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Nitrate Nitrogen Content and IAA Production Potential of the Rhizosphere Soil of Three Legumes in Relation to Nodulation

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ABSTRACT

An evaluation of the relationship between nitrate nitrogen in the rhizosphere soil and nodule number indicated a negative correlation between the two factors in greengram and sunnhemp, and a positive correlation in blackgram. A positive correlation between IAA production potential and nodulation was observed only in blackgram.

INTRODUCTION

It is evident that the environment greatly influences not only the growth and longevity of the nodule bacteria in the soil, but also the production and behaviour of nodules and development of the host plant (Hallsworth, 1958). In recent years, several attempts have been made to examine the possibilities of altering the rhizosphere environment by foliar application of chemicals. Although Thurber *et al.* (1958) and Galston (1959) presented evidences to show that nodulation could be changed due to foliar spray of growth regulators, not much information is available on factors influencing nodulation. While indole acetic acid (IAA) production in rhizosphere soil is an important phenomenon believed to cause curling of root hair and infection by rhizobium nodulation (Nutman, 1965), the inhibitory action of nitrate on nodule formation has been observed by many workers (Samuels and Landran, 1952; Pate and Dart, 1961; Cartwright and Snow, 1962; Darbyshire, 1966). The present work reports on the interrelationship between nitrate nitrogen content and IAA production potential of the rhizosphere soil in relation to nodulation following foliar spray of certain growth regulators.

MATERIALS AND METHODS

Greengram (*Phaseolus aureus*), blackgram (*P. muugo*) and sunnhemp (*Crotalaria iuncea*) plants grown in earthern pots were sprayed with gibberellic acid (GA) (50 and 100 ppm), 2.4-dichlorophenoxy acetic acid (2.4-D) (5 and 10 ppm) and indole acetic acid (IAA) (250 and 500 ppm) at weekly interval beginning from 15th day after sowing till 50 th day in the case of the former two and 70 th day in the latter. Sufficient precautions were taken to prevent drenching the soil

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with the growth regulators in run-off spray by covering the soil around the plants with alkathene sheets and absorbent cotton wool (Kandasamy and Rangaswami, 1967). The rhizosphere soil samples were collected for the estimation of nitrate nitrogen and IAA level on 24th, 38th and 52nd day for greengram and blackgram to represent the vegetative, flowering and pod bearing phases of the crop. But in the case of sunnhemp the samples were taken on 38th, 52nd and 72nd day to represent the above stages.

The nitrate nitrogen in the rhizosphere soil was estimated following the procedure described by Jackson (1967). Extraction of IAA in the rhizosphere soil was carried out as per the procedure described by Narayanaswami and Veerraju (1969). The IAA in the methanol extract was determined following the method of Gorden and Paleg (1957) using Salper's reagent (50ml of 35 per cent. perchloric acid and 1 ml of 0.5M ferric chloride). The colour development was allowed to proceed for 1 hr in dark and read in a Spectronic-20 colorimeter at 525 nm against reagent blank. The quantity of IAA was calculated from a standard graph. Detection of IAA in the extract was done chromatographically by spotting 50 μl of the extract on Whatman No.1 filter paper (28x35 cm) developing the paper in n-butanol : acetic acid : water :: 4 : 1 : 1 (V/V) solvent system ascendingly and sprayed with Salkowski reagent (50 ml of 5 percent perchloric acid and 1 ml of 0.5 M ferric chloride). The Rf value and colour of the spots were compared with the co-chromatographed authentic sample of IAA. The nodules from the plants sprayed with the growth regulators were counted at three different growth phases of the crop as indicated earlier.

RESULTS AND DISCUSSION

The results on the effect of foliar

TABLE I. Changes in the nitrate nitrogen content of rhizosphere soils of greengram, blackgram and sunnhemp due to foliar spray of growth regulators (expressed as ppm)

Treatment	Green gram			Black gram			Sunnhemp		
	A	B	C	A	B	C	A	B	C
GA — 50 ppm	3.27	8.26	13.32	11.15	20.29	24.31	9.33	17.50	20.54
GA — 100 ,,	7.66	11.45	20.11	15.26	16.40	19.85	13.55	15.46	21.39
2, 4—D — 5 ,,	2.71	4.17	6,42	5.91	12.61	17.78	10 64	21.73	20.65
2, 4—D — 10 ,,	6.26	7.70	10.05	9.10	13.25	15.45	16.20	23.82	21.50
AA — 250 ,,	11.67	10.62	12.89	17 20	19.92	20.31	15.07	19.55	23.23
AA — 500 ,,	14.25	12 41	13.66	14.35	17.56	23.56	10 85	17.00	17.90
Control (Distilled water spray)	5.75	6.20	9.42	6.72	7.82	12.43	5.92	9.25	14.10
Soil	8.66	10.05	9.60						

A—Vegetative stage; B—Flowering stage; C—Pod bearing stage
Mean of three replications

spray of growth regulators on nitrate nitrogen and IAA level in the rhizosphere soil of crop plants and nodule number are given in Tables I, II and III, respectively. An evaluation of the relationship between the nitrate nitrogen and nodule number indicated a negative correlation between the two factors in greengram and sunnhemp (Table IV). As for example, the spray of GA - 100 ppm and IAA (250 and 500 ppm) at vegetative stages of greengram and 2,4-D - 5 ppm and IAA (250 and 500 ppm) at vegetative and flowering stages of sunnhemp increased the nitrate nitrogen level in the rhizosphere soil but decreased nodulation. The depressing effect of nitrate nitrogen on nodulation and nitrogen fixation has been reported (Hallsworth, 1958). Subba Rao and Vasantha (1965), Fahraeus and Ljunggren (1968), Munns (1968 a & b) and Ljunggren (1969) and many other workers have observed the inhibitory effect of nitrate on infection thread formation and subsequent nodule formation. The mechanism of inhibition of nodule formation by nitrate nitrogen may act in more than one way. It has been suggested that the inhibition of nodulation by nitrate is mediated by the destruction of IAA necessary for the purpose. It has been shown that nitrite, formed by reduction of nitrate by rhizobia, catalysed the oxidation of IAA (Tonhazy and Pelczar, 1954; Tanner and Anderson, 1964) and also that nitrate added to the culture solution of rhizobia decreased the amount of IAA formed from tryptophan (Tanner and Anderson, 1963).

However, in the case of blackgram rhizosphere a positive correlation between the nitrate nitrogen content and nodulation was observed. Although this observation was in contradiction to the above findings

TABLE II. Indole acetic acid production potential in the rhizosphere soils of greengram, blackgram and sunnhemp as influenced by foliar spray of growth regulators (expressed in μg / g of rhizosphere soil)

Treatment	Greengram			Blackgram			Sunnhemp		
	A	B	C	A	B	C	A	B	C
GA — 50 ppm	45.83	70.85	68.20	52.06	82.76	87.48	18.84	18.34	37.63
GA — 100 ,,	42.29	68.61	61.41	47.04	76.83	74.12	27.69	49.29	55.07
2,4-D — 5 ,,	72.72	62.18	72.83	39.52	53.28	73.84	35.09	11.42	19.14
2,4-D — 10 ,,	45.88	35.24	22.34	39.11	96.57	43.73	23.64	13.84	20.28
IAA — 250 ,,	38.85	78.52	51.21	54.89	67.10	52.05	24.58	14.17	16.00
IAA — 500 ,,	43.49	68.95	43.75	77.64	68.86	61.06	27.37	10.35	4.55
Control (Distilled water spray)	32.73	56.14	55.53	64.15	67.57	56.24	24.08	7.16	6.27
Soil	28.61	29.12	28.86						

A— Vegetative stage; B — Flowering stage; C.—Pod bearing stage
Mean of three replications

TABLE III. Influence of foliar spray of growth regulators on the nodule number of three legume plant species (average of nodules from 10 plants)

Treatment	Greengram			Blackgram			Sunnhemp		
	A	B	C	A	B	C	A	B	C
GA — 50 ppm	23.6	13.6	14.6	23.0	32.6	35.6	50.0	52.0	62.6
GA — 100 ,,	15.0	17.3	22.3	11.3	40.6	29.3	57.1	83.6	58.3
2, 4—D — 5 ,,	42.3	62.0	27.3	20.6	32.0	38.6	32.3	39.3	55.6
2, 4—D — 10 ,,	14.3	21.3	33.6	43.0	79.0	57.6	51.3	39.0	60.3
IAA — 250 ,,	11.0	46.0	31.3	25.6	32.6	40.3	29.0	44.0	52.0
IAA — 500 ,,	10.3	51.6	37.6	65.0	38.3	44.6	25.3	30.0	33.6
Control (Distilled water spray)	29.0	32.0	17.6	40.0	54.0	46.0	48.3	68.3	35.6

	C. D. (P=0.05)		C. D. (P=0.05)
Stages	2.93	Control *vs* growth regulators	3.88
Crops	2.93	Between growth regulators	3.16
Treatments	4.48	Between concentrations within growth regulators	4.48

A—Vegetative stage; B—Flowering stage; C—Pod bearing stage

TABLE IV. Correlation between nitrate nitrogen content and IAA production potential and nodule number

Independent variable	Dependent variable	Coefficient of Correlation 'r'	Coefficient of regression 'b'	Prediction equation
Greengram				
Nitrate nitrogen	Nodule number	—0.637**	—5.713	Y=—5.713X + 65.92
IAA	,,	0.281 NS	—	—
Blackgram				
Nitrate nitrogen	,,	0.332**	0.881	Y= 0.881X + 25.48
IAA	,,	0.439*	0.430	Y= 0.430X + 12.36
Sunnhemp				
Nitrate nitrogen	,,	—0.355**	—1.075	Y=—1.075X + 65.74
IAA	,,	0.428NS	—	—

** Significant at 1%; * Significant at 5%; NS : Not significant

with greengram and sunnhemp, this evidently indicates the effect of plant species on the mechanism of action of nitrate on nodulation factors (Mac Connell and Bond, 1957; Gibson and Nutman, 1960). Partial alleviations of the inhibition by nitrate of root hair curling and infection thread formation

and development by the addition of IAA has been reported by Valera and Alexander (1965) and Munns (1968 b). In the present study a positive correlation between IAA production potential and nodulation was observed in blackgram. However, no such relationship existed in greengram and sunnhemp.

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