ×210. (B) 6 days after anthesis, ×350.

epi, Epidermis; *ip*, insoluble polysaccharides (starch grains); *iz*, inner zone; *mz*, middle zone; *oz* outer zone.







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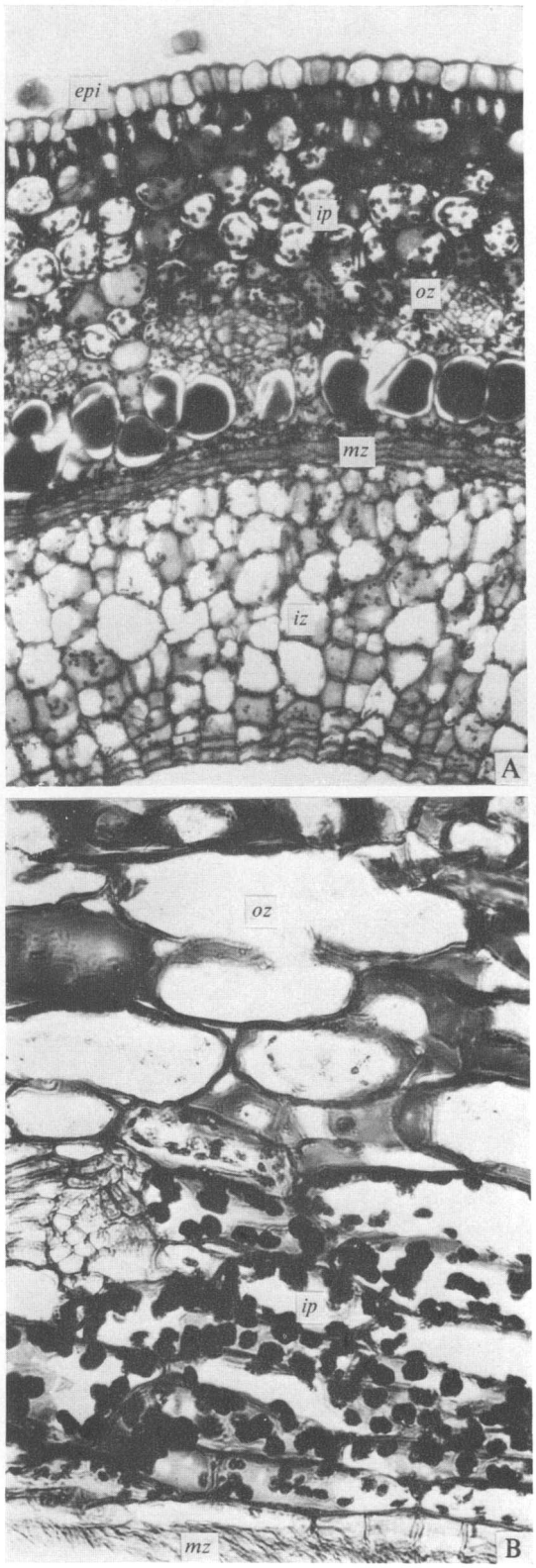
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