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Effect of brassinosteroid (TNZ-303), chloro indole acetic acid (CL-IAA) and GABA on vegetative growth of lentil

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Abstract

An experiment was carried out in the field and laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh to find out the effect of CI-IAA, TNZ-303 and GABA on vegetative growth in lentil (*Lens esculenta* Moench.) var. BM-3 and BM-4. Plant growth regulators were applied on seeds by soaking in aqueous solution of different concentration for 12 hours prior to sowing. Vegetative growth of variety BM-3 was better than that of BM-4. Results revealed that all the plant growth regulators selectively enhanced vegetative growth of both the varieties. Among the PGRs, the GABA performed the best on vegetative growth over other two plant growth regulators as well as over the control. The plant height, number of branches, number of leaves and number of nodules per plant significantly enhanced by GABA and the best result was at 0.664 ml L⁻¹. Among the other growth regulators TNZ-303 at 0.166 ml L⁻¹ and CI-IAA at 0.332 ml L⁻¹ was also good for better morphological growth of lentil. All plant growth regulators regulated morphological growth of both the varieties.

Keywords: CI-IAA, TNZ-303, GABA, Vegetative growth and Lentil

Introduction

Lentil (Lens esculenta Moench.) a legume, is one of the most important pulse crops in Bangladesh. It is a legume crop covers about 36 % of the total area under pulses and shares approximately 35 % of the total production of pulses in Bangladesh (BBS, 2001). It has occupied a unique position in the world of agriculture by virtue of its high protein content and capacity for fixing atmospheric nitrogen into soil. It is considered as the poor men's meat as it is the cheapest source of protein for the under privileged people. Lentil seeds contain about 25.1 % protein, 59 % carbohydrate, 0.5 % fat, 2.1 % minerals and good amount of vitamins (Gowda and Kaul, 1982). Beside these, the stover of lentil popularly known, as 'bhushi' in Bangladesh is a high protein concentrate feed for cattle, horse, pig and sheep. As lentil fixes atmospheric free nitrogen through root nodule it may reduce the pressure of additional nitrogenous fertilizer application to the crops and thus helps to increase the organic matter in the soil. The large import of pulse from abroad indicates the importance of increase production. Inspite of its importance and well adaptability in the agro-climatic condition of Bangladesh, the acreage production is decreasing gradually because of serious competition from other profitable cereals, especially irrigated boro rice in medium high land (Shaikh et al., 1981; Ahmed, 1984; Anonymous, 1987). Furthermore, increment in pulse production by expanding cropping area of pulse is almost impossible, as it affects other major crops. So, proper management and techniques are the alternative means to get the maximum pulse yield. Yield of lentil in Bangladesh (854 kg ha 1) is much lower than that of Egypt (1984 kg ha), USA (1370 kg ha⁻¹) and France (1279 kg ha⁻¹; FAO, 2000). Low vegetative growth is one of the main causes for low yield. Enhancement in vegetative growth may play an important factor to improve the quantitative and qualitative values of lentil. Various practices may help to achieve the target. Application of plant growth regulators (PGRs) seems to be one of the important means in enhancing growth as well as yield of lentil. Application of PGR is convenient, less expensive and required no extra labour. CI-IAA, GABA are newly formulated

PGRs produced in Japan (Kamuro, 1996). CI-IAA is also a growth promoting substance. GABA is a mixture of 1 % Gibberellic Acid (GA₃) and 0.05 % STC, has significant and stable effect on plant growth. TNZ-303 is an important Brassinosteroid, which contains 30 mg each of TS 303 and n-propyldihydrojasmonate (PDJ) per litre. PDJ itself promotes germination at a very low concentration and shows a synergistic effect with TS-303 (Kamuro *et al.*, 1996). GA₃ influences growth by elongation of internodes and stem in plants. Above mentioned PGRs have been using in different countries profitably. Prior to introducing them in Bangladesh, it is necessary to evaluate their effects on growth and yield of different crops under existing environmental conditions. Therefore, the present research work was designed to study the effects of CI-IAA, TNZ-303 and GABA on yield and yield contributing characters of lentil.

Materials and Methods

Two lentil varieties namely BARI masur 3 (BM-3) and BARI masur 4 (BM-4) were collected from Bangladesh Agricultural Research Institute, Joydebpur, and used in the present experiment. The experiment was conducted at the field of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh. Geographically the experimental field is located at 24°75' N latitude and 90°50' E longitude at the elevation of 18 m above the sea level (Khan, 1997). The crop was cultivated following the procedure of BARC (1997). The basal dose of fertilizers per hectare was incorporated @ 45 kg urea, 85 kg TSP, 35 kg MP and 6 tons cowdung applied at the time of final land preparation. Three growth regulators, namely Cl-IAA, TNZ-303 and GABA, used at 0.0 mlL-1 (control), 0.166 mlL-1, 0.332 mlL-1 and 0.664 mlL⁻¹ were applied for seed treatments in separate buckets. There were 24 treatments under three factorial experiments, was laid out in Randomized Complete Block Design (RCBD), where each treatment was replicated five times. The unit plot was 4.0 m x 2.5 m with a spacing 0.5 m between plots. The seeds were soaked in test solutions of different concentration of PGRs for 12 hours and sown by broadcast method. Two weeding and thinning were done at 15 DAS and 45 DAS respectively. Data were recorded on number of flowers plant⁻¹, pods plant⁻¹, and seeds plant⁻¹, seed yield plant⁻¹, 1000 seed weight and seed yield. Harvest index (HI) was calculated by the following formula (Donald and Humblin, 1976).

$$HI = \frac{\text{Economic yield (seed yield)}}{\text{Biological yield (TDM)}} X 100$$

Analysis of variance was calculated by using the computer software programme MSTAT following three factorial Randomized Complete Block Design and the treatment means were compared by Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results and Discussion

Plant height

The average plant height varied significantly between two lentil varieties during early growing period (15 DAS) to end of active flowering stage (60 DAS). The variety BM-3 was superior in plant height over BM-4 (Table 1). The plant growth regulators stimulated the plant height during whole growth periods (Table 2). Among the PGRs, GABA enhanced plant height significantly over other treatments. At early growing stage (15 DAS), the plant height was 8.2 cm with GABA, followed by 6.5 cm and 6.4 cm with Cl-IAA and TNZ-303 respectively. Similar trend in the plant height was continued in later growth stages. At 90 DAS, the highest plant height was 37.9 cm with GABA and the lowest 34.9cm with TNZ-303. The interaction effect between varieties and plant growth regulators on the plant height is presented in Table 3.

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Both the varieties were responded similarly with PGRs. The plant height of variety BM-3 was the highest (38.5 cm) with GABA and the lowest (35.3 cm) for TNZ-303. Similar trend in plant height was revealed in BM-4 during whole growth periods. The plant height enhanced differently with different concentrations of PGRs. All PGRs enhanced plant height significantly over the control (Table 4). At 15 DAS, among the different concentrations of PGRs, GABA at 0.664 mIL⁻¹ was the best, which significantly enhanced growth of plant height over other treatments. The 2nd highest plant height was for TNZ-303 at 0.166 mI L⁻¹, similar to that for GABA at 0.332 mI L⁻¹. The lowest plant height was for control. Sekh (2002) and Mian (2003) reported that GABA and TN2 303 enhanced plant height in rice and wheat respectively. Similar trend in plant height was continued up to 60 DAS, and thereafter, effects of PGRs on plant height were almost similar and significantly higher over respective controls.

Number of branches plant⁻¹

Number of branches plant⁻¹ increased gradually with the advancement of the growth of the plants at all growth stages (Table 1). At 90 DAS, variety BM-3 produced the maximum number of branch plant⁻¹ (31.13) over the BM-4 (30.22). The GABA, TNZ-303 and CI-IAA significantly increased the number of branches plant⁻¹ at 15 to 90 DAS. Table 2 shows that GABA produced the highest number of branches plant⁻¹ (31.82) and TNZ-303 produced the lowest branches plant⁻¹ (29.57) at 90 DAS. Both varieties were interacted similarly with plant growth regulators (Table 3). BM-3 produced the highest (32.66) and the lowest (29.8) number of branches plant⁻¹ with GABA and TNZ-303 respectively. Similar result was found in variety BM 4. Number of branches plant⁻¹ increased differently with different concentrations of plant growth regulators. Among different concentrations of PGRs 0.664 ml L⁻¹ of GABA produced the highest number of branches, similar to that for TNZ-303 at 0.166 ml L⁻¹ and followed by GABA at 0.332 and 0.166 ml L⁻¹ (Table 4). Among the different concentrations GABA was effective at higher concentration which was contrary for TNZ-303 in the present experiment.

Number of leaves plant⁻¹

Significant variation in number of leaves plant was revealed between varieties during growth periods (Table 5). The number of leaves plant was higher in variety BM 3 (222.7) than BM 4 and similar trend was continued up to harvest at 90 days. All the plant growth regulators had stimulatory effects on number of leaves compared to that in control (Table 6). The effect of GABA was the best over other PGRs in the present experiment. From early (15 DAS) to final (90 DAS) growing stages, the highest number of leaves plant was produced with GABA (223.16), followed by TNZ 303 and the lowest number was for CI-IAA (218.92). The significant interaction between varieties and PGRs indicated that the effects of PGRs were non-specific to varieties (Table 7). Interaction effect between plant growth regulators and their different concentrations on the number of leaves plant was significant at all the sampling periods (Table 8). GABA at 0.664 ml L⁻¹ and TNZ 303 at 0.166 ml L⁻¹ were enhanced number of leaves plant significantly over other treatments. After 60 DAS, when source sink relation is more important for reproductive growth the effect of GABA was superior for leaf production over other PGRs. The most effective concentration of GABA was 0.664 ml L⁻¹ for leaf growth. The TNZ-303 stimulated number of leaves plant at the lowest concentration (0.166 ml L-1) compared to the control and higher concentration of same. These results indicated that the concentrations of PGRs were an important factor for leaf production. GABA induced higher number of leaves in soybean (Abdullah, 2002) and in rice (Sekh, 2002). Present finding agrees well with the above reports.

Table 1. Performance of varieties for vegetative growth in lentil

Name of	Aver	age plant	height (c	m) at days	after so	wing	Average number of branches per plant at days after sowing							
varieties	15	15 30		60	75	90	15	30	45	60	75	90		
BM 3	7.4 a	14.8 a	22.0 a	28.4 a	35.2	36.8	1.98 a	4.29	9.97 a	21.2 a	27.04 a	31.1		
BM 4	6.6 b	13.8 b	20.9 b	27.2 b	34.5	35.8	1.74 b	4.14	9.03 b	20.1 b	25.45 b	30.2		

Table 2. Effects of plant growth regulators on vegetative growth of lentil

Name of	Ave	rage plant	height (c	m) at day	ys after so	owing	Average number of branches per plant at days after sowing							
PGRs	15	30	45	60	75	90	15	30	45	60	75	90		
CI-IAA	6.5 b	13.7 b	20.7 b	27.3 b	34.3 b	35.9 b	1.5 b	3.9 b	9.2 b	20.3 b	25.9 b	30.6 ab		
TNZ-303	6.4 b	13.3 b	20.4 b	26.1 b	34.2 b	34.9 b	1.6 b	3.9 b	9.1 b	19.8 b	25.2 b	29.6 b		
GABA	8.2 a	15.9 a	23.3 a	30.1 a	36.2 a	37.9 a	2.3 a	4.8 a	10.1 a	21.8 a	27.5 a	31.9 a		

Values with different letters within a column differ significantly at 5% level of significance by DMRT

Table 3. Interaction effects between varieties and plant growth regulators on vegetative growth of lentil

	Av	erage plar	nt height (c	m) at days	after sow	/ing	Average number of branches per plant at days after sowing							
Interaction	15	30	45	60	75	90	15	30	45	60	75	90		
BM 3 x CL-IAA	6.8a	14.0a	21.1 c	27.8 bc	34.4a	36.4a	1.53a	3.9a	9.5a	20.6a	26.6 b	30.9a		
BM 3 x TNZ-303	6.7a	13.6a	20.8 cd	26.5 cd	34.6a	35.3a	1.70a	4.1a	9.8a	20.2a	26.3 b	29.8a		
BM 3 x GABA	8.8a	16.8a	24.2 a	31.0 a	36.7a	38.5a	2.44a	4.9a	10.5a	22.7a	28.3 a	32.6a		
BM 4 x CI-IAA	6.1a	13.4a	20.3 cd	26.9 cd	34.2a	35.4a	1.44a	3.8a	8.8a	19.9a	25.3 bc	30.3a		
BM 4 x TNZ-303	5.9a	12.9a	19.9 d	25.7 d	33.7a	34.5a	1.58a	3.9a	8.4a	19.4a	24.2 c	29.3a		
BM 4 x GABA	7.6a	15.1a	22.4 b	29.1 b	35.7a	37.5a	2.21a	4.7a	9.9a	20.8a	26.8 b	31.1a		

Table 4. Interaction effects between plant growth regulators and their different concentrations on vegetative growth of lentil

Interaction of	Avei	age plan	t height (cm) at da	ys after so	owing	Average	number o	f branches	per plant at	days afte	r sowing
PGRs x concentration	15	30	45	60	75	90	15	30	45	60	75	90
CI-IAA x 0 ml/l	5.8 f	12.3 d	19.3 e	24.8 c	33.0 cd	34.2 bc	1.30 e	3.73 ef	8.77 cde	19.04 cd	24.50b	28.71 bc
CI-IAA x 0.166 ml/l	6.8 e	14.9 c	22.1 d	29.3 b	35.6 ad	37.9 ab	1.66 cd	4.12 de	9.77 bcd	21.88 ab	27.2a	32.45 a
CI-IAA x 0.332 ml/l	7.6 de	15.8 bc	22.9 cd	30.7 ab	36.6 ab	38.7 a	1.83 c	4.29 cd	9.95 abc	22.05 ab	28.4a	32.98 a
CI-IAA x 0.664 ml/l	5.6 f	11.9 d	18.5 e	24.5 c	31.9 d	32.9 c	1.14 e	3.40 f	8.29 e	18.07 d	23.7b	28.25 bc
	1											
TNZ-303 x 0 ml/l	5.8 f	12.3 d	19.3 e	24.8 c	33.0 cd	34.2 bc	1.30 de	3.73 ef	8.77 cde	19.04 cd	24.5b	28.71 bc
TNZ-303 x 0.166 ml/l	9.1 ab	17.2 ab	24.5 ab	31.5 ab	38.3 a	39.3 a	2.88 a	5.34 a	10.79 ab	30.09 ab	29.1 a	33.55 a
TNZ-303 x 0.332 ml/l	5.5 f	11.9 d	19.0 e	24.4 c	32.7 d	33.7 с	1.23 e	3.54 f	8.57 de	18.75 d	24.1b	28.27 bc
TNZ-303 x 0.664 ml/l	5.1 f	11.6 d	18.9 e	23.8 с	32.5 d	32.5 c	1.17 e	3.34 f	8.29 e	18.32 d	23.3 b	27.74 c
									•			
GABA x 0 ml/l	5.8 f	12.3 d	19.3 e	24.8 c	33.0 cd	34.2 bc	1.30 de	3.73 ef	8.77 cde	19.04 cd	24.5 b	28.71 bc
GABA x 0.166 ml/l	8.0 cd	15.9 bc	23.6 bc	30.8 ab	36.4 abc	38.2 a	2.33 b	4.79 bc	10.28 ab	21.05 bc	27.8 a	33.15 a
GABA x 0.332 ml/l	8.9 bc	17.2 ab	24.5 ab	31.8 ab	37.3 a	39.3 a	2.68 ab	5.14 ab	10.64 ab	23.06 ab	28.4a	31.62 ab
GABA x 0.664 ml/l	10.0 a	18.3 a	25.7 a	32.9 a	38.1 a	40.3 a	2.99 a	5.45 a	11.05 a	24.04 a	29.4a	34.06 a

Table 5. Performance of varieties for vegetative growth of lentil

Name of	Avera	ge numbei	of leaves	per plant at	days after	Average number of nodules per plant (days after sowing)							
varieties	15	30	45	60	75	90	15	30	45	60	75	90	
вм з	6.76a	22.47 a	61.86 a	152.45 a	212.03 a	222.7 a	4.23 a	7.45 a	16.59 a	20.55 a	16.7 a	12.3 a	
BM 4	5.49b	21.53 b	60.18 b	150.39 a	208.13 b	219.1 b	3.80 b	6.96 b	15.73 b	19.99 b	14.7 b	11.9 b	

Table 6. Effect of plant growth regulators on vegetative growth of lentil

Name of PGRs	Averag	ge number	of leaves	per plant at	days afte	Average number of nodules per plant at days after sowing							
	15	30	45	60	75	90	15	30	45	60	75	90	
CI-IAA	5.58 b	20.50 с	59.16 c	149.79 b	207.9 b	218.9 b	3.4 b	6.5 b	15.2 b	19.61 b	14.7 c	11.4 b	
TNZ-303	5.84 b	21.98 b	60.79 b	150.61 b	209.7 b	220.7 ab	3.9 ab	7.2 ab	15.8 b	20.3 ab	15.4 b	11.9 b	
GABA	6.95 a	23.50 a	63.11 a	153.86 a	212.6 a	223.2 a	4.6 a	7.9 a	17.4 a	20.9 a	17.1 a	12.8 a	

Table 7. Interaction between varieties and plant growth regulators on vegetative growth of lentil

Interaction of	Averag	e number	of leaves p	er plant a	t days afte	r sowing	Average number of nodules per plant (days after sowing)							
Var. x PGRs	15	30	45	60	75	90	15	30	45	60	75	90		
BM 3 x CI-IAA	6.07a	20.82a	59.9 c	150.6a	209.6a	220.8a	3.61a	6.81a	15.69a	19.9a	15.5 c	11.5 c		
BM 3 x TNZ-303	6.37a	22.51a	61.4 b	152.1a	211.4a	222.4a	4.20a	7.41a	16.22a	20.5a	16.3 b	12.2 abc		
BM 3 x GABA	7.83a	24.07a	64.2 a	154.7a	215.1a	225.1a	4.88a	8.12a	17.87a	21.2a	18.4 a	13.1 a		
BM 4 x CI-IAA	5.09a	20.19a	58.4 d	149.0a	206.3a	217.1a	3.26a	6.29a	14.80a	19.3a	13.9 e	11.3 c		
BM 4 x TNZ-303	5.31a	21.45a	60.1 c	149.2a	208.1a	219.1a	3.71a	6.92a	15.44a	20.0a	14.6 d	11.7 bc		
BM 4 x GABA	6.08a	22.94a	62.0 b	153.0a	210.0a	221.2a	4.44a	7.66a	16.95a	20.6a	15.7 c	12.5 ab		

Table 8. Interaction effects between plant growth regulators and their different concentrations on vegetative growth of lentil

Interaction of	A	verage num	ber of leaves	per plant at	days after so	owing	Avera	ge numbe	number of nodules per plant (days after						
PGRsxconcentration	15	30	45	60	75	90	15	30	45	60	75	90			
CI-IAA x 0 ml/l	5.55 cd	21.69 de	59.88 def	150.40 bc	208.80 bc	219.81 ab	3.73 abc	6.94 ab	14.96 ef	20.03 bc	15.84 d	11.71 cd			
CI-IAA x 0.166 ml/l	5.75 cd	22.15 de	61.14 cde	151.54 ab	209.98 bc	221.56 ab	3.78 abc	7.04 ab	15.71 de	20.10 abc	16.20 cd	11.80 cd			
CI-IAA x 0.332 ml/l	6.44 bc	22.38 cde	61.64 cd	152.07 ab	210.55 bc	222.16 ab	3.84 abc	7.10 ab	16.57 cd	20.19 abc	16.84 c	11.95 bcc			
CI-IAA x 0.664 ml/l	4.59 d	15.80 f	54.01 g	145.17 c	202.52 d	212.16 c	2.39 c	5.12 c	13.75 f	18.11 d	9.92 f	10.06 e			
						·									
TNZ-303 x 0 ml/l	5.55 cd	21.69 de	59.88 def	150.40 bc	208.80 bc	219.81 ab	3.73 abc	6.94 ab	14.96 ef	20.03 bc	15.84 d	11.71 cd			
TNZ-303 x 0.166 ml/l	8.08 a	24.22 ab	64.91 ab	152.42 ab	213.83abc	224.83 ab	5.27 a	8.48 a	19.09 a	21.57 ab	18.88 b	13.25 ab			
TNZ-303 x 0.332 ml/l	5.07 cd	21.21 e	59.40 ef	150.10 bc	209.32 bc	219.33 ab	3.64 abc	6.85 ab	14.87 ef	19.94 bc	13.75 e	11.62 d			
TNZ-303x0.664 ml/l	4.67 d	20.81 e	58.99 f	149.52 bc	207.92 c	218.92 b	3.19 bc	6.40 bc	14.42 ef	19.49 cd	13.30 e	11.17 de			
GABA x 0 ml/l	5.55 cd	21.69d e	59.88 def	150.40 bc	208.80 bc	219.81 ab	3.73 abc	6.94 ab	14.96 ef	20.03 bc	15.84 d	11.71 cd			
GABA x 0.166 ml/l	6.45 bc	23.14b cd	62.33 c	152.85 ab	210.75 bc	221.75 ab	4.64 ab	7.87 ab	17.08 bc	20.76 abc	16.06 d	12.52 a-d			
GABA x 0.332 ml/l	7.30 ab	23.94 abc	64.15 b	155.08 ab	214.23 ab	224.57 ab	4.95 a	8.22 a	17.87 b	21.27 ab	16.83 c	13.14 abo			
GABA x 0.664 ml/l	8.50 a	25.25 a	66.09 a	157.11 a	216.51 a	226.52 a	5.32 a	8.55 a	19.73 a	21.70 a	19.49 a	13.88 a			

Number of nodule plant⁻¹

Number of nodule plant differed significantly among the varieties at all the sampling stages (Table 5). Variety BM 3 produced the highest number of nodules plant (20.55) over BM 4 and this trend was continued up to 90 DAS. The number of nodule plant 1 gradually increased up to 60 DAS in both varieties and decreased thereafter. At 60 DAS, the highest number of nodule was 20.5 and 19.9 plant 1 in BM 3 and BM 4 respectively. However, at maturity, BM 3 produced 12.3 nodules plant which is significantly higher over BM 4. All plant growth regulators stimulated the number of nodule plant from early to maturity stages (Table 6). GABA enhanced the number of nodule plant significantly over other PGRs. At 60 DAS, GABA produced the highest number of nodules plant⁻¹ (20.9), followed by TNZ-303 (20.3) and CI-IAA (19.6). Interaction effect between varieties and plant growth regulators on the nodule plant⁻¹ was significant from 75 to 90 DAS but not in early growth periods (Table 7). Different concentrations of PGRs affected number of nodule plant differently (Table 8). The number of nodule plant increased significantly with GABA at 0.664 ml L-1, followed by 0.332 ml L⁻¹ of same and 0.166 ml L⁻¹ of TNZ 303 in early growth periods (15-30 DAS). At 90 DAS, GABA at 0.0, 0.166, 0.332 and 0.664 ml L⁻¹ were produced 11.7, 12.5, 13.1 and 13.9 nodules plant 1 respectively. The lower concentration (0.166 ml L 1) of TNZ-303 was more effective in nodule production than higher (0.664 ml L⁻¹) concentration which was adverse for GABA in the present experiment.

The morphological features studied in the present experiment are important yield contributing characters in lentil. Height of a plant and number of branch plant are directly related to vield. Similar number of leaves per plant indicates photosynthetic potentiality of plants. Results indicated that vegetative growth enhanced by the application of PGRs selectively, which further enhanced the probability of seed yield increment. Stover of lentil (bhushi) is used as cattle feed and therefore, higher vegetative growth of plants in lentil will increase the production of bhushi. On going research will confirm the seed production performance of higher vegetative growing plants. Growth of both the varieties of lentils was enhanced in the present experiment. The higher growth might be due to earlier germination of treated seeds or due to activation of vegetative growth of seedlings induced by plant growth regulators. Genetic potentiality is one of the key factors in these regards. In the present study. BM 3 was more potential than BM 4. Higher plant height enhanced canopy area as well as canopy architecture in lentil, which enchanted number of branches and leaves in a plant. Higher number of branches and leaves increase the number of flower production, which ultimately enhanced yield of lentil. Number of root nodule plant enhanced due to application of PGRs selectively. Root nodule directly related to plant growth and yield. Further more, it adds more nitrogen in soil, which reduced need of N-fertilizer for subsequent crop. Considering these aspects, application of PGRs in adequate concentration is proved very useful and effective and therefore, might be recommending for farmers' use, tails may be made at different regional stations for its broad based performance.

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