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CHANGES OF EDAPHIC FACTORS DUE TO METABOLISM OF INDOLE ACETIC ACID WITHIN THE ROOT NODULES OF *CICER ARIETINUM* L.

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Abstract

The mature root nodules of *Cicer arietinum* L., a pulse legume, contained higher amount of indole acetic acid (IAA) and total phenol than non-nodulated roots. Higher amount of tryptophan pool both in the root nodules and the roots were found as the precursor molecule of IAA. Presence of IAA oxidase and peroxidase, the IAA metabolising enzymes, in the nodules and roots indicated the metabolism of IAA in the nodules and roots. Metabolism of phenol by enzymes in both root nodules and roots controlled the IAA production in those tissues. Decomposition of the mature root nodules were increased the percentage of average organic carbon and total nitrogen over control as the root nodules were mixed up with the soil after decomposition. The important physiological implication of the IAA production in the legume-*Rhizobium* symbiosis is discussed.

Key words : *Cicer arietinum*, root nodule, indole acetic acid, edaphic factors, symbiosis.

Introduction

Leguminous plants (under the family Fabaceae), which is the third largest (after Orchidaceae and Asteraceae) family of angiosperms (Mabberley, 1997), include approximately 730 genera and about 20000 species worldwide (Dzoyem *et al*., 2014). Rhizobia, which include 76 species within 13 genera (Weir, 2010), are bacteria capable of fixing atmospheric nitrogen and forming root nodules in plants of the family Fabaceae. Only 20% of the leguminous plants have been examined for nodulation (Hopkins, 1999) and only 0.5% of the legumes have been studied in relation to the nodule bacteria (Jordan, 1984). The appreciable amount of different phytohormones present in the root nodules play important roles in genesis and development (Nutman, 1977; Verma *et al*., 1992) and formation (Kefford *et al.*, 1960) of root nodules. A successful symbiosis is the result of a complex series of interactions between the host and the symbiont (Pellock *et al*., 2000). The successful symbiotic establishment with leguminous host plants depends on the production of Nod factor signals and exposure of the correct surface and/or extracellular polysaccharide (EPS), which involved in root hair infection and nodule formation (Phillip-Hollingsworth *et al*., 1989). Leguminous plants possessed large numbers of microbes in its rhizospheric area including *Rhizobium* sp. which help in the development of root nodules. Several factors like soil pH, soil moisture, C and N ratio, organic matter content, numbers of nodule etc. were found to influence the rhizosphere of the legume. *Rhizobium* sp. could increase the percentage of organic carbon into soil in several ways including the decomposition of mature root nodules, besides increasing the soil nitrogen content.

Cicer arietinum L. (Chickpea), commonly known as Bengal gram, is a cholesterol free and good source of protein, carbohydrates, minerals and vitamins, dietary fibres, folate, β-carotene and health-promoting fatty acids (Jukanti *et al*., 2012). This important leguminous pulse has been consumed all over the World especially in African and Asian Countries. It has several beneficial effects on human diseases including digestive diseases and some cancers (Jukanti *et al.*, 2012).

The present work has shed some light on changes of the edaphic factors of soil surrounding root system of chickpea. The changes take place in the content of nitrogen, carbon, phosphorus, soil moisture etc. during the growth and nodulation of the plant in the soil and IAA metabolism in root and nodule.

Materials and Methods

The pulse legume *Cicer arietinum* L. (Chickpea) was selected for study. The certified seeds were purchased from a reputed seed-selling centre at Burdwan, WB. The plants were grown in experimental plots, soil samples were collected from different places in experimental plots before plantation and after the decomposition of nodules. Soil samples were dried in hot air oven for 2-3 h at 75-80°C. Dried soil samples were stored in sterile plastic bags till analyzed.

Estimation of nitrogen, carbon and phosphorus in soil sample

Total nitrogen and carbon was estimated by following methods of Vogel (1961) and Black (1965) respectively. Available phosphorus was estimated by following method of Jackson (1968).

Determination of soil moisture and pH

Soil moisture and soil pH were determined by the methods of Kapur and Govil (2000) and Jackson (1968) respectively.

Extraction and estimation of IAA, tryptophan and total phenol from root nodules and roots

Mature, fresh nodules and young roots are selected for the experiment. IAA was extracted from about 5 g of fresh tissue of root nodule and purified by repeated thin layer chromatography (TLC) following Sinha and Basu (1981) with slightly modification by Ghosh and Basu (2006) and was estimated colorimetrically following Gordon and Weber (1951) using a standard curve prepared from authentic IAA. Tryptophan was extracted according to Nitsch (1955) and estimated colorimetrically following Hassan (1975). The total phenol content was extracted and estimated after Bray and Thorpe (1954).

Extraction and estimation of IAA and phenol metabolizing enzyme

IAA oxidase was extracted and estimated following Sinha and Basu (1981). Oxidation of IAA by peroxidase was estimated following Kokkinakis and Brooks (1979) with modification by Datta and Basu (1998). Peroxidase and polyphenol oxidase were extracted and estimated according to Kar and Mishra (1976). The protein content in the enzyme was estimated following Lowry *et al.*, 1951.

Statistical analyses were carried out following Panse and Sukhatme (1985).

Results and Discussion

The plant *Cicer arietinum* L. produced plenty of root nodules after 30 days of growth. Out of total number of nodules near about 29% of the nodules were immature table 1 and the mature nodules were small, spherical and pinkish in colour.

The mature root nodules contained higher amount of IAA than young roots table 2. A tryptophan pool was found in both mature nodules and young roots. Mature nodules contained higher level of total phenol than of young roots table 2. The IAA might transport from nodules to other parts of the plant. It was also expected that free phenols should be more in nodules of the plants because the nodules possess higher IAA and lower IAA catabolising enzyme activity than roots. Higher levels of total phenol in the nodules than root would result from the lower peroxidase and polyphenol oxidase activity in the nodules (Stafford, 1974) than the roots.

Presence of IAA metabolising enzymes in both root nodules and roots indicated the metabolism of IAA in these tissues. The root always contained higher amount of IAA oxidase and peroxidise, the IAA metabolising enzymes, than the root nodules table 3. The presence higher amount of IAA metabolising enzymes in roots resulted the lesser amount of IAA in that tissues. The amount of IAA was controlled as per the necessity by the tissues accordingly and plays an important role for the persistence of functional root nodules (Badenoch-

Table 1: Total number of Root nodules (mature and immature) found in the plant *Cicer arietinum* L.

Plant[1]	Mature root root nodules	Immature root nodules	Total
Plant-1	36	11	47
Plant-2	39	18	57
Plant-3	17	08	25
Plant-4	43	16	59
Plant-5	42	17	59
Plant-6	32	18	50
Plant-7	25	10	35
Plant-8	28	11	39
Plant-9	30	11	41
Plant-10	28	10	38
Total	320	130	450
Average	32	13	45

[1]Age of all plants- 42 days
Size of plants- 09-12 inches
Time of year data taken- May
Whether manure given or not- not given
Flowering came or not- before flowering

Jones *et al*., 1983).

The activity of the peroxidase and polyphenol oxidase, phenol metabolising enzymes, was lesser table 4 due to the presence of higher levels of total phenols in the nodules than the roots table 2. It was also expected that free phenols should be more in nodules of the plants because the nodules possess higher IAA and lower IAA catabolising enzyme activity than roots.

Higher amount of tryptophan, acts as precursor of IAA, present in the root nodules might be helped in higher IAA production in those tissues than that of roots. The IAA metabolising enzymes, IAA oxidase and peroxidase might actively controlled the IAA production in tissues. The cause of difference of phenol levels in different parts would also be due to the variation in the metabolism and synthesis of phenols in different tissues by phenylalanine-ammonia lyase and tyrosine-ammonia lyase (Stafford, 1974, Vance, 1978).

The percentage of average organic carbon and total nitrogen were increased as the root nodules of this plant were mixed up with the soil after decomposition table 5. The percentage of available phosphorus was also increased after decomposition table 5. The percentage of soil moisture was decreased gradually with the gradual decrease of soil depth from surface level table 6.

The organisms present in the nodule have the capacity to increase the organic matter besides nitrogen fixation. The percentage of available phosphorus remains same after decomposition of the root nodules into the soil. It was might be due to the use of the soil phosphorus by the soil organisms. It has been reported that C:N ratio was affected by soil properties such as moisture content, texture, pH (Anderson and Domsch, 1980, Campbell *et al*., 1991) and the C:N ratio is often used to describe the structure and the state of the microbial community (Kaushik *et al*., 2006). The gradual decrease of soil moisture with depth was due to the root system of the plant as the rootlets and roots were spread in the soil just below the surface level and hold the soil water. Mature plants contained numerous root nodules and caused by the native rhizobia already present into the soil.

Percentage of organic carbon, total nitrogen, available phosphorus before and after plantation of *Cicer arietinum.* Data presented here were the mean of three experimental sets.

Percentage of soil moisture at different depths of soil in the field during cultivation of *Cicer arietinum*. Data presented here were the mean of three sets.

Table 2: Maximum level of Indole acetic acid (IAA), tryptophan and total phenol found in nodules and roots of *Cicer arietinum* L. (IAA, tryptophan and total phenol were estimated colorimetrically).

Plant Parts	IAA	Tryptophan	Phenols
	(µg/g fresh tissue)		
Nodule	18.25	1637	1440
Root	2.57	335	950
Critical Difference at P = 0.05	0.07	5.07	14.36

Table 3: Catabolism of IAA by IAA oxidase and peroxidase in nodules and roots of *Cicer arietinum* L.

Plant parts	IAA oxidase (mg IAA oxidised/ mgprotein/h)	Peroxidase (mg IAA oxidised/ mgprotein/h)
Nodule	12.24	8.90
Root	47.45	13.15
Critical difference at P=0.05	0.38	0.12

Table 4: Levels of total peroxidase and polyphenol oxidase in nodules and roots of *Cicer arietinum* L.

Plant parts	Peroxidase (mg purpurogallin formed/mg protein/min)	Polyphenol oxidase (mg purpurogallin formed/mg protein/min)
Nodule	142.17	129.50
Root	177.34	148.47
Critical difference at P=0.05	1.08	0.77

Table 5: Changes of carbon, nitrogen and phosphorus before and after plantation of *Cicer arietinum* L.

Place of collection of soil sample	% of organic carbon(C)	% of total nitrogen(N)	C : N	% of available phosphorus
Soil before plantation	0.43	0.12	3.58	0.0091
Soil having nodulated plant(after decomposition)	1.29	0.67	1.93	0.0112

Table 6: Changes of soil moisture content.

Soil depth (cm)	Fresh weight of soil sample(g)	Dry weight of soil sample(g)	Weight of water(g)	Soil moisture (%)
0-5	100	78.0	22.0	28.21
5-10	100	80.1	19.9	24.84
10-15	100	81.7	18.3	22.40

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