Proc. Indian natn. Sci. Acad. **B46** No. 3 pp 356–360 (1980)

Significance of Bound Auxin in Developing Seeds and Pods of Certain Legumes

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(Received 28 May 1979)

Changes in free and bound IAA levels in developing seeds and pods of *Cice arietinum* T3 *Pisum sativum* Bonnevile and *Vicia faba* have been studied. A positive correlation seems to exist between growth increments and bound auxin. IAA seems to play an important role in the mobilization of precursors and growth of developing seeds. IAA-oxidase may regulate growth through the control of endogenous IAA level in growing tissues.

Key Words: **Legumes, Seed development, Endogenous IAA, IAA oxidase**

Introduction

Indole-3-acetic acid (IAA) occuıs in the free and bound states in plant tissues. Several workers have proposed that the physiologically active form of auxin is the bound form (Fransson & Ingestad 1955, Bonner & Foster 1956, Bentley 1961). Further, IAA has been thought to play a significant role in the mobilization of precursors to and the growth of developing seeds (Titova 1969, Eeuwens & Schwabe 1975). As the developing seeds and pods constitute a fairly suitable system for studying the correlations between growth and biochemical changes (Rauf 1978, Rauf & Banerji 1978), we studied the changes in endogenous levels of free and bound IAA in the developing seeds and pods of *Cicer arietinum*, *Pisum satitvm* and *Vicia faba*.

Materials and Methods

Seeds of *Cicer arietinum* T3 *Pisum sativum* Bonneville and *Vicia faba* were procured respectively from the Indian Agricultural Research Institute, New Delhi; Sutton and Sons, Calcutta; and Agarwal Seed Stores, Meerut. The plants were grown in the experimental plots of the University Botanical Garden. Flowers were tagged on the day of anthesis (DAA) and pods were collected at different stages of development. In *Pisum* the samples were taken at 7, 14 and 21 DAA and in *Cicer* and *Vicia* at 14, 21 and 28 DAA. The period between the first and second stages has been described as the 1st phase and that between the second and third stages as the 2nd phase. At each stage, the pods and seeds were analysed for fresh weight, dry weight, endogenous Indole-3-acetic acid (IAA) level and IAA-oxidase activity.

Extraction and estimation of free and bound IAA

The indoles were extracted by the procedure of Tamura et al. (1970). Twenty grams of frozen material were homogenized in chilled ethanol (96%), centrifuged (1000 × g, 15 min) and the supernatant and the residue processed respectively to extract free and bound IAA respectively. For extraction of free IAA, the supernatant was evaporated to its aqueous residue, adjusted to *p*H 2.5 with 1 N HCl and partitioned against ethylacetate to yield the organic and aqueous fractions. The ethylacetate fraction was evaporated to dryness and the residue dissolved in 4 ml ethanol. The aqueous fraction was neutralized with sodium bicarbonate and the volume reduced by evaporation to 4 ml. Thus, 4 ml each of an organic and aqueous fractions were obtained. For bound IAA extraction, the residue was hydrolysed in 1N NaOH at 15 lb/sq. inch pressure for 30 min, to release the bound indoles (Bentley 1961). The indoles were extracted from the hydrolysate by mixing with ethanol (90%) at 25°C for 24 hr and the extract processed as in the case of free indoles to yield the organic and aqueous fractions.

The organic and aqueous fractions were then subjected to ascending and/or circular chromatography on Whatman No. 1 paper with *n*-butanol : acetic acid : water (12:3:5v/v) solvent mixture. The spots were developed with Ehrlich reagent (10% *p*-dimethly amino benzaldehyde in conc. HCl : acetone, 1 : 9). The endogenous IAA spots were identified on the basis of Rf value and colour reaction and the quantity of IAA determined with the help of spot area/or densitometric standard curves.

Isolation and assay of IAA-oxidase

IAA-oxidase was extracted and assayed according to the procedure of Rabin and Klein (1975). Two grams of seeds/pods were homogenised in cold distilled Water, filtered through cheese cloth and Whatman No. 1 paper and the enzyme precipitated by addition of acetone after evaporation and dissolved in 10 ml of phosphate-citrate buffer (*p*H 5.6) to constitute the crude enzyme preparation. Two ml of the crude enzyme were mixed with 0.5 ml IAA solution (200 μg/ml) and 5 ml buffer and incubated at 255 °C for 50 min. After incubation, 2 ml aliquot of the reaction mixture was reacted with 4 ml Salkowski reagent (2% v/v 0.5 M ferric chloride in 35% perchloric acid) for 30 min and OD a 525 nm was read against a blank. The μg of IAA degraded was calculated from the standard curve.

Results

The results are given in figures 1 and 2 which indicate the parallelism between the rates of growth and rise in bound auxin in the developing seeds and pods of the legumes studied. In *Cicer* and *Vicia*, IAA was present only in the organic fraction and in *Pisum* even in the aqueous fraction.

Seed development

In *Cicer* both fresh weight and dry weight increased by *ca.* 2 times in the 1st phase and *ca.* 2.5 times in the 2nd phase (figure 1a). The free IAA level doubled in the 1st phase, followed by a steep decline. However, the changes in the bound IAA level follow the pattern of growth increase, the rise in the 1st and 2nd phases being *ca.* 1.6 and 3 times

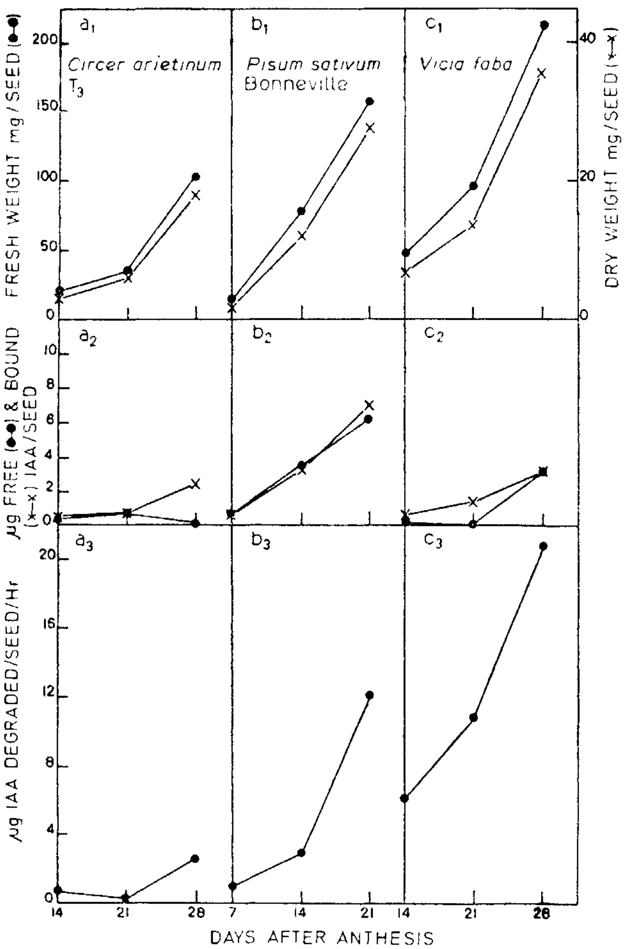

Figure 1 Change in free IAA, bound IAA and IAA-oxidase activity accompanying seed development in certain legumes

respectively (figure $1a_2$). In *Pisum*, the growth increments are *ca.* 6 times and 2 times in the 1st and 2nd phases respectively (figure $1b_1$) and the increases in free and bound IAA are nearly at similar rates, being *ca.* 2 times in the 1st phase and 2 times in the 2nd phase (figure $1b_2$). In *Vicia*, the growth increments are at similar rates, being *ca.* 2 times in both the 1st and 2nd phases (figure $1c_1$). The free auxin level is very low in the first phase and a rise is observed in the 2nd phase. Again, the rate of increase in the bound IAA level parallels the growth rate, the content nearly doubling in both the 1st and 2nd phases (figure $1c_2$). Thus, some correlation seems to exist between the increment in growth, both in terms of fresh and dry weights, and the level of bound auxin in the developing seeds.

IAA-oxidase activity rises in the developing seeds of all the legumes studied. In *Cicer* (figure $1a_3$), there is a slight decline in the 1st phase followed by about two fold-increase in the 2nd phase. In *Pisum* (figure $1b_3$) and *Vicia* (figure $1c_3$) there is a continuous rise. However, while in *Pisum* the increase is more in the 2nd phase, in *Vicia* the rise in IAA-oxidase activity is at nearly similar rates in both the 1st and 2nd phases. In *Cicer*, there is an inverse correlation between IAA-oxidase levels and endogenous free IAA content. Such a negative correlation is not observed in *Pisum* (both phases), in *Vicia* (2nd phase), and in the bound IAA pattern.

Pod development

In *Cicer*, the fresh weight of the pod shows a slight increase of 13% and 6% in the 1st and 2nd phases respectively (figure $2a_1$). The dry weight rises by 40% in each phase. The free IAA level, after an increase of 21% in the 1st phase, falls to trace level. However, the changes in bound IAA level parallel the fresh weight change (figure $2a_2$). The pod of *Pisum*, registers an increase in fresh weight of 65% and 14% and in dry weight of 93% and 282% in the 1st and 2nd phases respectively (figure $2b_1$). In this case again change in auxin level tallies with fresh weight, the free IAA level rising by 62% and 13% and bound auxin by 65% and 90% in the two successive phases (figure $2b_2$). In *Vicia*, the pod fresh weight rises by 50% and 30% in the 1st and 2nd phases respectively and the dry weight by 57% and 37% (figure $2c_1$). The free IAA level falls continuously during the developmental phases. The bound auxin, however, rises again by *ca.* 40% and 25% in the 1st and 2nd phases, indicating again a parallelism with the pattern of fresh weight change (figure $2c_2$). Thus, in the case of pods also, 2 close parallelisms exist between growth and bound auxin level.

As in the case of the seed, IAA-oxidase activity rises with pod development. While an enhanced rise in the 2nd phase overlaps with the decline in free IAA level in *Cicer* (figure $2a_3$), in *Vicia* a relatively high initial level and subsequent high activity are associated with continuous loss of free IAA during pod development (figure $2c_3$). The extent of IAA-oxidase activity in ***Pisum*** pod is intermediate with regard to *Cicer* and *Vicia* and rises with pod development (figure $2b_3$).

Discussion

Several workers have proposed that IAA plays a major role in the growth and translocation of assimilates in the developing seeds of wheat (Titova 1962), pea (Euewens & Schwabe 1975), pigeon pea (Rao & Rao 1975) etc. Although Wardlaw and Moncur (1976) could not obtain any correlation between auxin production and

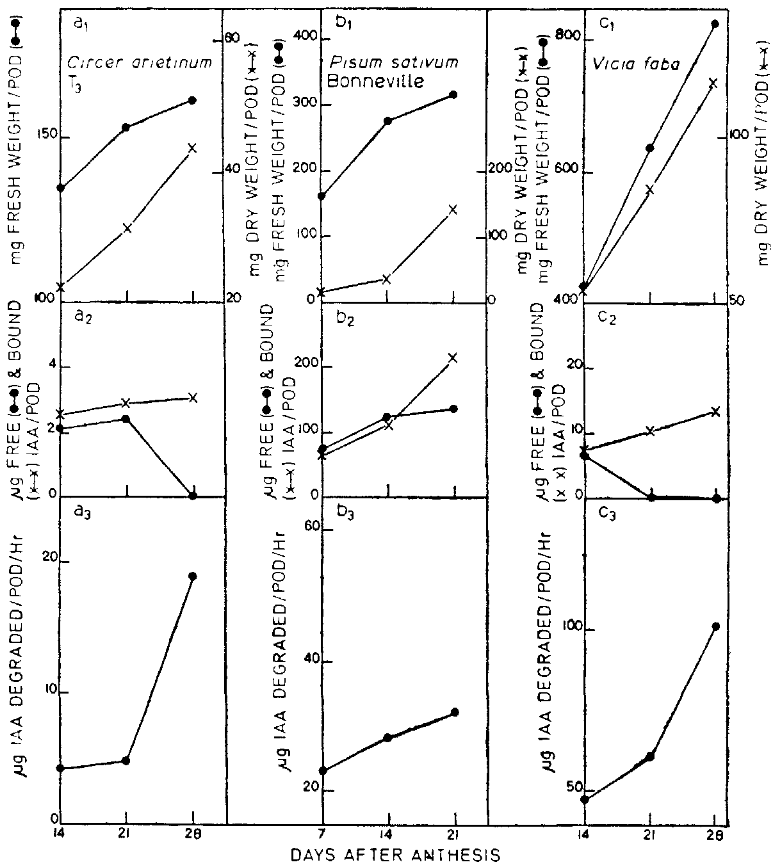

Figure 2 Change in free IAA, bound IAA and IAA-oxidase activity accompanying pod development in certain legumes

assimilate translocation in wheat, the parallelism observed here between increase in fresh weight, dry weight and endogenous IAA level in the legumes studied, provides support to the contention of earlier workers. The exact role of IAA-oxidase in growing tissue remains unclear although some involvement in the maintainence of optimal level seems possible. The noteworthy feature is the correlation between growth increment on freshweight basis, in both the developing seeds and pods, with the bound auxin levels. This observation bears out the contention that bound auxin is the physiologically active form. (Fransson & Ingestad 1955, Bonner & Foster 1956, Bentley 1961)

Acknowledgements

We are thankful to Professor Y S Murty for encouragement and one of us (A A) acknowledges the award of the UGC Teacher Fellowship.

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