Seasonal Changes and Metabolism of Plant Hormones in Root Nodules of *Lens* sp.

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Abstract. The mature nodules of *Lens esculenta* MOENCH. contained higher levels of indolyl acetic acid (IAA), cytokinins (CK), gibberellic acid (GA)-like substances and more active in nitrogenase (N_2-ase) activity than young or old ones. Synthesis of IAA and its metabolism was found to be controlled by tryptophan (tryp) and phenol metabolism, respectively, in nodules of different ages. An abscisic acid (ABA)-like substance being a 'late growth phase' hormone, was highest in old nodules.

IAA and CK were highest in winter when N_2-ase activity was also highest but then GA and ABA were low. The IAA metabolic pattern of both roots and nodules was regulated by phenols. The hormones hardly changed seasonally in the roots and showed higher activities of IAA oxidase, MeOx reductase, peroxidase and polyphenol oxidase than nodules.

The nodular anabolic changes are more pronounced in winter as lentil is a winter crop. The size of nodules at a particular age was the same in different seasons, even though their hormone content varied with the season showing that the nodular hormones were not solely utilized for nodule development and growth.

Among leguminous plants, root nodules of only few pulses like *Pisum sativum* and *Phaseolus vulgaris* were studied for gibberellin content (RADLEY 1961), as well as other herbs as *Lupinus luteus* were studied for IAA (DULLAART 1970a). CK content in the nodules were studied in different leguminous nodules, among which plants like *Vicia faba* (HENSON and WHEELER 1976), *Pisum sativum* (SYONO et al. 1976, NEWCOMB et al. 1977) may be mentioned. But all these studies were unable to give a clear picture of the nodular physiology through different seasons because only a few studies were focussed on the seasonal changes. The aim of the present study was to relate the hormone status with age of nodules, and seasonal variation. Attempts were also directed to check the content and seasonal variation of ABA in root nodules, which was absolutely lacking. So this study enriches the knowledge of leguminous nodules with new and unique information.

MATERIAL AND METHODS

Fresh nodules and roots of lentil (*Lens esculenta* MOENCH.) were used for study. The nodules were divided into three categories on the basis of age

of the nodules. The young nodules were 15—22 days old, slightly elongated and unbranched; mature nodules were 25—32 days old, elongated, branched and available from preflowering to fruit-setting stage of the plant. Old ones were 35—38 days of age without further change in morphology after maturity. The hormonal studies in young, mature and old nodules were performed in winter, seasonal studies were performed with mature nodules only.

IAA was extracted, identified by wheat coleoptile elongation bioassay and estimated spectrophotometrically according to SINHA and BASU (1981). GA was extracted following OBATA-SASAMATO and SUZUKI (1979), identified and estimated as GA_3 equivalents according to FRANKLAND and WAREING (1960) using lettuce hypocotyl elongation assay. The lower limit of detection by this method was 1.5 ng ml^{-1}. CK was extracted following OBATA-SASAMATO and SUZUKI (1979), identified by chlorophyll synthesis in etiolated cucumber cotyledons (FLETCHER and McCULLAH 1971) and estimated as kinetin equivalent by UV absorption at 268 nm (GLENN et al. 1972). ABA was extracted according to RUDICH et al. (1972) and identified by wheat coleoptile negative elongation assay (WRIGHT and HIRON 1972) and estimated by UV absorption at 245 nm as abscisic acid equivalents (GLENN et al. 1972). Total free phenol was extracted and estimated spectrophotometrically using ferulic acid as standard following BRAY and THORPE (1954). Tryp was extracted according to NITSCH (1955) and estimated spectrophotometrically at 360 nm (HASSAN 1975).

IAA oxidase and MeOx reductase were extracted and estimated using tissue extracts according to SINHA and BASU (1981). IAA oxidase, partially purified through 95% $(NH_4)_2SO_4$ saturation and dialysis, was also used to study any change in activity after removing other substances from the crude enzyme extract. Peroxidase and polyphenol oxidase enzymes were extracted and estimated according to KAR and MISHRA (1976). N_2-ase activity was estimated spectrophotometrically at 412 nm in terms of nmol ethylene produced g^{-1} (nodule) h^{-1} following LARUE and KURZ (1973). According to the authors, the method is as efficient as GLC. Protein contents were estimated following LOWRY et al. (1951). Each of the above experiments were repeated five times.

RESULTS

IAA and GA in mature nodules were higher than in young or old ones (Fig. 1A). IAA content came down in old nodules to about 1/3rd and 1/5th of young or mature nodules, respectively. Variation of GA with age was much smaller. CK content was the same in young or mature nodules and came down to about 1/3rd in the old nodules (Fig. 1A). ABA increaseded sharply up to maturity, then its increase slowed down and reached the highest in the old nodules (Fig. 1A).

The changes of tryp (Fig. 1B) levels followed the same pattern as those of IAA and GA. Phenol content (Fig. 1B) was highest in young nodules and gradually came down to the lowest level in the old ones.

IAA oxidase activity increased up to maturity and then declined in the old nodules (Fig. 2A). MeOx reductase activity was the same in young and mature nodules but it fell down to about 1/7th of the above in the old nodules (Fig. 2A). N_2-ase activity showed a sharp rise with age up to maturity of the nodules and then decreased to about 1/5th in the old nodules
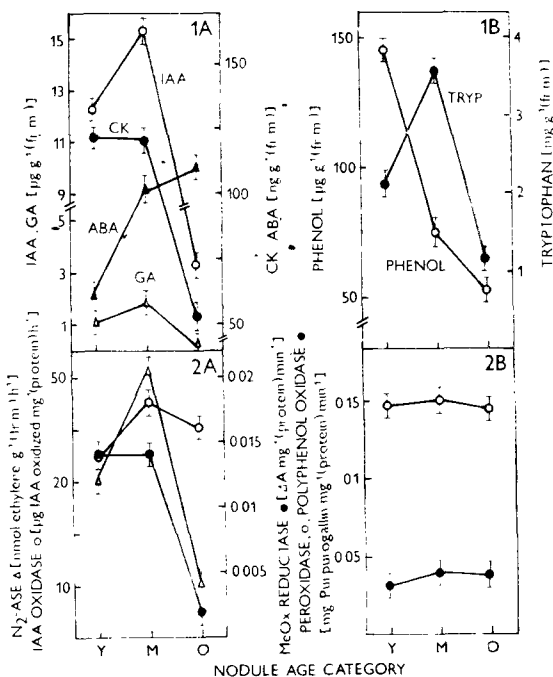
Fig. 1. Variation in content of (Fig. 1A) IAA (○), GA (△), CK (●), ABA(▲); (Fig. 1B) phenol (○) and tryptophan (●) in young (Y), mature (M) and old (O) nodules of *L. esculenta*. Each value is the mean of 5 replicates and bars on points indicate ± SE.
Fig. 2. Changes of activities of (Fig. 2A) N_2-ase, IAA oxidase, MeOx reductase; (Fig. 2B) peroxidase and polyphenol oxidase in nodules of *L. esculenta*. Symbols of ages and bars on points *etc.*, are as in Fig. 1.

(Fig. 2A). Both polyphenol oxidase and peroxidase activities were almost the same from young to mature nodules (Fig. 2B). Peroxidase activity was always higher than polyphenol oxidase activity (Fig. 2B).

Seasonal changes of N_2-ase activity (Fig. 3) in mature nodules were highest in December (winter) and lowest, about 1/5th, in May (summer).

The seasonal changes of IAA and CK were exactly the reverse of those of GA and ABA in the mature nodules (Fig. 4). The former two hormones increased gradually from the lowest level of the pre-rainy season (May—June) to the highest peak in December and January, respectively, and then decreased again. But the levels of GA and ABA were lowest in winter and highest in the rainy season. None of the above hormones tend to change significantly in roots throughout the year (Fig. 4).

Changes of IAA oxidase activity was opposite to IAA content. Both in nodules and roots the enzyme activity was lowest in December (winter) and then increased gradually to a high activity level in other seasons of the year (Fig. 5). MeOx reductase activity in roots showed a seasonal variation, though in nodules the activity changed very little except a slight fall in winter (Fig. 5). Activities of the enzymes were always higher in the roots than in nodules (Fig. 5). Tryp content was much higher in nodules than in roots and changed in negative relations with IAA oxidase activity (Fig. 5).

Activities of peroxidase and polyphenol oxidase in roots were lowest in winter (January) and increased in other seasons (Fig. 6). In nodules peroxidase followed the same pattern as in roots, whereas polyphenol oxidase activity was very low throughout the year (Fig. 6). Activities of both the enzymes were always higher in roots than in nodules. The phenol content had
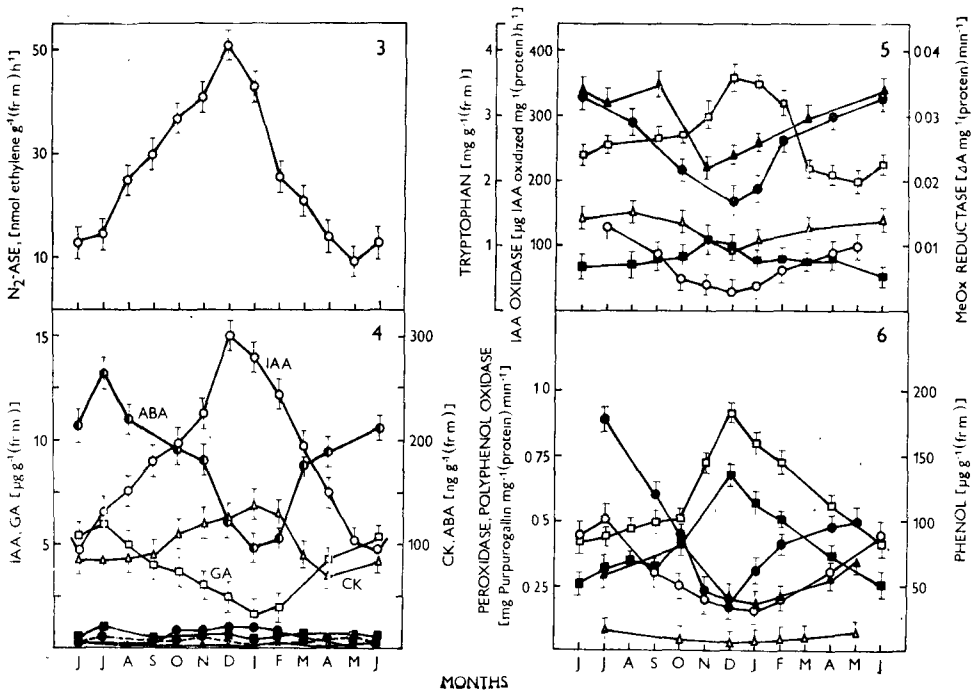
Fig. 3. Seasonal variation of nitrogenase activity in mature nodules of *L. esculenta*. Months are represented by respective first letter. Each value is the mean of 5 replicates and bars on points indicate ± SE.

Fig. 4. Seasonal variation of IAA (circle), CK (triangle), GA (square) and ABA (circle half-open) in mature nodules (open symbol) and roots (closed symbol) of *L. esculenta*. Other conditions are the same as in Fig. 3.

Fig. 5. Seasonal variation of activity of IAA oxidase (circle), MeOx reductase (triangle) and tryptophan (square) in mature nodules (open symbol) and roots (closed symbol). Other conditions are the same as in Fig. 3.

Fig. 6. Seasonal variation of activity of peroxidase (circle), polyphenol oxidase (triangle) and phenol (square) content in nodules (open symbol) and roots (closed symbol). Other conditions are the same as in Fig. 3.

negative relations with the activities of the above two enzymes. It was highest in December and became lowest in the rainy season (Fig. 6). Phenol content of nodules was always higher than in roots throughout the year.

DISCUSSION

Root nodules of *Lens esculenta* were very rich in IAA, GA, CK and also contained some ABA (Fig. 1A); in addition, they contained some of their metabolic enzymes showing most active participation of the nodules in hormone metabolism (Fig. 2A). Highest tryp (Fig. 1B) in mature nodules explained the highest IAA level (Fig. 1A) through its synthesis from tryp. The nodules of this plant were branched at maturity. Nodular CK probably have a positive role in cell division and branching which could be the cause of high levels of CK both in young and mature nodules and a decline in old

ones when that function was over (Fig. 1A). ABA being a late phase hormone, its level in nodules increased gradually with age (Fig. 1A). The N_2-ase activity was also highest in mature nodules; the time coincides with the time when the plant flowers. It continued up to fruit development, after which nitrogen requirement declined and N_2-ase activity also declined (Fig. 2A).

The high IAA level of the mature nodules (Fig. 1A) was metabolized by IAA oxidase and MeOx reductase (BASU and TULI 1972) whose level also remained high (Fig. 2A). The activity of the enzymes lowered in old nodules when the level of IAA also became low, probably due to a decrease in its rate of synthesis from tryp whose level was also low in old nodules (Fig. 1B). The activity of IAA oxidase was probably controlled by phenol of the nodules (Fig. 1B). Partially purified and dialysed IAA oxidase from roots and nodules had increased the activity 7 and 4 times, respectively (data not shown), showing a regulatory effect of some inhibitors *in vivo*. As phenol metabolizing enzymes, peroxidase and polyphenol oxidase showed little variation in the nodules with age, it could be expected that the level of the free phenols was controlled by their synthesis from phenylalanine (VENCE 1978). The level of phenol in the nodules declined sharply from young to old age (Fig. 1B) due to less synthesis.

Seasonal changes of N_2-ase (Fig. 3) together with IAA and CK (Fig. 4) were highest in winter (December and January) but then GA and ABA (Fig. 4) were lowest. On the contrary, N_2-ase, IAA and CK were low in spring (March—May) and the rainy season (June—August). The changes of hormone levels in roots are not significant. A positive relation of N_2-ase with IAA and CK and negative relation with GA and ABA could be observed. WHEELER *et al.* (1979) observed such positive and negative relations of N_2-ase with CK and GA respectively in actinomycete nodules of *Alnus*. But DULLAART (1970b) failed to show any change either in IAA level or such a relation with N_2-ase in nodules of *Alnus*. ABA may also have a similar regulatory effect to GA on nodule growth and development.

The high IAA oxidase and MeOx reductase activity and low IAA and tryp content in roots (Fig. 5) strengthened the fact that IAA is less in them due to its low synthesis from tryp, which is also low, together with a higher rate of degradation. In nodules the picture is just reversed, they contained more tryp and less IAA oxidase and MeOx reductase activity (Fig. 5).

The nodules and roots possessed more phenol in winter (Fig. 6) when IAA level is high (Fig. 4) and *vice versa*. More phenol provided more di- and poly-phenols as inhibitors of IAA oxidase which superseded the activity of monophenols having some promotive effect on the enzyme. Peroxidase and polyphenol oxidase changed inversely with the phenol level both in nodules and roots and the enzymes were less active in nodules, the condition which favoured the higher phenol level in the nodules. This coincided with the observation by VENCE (1978) in alfalfa. The roots of the plant possessed threshold levels of all the hormones to continue the metabolic regulation but never changed significantly.

LIBBENGA and BOGERS (1974) suggested that nodular hormones are utilized for development and growth. It was observed here that the size of nodules of an age throughout the year remained the same for that age, though a high seasonal variation of all the hormones were observed. This clearly indicated that though a part of the hormones functions for the de-

velopment and growth of the nodules, an excess of the hormones might be transported to the other plant parts, as suggested by WHEELER *et al.* (1979).

In culture the *Rhizobium* spp. were able to produce IAA (SINHA and BASU 1981), GA (LIBBENGA and BOGERS 1974) and CK (PHILLIPS and TORREY 1970), and showed N_2-ase activity (PAGAN *et al.* 1975). It is expected that nitrogen supply may not be the only role of *Rhizobium* in symbiosis. As the symbiont of this plant also possessed such characters (data not shown) its multipurpose function could also be expected.

Acknowledgement

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BOOK REVIEW

Dieses Buch erscheint als erster Band einer Reihe unter dem Sammeltitel „Ökologie der Erde". Es werden noch Band 2: Spezielle Ökologie der Tropischen und Subtropischen Zonen und Band 3: Spezielle Ökologie der Gemässigten und Arktischen Zonen folgen.

Die Verfasser haben sich in diesem ersten Band der Reihe die Aufgabe gestellt den Studierenden eine Synthese oder eine Darstellung der grossen Zusammenhänge vom Ganzen in der Ökologie zu bieten. Sie sind der Meinung, dass die heutige biologische Wissenschaft ein Überangebot an von den verschiedensten Spezialisten erarbeiteten Einzelheiten bietet, die jedoch das Erfassen des Ganzen sehr erschwert, besonders im Falle von Studierenden. — Die Verfasser klassifizieren zuerst die einzelnen Forschungsbereiche der Ökologie und die Beziehungen zwischen Geo- und Hydro-Biosphäre und schildern die Geo-Biosphäre in der geologischen Vergangenheit: die Entstehung der Kontinente und deren Besiedlung durch Organismen. Es folgt eine präzise ökologische Gliederung der Geo-Biosphäre und die Charakterisierung der Besonderheiten der terrestrischen Ökosysteme: des kurzen und langen Kreislaufs demonstriert am Beispiel eines Laubwaldes, Ökosysteme mit krautiger Vegetation, Ökosysteme der Wüsten usw. Des weiteren behandeln die Autoren die absoluten Grenzwerte der Grundvoraussetzungen für aktive Lebensvorgänge: der Temperatur und der Hydratur, d. i. eines bestimmten Aktivitätszustandes des Wassers im Protoplasma, und die dementsprechende Einteilung der Pflanzentypen. Nach der eingehenden Erläuterung der in der Pflanzenökologie so wichtigen Begriffe wie Wettbewerbsfaktor und Wurzelkonkurrenz folgen die Grundlagen der Sukzessionslehre und Betrachtungen über die Primärproduktion und den Assimilathaushalt in ökologischer Sicht. Weiter behandelt wird das Gesetz der relativen Standortskonstanz und des Biotopwechsels und die Ausbildung von Ökotypen und Ökoklinen. Das Buch schliesst mit einem Kapitel, in dem ein Vegetationsbeispiel in den Tropen (Venezuela) durchgearbeitet wird. Das Buch ist mit einem Literaturverzeichnis und einem vereinigten Sach- und Pflanzenregister ausgestattet, ist reich illustriert (132 Abbildungen und 24 Tafeln) und technisch sehr gut ausgeführt.

Das Buch stützt sich auf die tiefen Erfahrungen, die Prof. Dr. Walter in seiner mehr als sechzigjährigen ökologischen Forschung gesammelt hat, und die er nun als reife Synthese Studenten, denn es handelt sich um ein Lehrbuch, und allen weiteren Interessenten darbietet. In einer Neuauflage sollten wohl konsequenter die SI-Einheiten benützt werden, also z. B. nicht mehr Lux für den Lichtkompensationspunkt (S. 148 bis 156).

INGRID TICHÁ (*Praha*)