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RESPONSE OF CHICKPEA TO PLANT GROWTH REGULATORS ON NITROGEN FIXATION AND YIELD

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Abstract

The study was conducted to compare the effects of three plant growth regulators and *Rhizobium leguminosarum* on the growth, yield parameters and N_2 fixation of chickpea under natural condition. The plant growth regulators viz., kinetin, indole 3-acetic acid (IAA) and abscisic acid (ABA) were applied at concentration of 10^{-5}M as seed soaking and 10^{-6} M as foliar spray, alone and in combinations with *Rhizobium* inoculum (strain TAL 1148 and TAL 620). Kinetin was found to be the most affective in increasing growth parameter (viz. root/shoot biomass, grain yield) and nitrogen fixation (viz., specific nitrogenase activity of nodules, and total N – fixed plant^{-1}) of chickpea. The IAA seed soaking was least effective. The ABA seed soaking as well as foliar spray treatments significantly decreased nodules weight, nitrogenase activity of nodules, specific nitrogenase activity of nodules and total N – fixed plant^{-1}. The kinetin seed soaking (10^{-5} M) and ABA foliar spray (10^{-6} M) were more effective in increasing the grain weight as compared to control. Application of *Rhizobium* inoculum, generally increased growth yields components and nitrogen fixation. The TAL 1148 strain was more effective than TAL 620. The pattern of response to hormone and *Rhizobium* inoculum was consistent in the three consecutive years. It would suggest that that both the efficiency and the longevity of the nodules seem to be favorably affected by kinetin application.

Introduction

Legume yield and N accumulation are directly related to the magnitude and efficiency of symbiotic N_2 fixation occurring in root nodules (Schubert, 1982). The interaction between roots of leguminous plants and bacteria leads to the formation of unique plant organ, the root nodule, in which the differentiated bacteria fix atmospheric nitrogen. It is generally believed that plant growth hormones viz., kinetin and indole acetic acid are involved in triggering the initiation of root nodules and that hormonal balance is an important factor in the control of nodule development, maintenance and senescence (Hirsch *et al*., 1997). Information is scanty on the effects of growth regulators on the process of nodulation, nitrogen fixation and total nitrogen fixed by chickpea. The present study attempts to investigate the effects of growth regulators on the process of nodulation, nitrogen fixation and total nitrogen fixed by chickpea.

Materials and Methods

Plant materials and growing conditions: The seeds of chickpea cv. CM-88 obtained from National Agricultural Research Centre (NARC) Islamabad, were surface sterilized in aqueous solution of Mercuric chloride (0.1%) for 2 min., and thoroughly rinsed with distilled water. Thereafter, seeds were soaked in distilled water (treated as control) and aqueous solution of IAA, ABA and kinetin (each at 10^{-5} M) for 6 h. The seeds were

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inoculated with *Rhizobium leguminosarum* strain TAL 620 or TAL 1148 and were sown in earthen pots. The phosphorus was applied as single super phosphate, and mixed well in soil at the time of pot filling . Four plants pot^{-1} were allowed to grow during mid November in natural environment. Three weeks after germination the foliar spray of each hormone were made. The seedlings/plants were irrigated with half strength Hoagland nutrient solution throughout the course of experiment. This experiment was repeated for three consecutive years.

At maximum flowering stage plant samples were collected from each pot for collection of nodules to determine, nodule mass/weight plant^{-1}, specific nitrogenase activity, volume of pink bacteroid tissue, etc. At maturity, plants were harvested and samples of roots and shoots were collected to determine above ground biomass (weight of shoot), below ground biomass (root weight), plant N – content and δ^{15}N.

Determination of nitrogenase activity of nodules: The nitrogenase activity of nodules was recorded following the method of Larue & Kurz (1973). Nitrogenase activity was estimated at two stages, one at the time of 50% flowering (DAP) and second at the time of pod filling (DAP).

Measurements of volume of pink bacteroid tissue of nodules: The nodules were taken from roots of chickpea plants at two different stages, (i) at 50% flowering stage and (ii) at pod filling stage. Thin sections of nodules (5μ) were made with the help of razor blade and the volume of pink bacteroid tissue containing leghaemoglobin present in the nodule cortex were measured under light microscope (Nikon Research Microscope, optiphat with HFX- II Camera) at 4X (Gretchen 1967).

Determination of specific nitrogenase activity of nodules: Specific nitrogenous activity of nodules is expressed as ηmoles C_2H_4 pink bacteroid tissue mm^3 plant^{-1} h^{-1} =

$$\frac{C_2H_4 \text{ nmoles g}^{-1} \text{ nodule plant}^{-1} \text{ h}^{-1}}{\text{Pink bacteroid tissue mm}^3 \text{ plant}^{-1} \text{ h}^{-1}}$$

Assessment of N_2 fixation by ^{15}N natural abundance technique: The assessment of natural nitrogen fixation by ^{15}N abundance in shoot was recorded at harvesting following the method of Peoples (1996).

Nitrate and total nitrogen content of soil: At harvesting, the nitrate nitrogen and total nitrogen of soil was determined following methods of Winkleman *et al*. (1985) and Jackson (1962).

The data were analyzed statistically by Analysis of Variance technique and comparison among treatment means was made by Duncan's Multiple Range test (DMRT) using MSTAT-C version 1.4.2.

Results and Discussion

Nodule weight, nitrogenase activity, volume of pink bacteroid tissue and specific nitrogen fixation: The results of DMRT showed significant ($\propto$= 0.05) difference in nodule weight plant^{-1} among the treatments. The result revealed that maximum nodule weight was recorded in kinetin seed soaking for plants inoculated with strain A. IAA seed soaking made to plants inoculated with strain A performed well as compared to control. The least increase was recorded in ABA and IAA treatments made as foliar spray. There were significant ($\propto$=0.05) differences among treatments regarding their effects on the volume of pink bacteroid tissue, nitrogenase activity and specific nitrogenase activity of nodules both at 50% flowering and pod filling stages. The maximum increase was recorded in kinetin treatment followed by IAA and ABA. Foliar spray proved better as compared to seed soaking. *Rhizobium* strain TAL 1148 performed better than TAL 620. However value of all these plants parameters was higher at flowering stage. At pod filling stage nodules were fragile and shriveled due to ABA treatment, the volume of pink bacteroid tissue could not be measured and ultimately specific nitrogenase activity of nodules plant^{-1} could not be calculated. The maximum specific nitrogenase activity was recorded in kinetin seed soaking treatment for plants inoculated with strain A. The response of IAA (seed soaking) was less than that of kinetin but better than ABA and control. The pattern of response to hormone and inoculum was consistent during three consecutive years. The observed increases in nodule weight plant^{-1} in kinetin treatment indicate the distinct role of cytokinin in nodule morphogenesis (Syono *et al.* 1976, Hirsch & Fang 1994). Kinetin is a growth-promoting hormone, it increases the chlorophyll content in leaves and delayed senescence process. Hardy (1977) observed that delay of nodule senescence is one of the factor(s) that contribute significantly to nitrogen fixation in legume. Kinetin caused increase in leghaemoglobin content and nodule bacteroid region over the control (Singh, 1993). Dayal & Bharti (1991) and Garg *et al*., (1995) also observed kinetin-induced increase in nodule dry weight as well as in the nitrogenase activity. Previous work of Bano (1986), Zarrin & Bano (1998) and Zarrin *et al*., (1998) demonstrated the positive role of cytokinin in nodulation. The nitrogenase activity of nodules was high at flowering stage as compared to podfilling stage (Vessey, 1992). Seed soaking treatment with IAA and inoculation with *Rhizobium* strain TAL 1148 was better than the control both at flowering and pod filling stages. Singh (1993) observed that growth regulators like IAA, NAA, 2, 4-D, GA and kinetin used as foliar spray brought considerable variations in nodulation. The size of nodule (wt.) was significantly decreased due to ABA treatment in inoculated plants but inoculation mitigated the suppressive effect of ABA indicating that growth promoting hormones produced in higher concentration following inoculation might have suppressed effect.

Fresh/dry weight of shoots and roots plant^{-1}: Applications of hormones significantly increased fresh and dry weight of shoot except foliar spray with IAA, which did not show significant effect over control. The maximum increase was recorded in kinetin treatments both as foliar spray and seed soaking. ABA as foliar spray application proved to be more effective in increasing shoot and root fresh weight. Application of *Rhizobium* inoculum whether alone or in combination with growth regulators significantly increased plant shoot and root fresh weight TAL 1148 strain being clearly more effective as compared to TAL 620. *Rhizobium* inoculum was effective in increasing root dry weight; although magnitude of increase was less as compared to their effect on shoot dry weight.

Among methods of hormones application seed soaking was significantly more effective in case of kinetin and IAA but there was no difference among seed soaking and foliar spray in case of ABA. The maximum shoot/root weight was recorded in kinetin treated plants, this increase could be attributed to cytokinin regulation of photosynthetic capacity and delay in the senescence. Rabie (1996) reported that benzyladenine (BA) increased plant height total numbers of tillers, spikes plant^{-1}. Liu *et al*., (2000) suggested that exogenous kinetin showed to promote tiller growth in wheat, oat and barley. Exogenously applied ABA inhibits the shoot growth and this reduction in growth was significantly correlated with decreased net assimilation rate. Cramer *et al*., (1998) reported that the effect of ABA on soybean root systems is to reduce the hydraulic conductance about 4 h after the application of ABA. Indirect evidence indicated by Tie & Grant (1996) indicated that the decreased photosynthesis might have resulted from feedback inhibition of the decreased growth.

Seed weight plant^{-1}: In this study the maximum grain weight plant^{-1} was recorded in ABA (foliar spray) and kinetin (seed soaking) treatment. Maximum seed weight in kinetin treatment may be attributed to the kinetin-induced translocation of assimilates from vegetative to reproductive parts. Kim *et al*., (1993), observed significant increase in soybean seed yield which may be attributed to increase in amino acid and mineral contents. This increase in seed of soybean may due to BA treatment. Reinbott and Blevins (1998) observed increased in seed numbers and pod of soybean due to synthetic cytokinin (BAP). This view is also supported by Peterson *et al*., (1990), who concluded that BAP significantly reduced pod abscission and subsequently increased the total number of pods and seeds. The significant increase in grain yield obtained in this study due to ABA treatment has been previously reported by Amzallag *et al*. (1990), who found increased number of grains per panicle due to ABA treatment. Exogenous supplies of ABA may influence assimilate distribution (Patrick & Wareing 1982). In this study the minimum grain yield was observed in IAA both in seed soaking and foliar spray treated plants as IAA delayed the maturation (Salisbury & Ross, (1992); Zarrin & Bano, (1998); Zarrin *et al*., (1998).

N_2 fixation by ^{15}N natural abundance technique: Results of percent nitrogen fixed (pfix) by the plants were more varied and complex. In general, application of plant hormones increased pfix values but there were no clear differences among the treatments. The significant increase in total nitrogen fixed was in kinetin with strain TAL 1148 both in seed soaking as well as in foliar spray treatment as compared to control. The total nitrogen fixed is correlated with shoot biomass. The increased in total biomass due to kinetin treatment is related with increase in photosynthesis activity. Caers & Vendrig (1986) reported that application of cytokinins promote photosynthetic activity mainly by means of increase in leaf chlorophyll content. Hardy & Havelk (1975) and Bethlenfalvay & Phillips (1978) reported the pivotal role of photosynthesis in nitrogen fixation. Kinetin being a growth-promoting hormone increased the nitrogenase activity of root nodules of chickpea (Dayal & Bharti 1991) and by increasing in the volume of pink bacteroid tissue, it also increased in leghaemoglobin contents and nodule bacteroid regions over control. The efficiency and the longevity of nodules seem to be favourably affected by kinetin application due to the similar reason. A higher nitrogen status of the plants was always correlated with a higher endogenous level of cytokinin (Wagner & Beck 1993). Rao (1984) reported that stimulatory effect of kinetin is to increase the efficiency of nitrogen fixation. The minimum total nitrogen fixed was recorded in ABA treatment as ABA decreased photosynthesis due to depressed intracellular CO_2 levels. ABA treatment inhibits nitrogen fixation (Sood *et al.* 1996). ABA also affects the nitrogen fixing ability of the nodule by delaying bacteroid tissue formation (Bano *et al*., 1983).

Table 1. Plant physicochemical parameters as effected by plant growth regulators and *Rhizobium* inoculation TAL 1148 (A) and TAL 620 (B) on *Cicer arientinum* L. cv. CM 88.

Treatments	Inoculated A	Inoculated B	Uninoculated	Mean	Inoculated A	Inoculated B	Uninoculated	Mean	Inoculated A	Inoculated B	Uninoculated	Mean
	Nitrogenase activity (nmol C_2H_4 plant^{-1} h^{-1}) of nodules at flowering stage				**Volume of pink bacteroid tissue (mm^3 plant^{-1} h^{-1}) of nodules at flowering stage**				**Specific nitrogenase activity (nmol C_2H_4 mm^{-3} pink bacteroid tissue plant^{-1} h^{-1}) of nodules at flowering stage**			
ABA (SS)	982 k	943 m	849 t	925 E	1.80 g	1.76 hi	1.71 j	1.76 D	546 j	535 m	504 p	529 G
ABA (FS)	935 n	926 o	857 s	906 G	1.72 j	1.72 j	1.64 k	1.69 F	543 k	542 kl	532 n	539 E
IAA (SS)	1086 g	1049 h	1021 i	1052 C	1.85 d	1.84 de	1.83 ef	1.84 C	587 g	572 h	558 i	572 C
IAA (FS)	993 j	963 l	882 q	946 D	1.82 f	1.77 h	1.64 k	1.74 E	547 j	543 k	536 m	542 D
Kinetin (SS)	1216 a	1178 c	1143 e	1179 A	1.95 a	1.92 b	1.90 c	1.92 A	624 a	614 c	601 e	613 A
Kinetin (FS)	1198 b	1148 d	1128 f	1158 B	1.94 a	1.90 c	1.88 c	1.91 B	618 b	603 d	598 f	606 B
Control	946 m	915 p	867 r	909 F	1.75 i	1.71 j	1.64 k	1.70 F	541 l	535 m	528 o	534 F
Mean	1047 A	1008 B	917 C		1.83 A	1.80 B	1.75 C		572 A	563 B	551 C	
	Nitrogenase activity (nmol C_2H_4 plant^{-1} h^{-1}) of nodules at pod filling stage				**Volume of pink bacteroid tissue (mm^3 plant^{-1} h^{-1}) of nodules at pod filling stage**				**Specific nitrogenase activity (nmol C_2H_4 mm^{-3} pink bacteroid tissue plant^{-1} h^{-1}) of nodules at pod filling stage**			
ABA (SS)	133.0 ij	118.5 k	74.7 n	108.7 F	0.000 i	0.000 i	0.000 i	0.000 F	0 l	0 l	0 l	0 F
ABA (FS)	137.0 i	130.3 j	85.3 m	117.6 E	0.000 i	0.000 i	0.000 i	0.000 F	0 l	0 l	0 l	0 F
IAA (SS)	169.8 f	158.7 g	150.0 h	159.5 C	0.383 c	0.377 c	0.369 cd	0.376 C	443 f	420 g	407 h	423 C
IAA (FS)	137.3 i	127.0 j	117.0 kl	127.1 D	0.341 e	0.323 f	0.300 g	0.321 D	400 hi	393 ij	390 j	394 D
Kinetin (SS)	299.0 a	284.5 b	218.5 d	267.3 A	0.447 a	0.447 a	0.357 de	0.417 A	674 a	637 c	612 d	641 A
Kinetin (FS)	287.0 b	269.3 c	205.7 e	254.0 B	0.441ab	0.426 b	0.344 e	0.404 B	651 b	632 c	599 e	627 B
Control	119.8 k	111.7 l	89.8 m	107.1 F	0.297 g	0.289 g	0.257 h	0.281 E	404 h	387 j	345 k	379 E
Mean	183.3 A	171.4 B	134.4 C		0.273 A	0.266 B	0.232 C		367 A	353 B	336 C	
	Weight of Nodules (g plant^{-1})				**Shoot Fresh Weight (g plant^{-1})**				**Shoot Dry Weight (g plant^{-1})**			
ABA (SS)	1.20 c-f	1.29 b-e	0.55 k	0.99 B	29.1 a-e	23.9 c-f	22.3 d-g	25.1 BC	14.67 c-e	11.93 e-i	11.23 e-i	12.61 B
ABA (FS)	1.29 b-e	1.07 e-g	0.68 jk	1.43A	30.3 a-e	27.2 b-f	20.3 d-g	25.9 BC	15.13 b-e	13.40 d-h	10.13 f-j	12.89 B
IAA (SS)	1.41 a-c	0.89 g-j	0.81 h-j	1.34 A	31.0 a-d	25.0 c-f	27.6 b-f	27.9 B	15.50 b-e	12.50 e-h	13.80 d-g	13.93 B
IAA (FS)	1.20 c-f	1.02 f-h	0.76 I-k	0.96 B	23.1 c-g	18.9 e-g	18.7 e-g	20.2 CD	11.57 e-i	9.43 g-j	9.37 h-j	10.12 C
Kinetin (SS)	1.57 a	1.40 a-c	1.31 b-e	1.43 A	40.9 a	37.3 ab	40.3 a	39.5 A	20.43 a	18.63 a-c	14.13 d-f	17.73 A
Kinetin (FS)	1.48 ab	1.34 a-d	1.20 c-f	1.34 A	38.5 ab	34.7 a-c	31.0 a-d	34.7 A	19.27 ab	17.33 a-d	15.50 b-e	17.37 A
Control	1.14 d-f	0.97 f-i	0.76 i-k	0.96 B	26.8 b-f	15.8 fg	12.0 g	18.2 D	13.33 d-h	7.90 ij	6.00 j	9.08 C
Mean	1.33 A	1.14 B	0.87 C		31.4 A	26.1 B	24.6 B		15.70 A	13.02 B	11.45 C	

All such means which share a common English letter are statistically similar, otherwise they are different at $\propto = 0.05$.

Table 2. Plant physicochemical parameters as effected by plant growth regulators and *Rhizobium* inoculation TAL 1148 (A) and TAL 620 (B) on *Cicer arietinum* L. cv. CM 88.

Treatments	Inoculated A	Inoculated B	Uninoculated	Mean	Inoculated A	Inoculated B	Uninoculated	Mean	Inoculated A	Inoculated B	Uninoculated	Mean
	Root fresh weight (g $plant^{-1}$)				Root dry weight (g $plant^{-1}$)				Grain weight (g $plant^{-1}$)			
ABA (SS)	13.6 d-f	13.6 d-f	11.9 ef	13.1 D	6.98 g-i	6.52 ij	6.12 j	6.54 D	3.22 c	2.80 de	2.12 g	2.71 C
ABA (FS)	15.2 de	14.3 d-f	13.5 d-f	14.3 D	7.53 fg	7.10 f-h	6.81 hi	7.15 C	3.74 ab	3.13 cd	2.30 fg	3.06 B
IAA (SS)	16.6 cd	20.6 bc	12.4 d-f	16.6 C	8.32 e	7.58 f	6.14 j	7.35 C	1.94 g	1.55 h	0.92 i	1.47 D
IAA (FS)	13.6 d-f	13.0 d-f	10.2 f	12.3 D	6.81 hi	6.50 ij	5.07 k	6.13 E	0.49 j	0.30 j	0.17 j	0.32 E
Kinetin (SS)	27.1 a	20.7 bc	19.6 c	22.4 A	13.47 a	10.28 c	9.79 cd	11.18 A	4.09 a	3.92 ab	2.65 ef	3.55 A
Kinetin (FS)	23.9 ab	19.4 c	14.5 d-f	19.2 B	11.93 b	9.68 d	7.19 f-h	9.60 B	3.61 b	2.89 c-e	2.62 ef	3.04 B
Control	14.6 de	12.5 d-f	10.2 f	12.4 D	7.25f-h	6.22 j	5.01 k	6.16 E	0.49 j	0.28 j	0.16 j	0.31 E
Mean	17.8 A	16.3 B	13.2 C		8.90 A	7.70 B	6.59 C		2.51 A	2.12 B	1.56 C	
	Nitrogen content in dry shoot (%$plant^{-1}$)				% pfix in dry shoot ($plant^{-1}$)				Total nitrogen fixed (g $plant^{-1}$)			
ABA (SS)	2.13 ab	2.18 ab	2.15 ab	2.15 A	63.7 c-f	72.0 a-e	73.7 a-e	69.8 BC	0.402 d-g	0.338 f-h	0.359 e-g	0.366 CD
ABA (FS)	2.14 ab	2.22 ab	2.22 ab	2.19 A	75.0 a-c	81.0 a	74.7 a-c	76.9 A	0.491 a-e	0.481 b-f	0.336 e-h	0.436 C
IAA (SS)	2.45 a	2.31 ab	2.24 ab	2.34 A	80.3 a	79.7 a	77.0 ab	79.0 A	0.639 a-c	0.513 a-e	0.483 b-f	0.545 AB
IAA (FS)	2.23 ab	2.29 ab	2.38 ab	2.30 A	71.3 a-e	64.3 c-f	61.7 ef	65.8 C	0.370 e-g	0.281 f-h	0.275 gh	0.309 DE
Kinetin (SS)	2.26 ab	2.23 ab	2.40 a	2.30 A	74.3 a-d	77.3 ab	74.0 a-d	75.2 AB	0.688 a	0.653 ab	0.502 a-e	0.614 A
Kinetin (FS)	2.30 ab	2.23 ab	1.97 b	2.17 A	62.3 d-f	53.7 f	52.3 f	56.1 D	0.579 a-d	0.449 c-g	0.372 e-g	0.467 BC
Control	2.24 ab	2.39 a	2.32 ab	2.32 A	53.3 f	65.7 b-e	53.0 f	57.3 D	0.308 e-h	0.252 gh	0.153 h	0.238 E
Mean	2.25 A	2.26 A	2.24 A		68.6 A	70.5 A	66.6 A		0.497 A	0.424 B	0.355 C	
	Nitrate–nitrogen content in soil (mg pot^{-1})				Total nitrogen content in soil (% pot^{-1})							
ABA (SS)	4.74 fg	4.28 gh	4.20 gh	4.41 D	0.049 a-c	0.050 a-c	0.050 a-c	0.049 AB				
ABA (FS)	3.97 hi	3.27 jk	3.02 kl	3.42 F	0.051 a-c	0.050 a-c	0.042 bc	0.048AB				
IAA (SS)	5.24 ef	5.30 ef	5.02 ef	5.19 C	0.05 a-c	0.041 bc	0.038 bc	0.043 B				
IAA (FS)	5.10 ef	3.83 h-j	3.50 i-k	4.14 DE	0.041 bc	0.044 bc	0.036 bc	0.041 B				
Kinetin (SS)	8.99 a	7.92 b	5.17 ef	7.36 A	0.065 a	0.053 ab	0.043 bc	0.054 A				
Kinetin (FS)	7.16 c	5.99 d	4.96 ef	6.04 B	0.044 bc	0.043 bc	0.037 bc	0.042 B				
Control	5.49 de	3.58 i-k	2.69 l	3.92 E	0.045 bc	0.040 bc	0.032 c	0.039 B				
Mean	5.81 A	4.88 B	4.08 C		0.049 A	0.046 A	0.04 B					

All such means which share a common English letter are statistically similar, otherwise they are different at $\propto = 0.05$.

Soil nitrate and total nitrogen content: Soil nitrate and total nitrogen content of soil was analyzed to determine the effects of increased plants biomass and nitrogen fixation on soil N fertility. Effects of increased plant biomass and N_2 fixation were reflected in the form of increased soil nitrate and total nitrogen. In fact it was one of the main objectives of the study. Soil nitrate was the highest in case of kinetin treatments especially when this hormone was applied as seed soaking along with *Rhizobium* inoculum, particularly TAL1148. This was same treatment that had highest plant biomass, grain yield and N_2 fixation. This was followed by seed soaking treatments of IAA and ABA. Foliar spray treatments of IAA and ABA had the least effect, if any, on soil nitrate. In general, seed soaking treatments had profound effects in increasing soil nitrate. *Rhizobium* inoculation increased soil nitrate content over control. TAL 1148 inoculated plants performed better than TAL 620. Soil nitrate was lower than that of control when ABA was applied, the reason for increasing nitrate content in soil during the growth season are reduced uptake rates of nitrate, or relative contribution of both N (nitrate-nitrogen and total nitrogen) source, mainly influenced by the amount and availability of inorganic nitrogen in the soil. The absolute amount of nitrogen fixed by chickpea is normally decreased with increased availability of mineral nitrogen (Kage 1995; Zarrin *et al.* 1998).

Through these experiments it could be concluded that both the efficiency and the longevity of the nodules seem to be favorably affected by kinetin application. Its performance in volume of pink bacteroid tissue, specific nitrogenase activity of nodules, nitrate nitrogen and total nitrogen content in soil and total nitrogen fixed was better than ABA and IAA. Among methods of hormones application seed soaking treatments was significantly more effective than foliar spray. In all treatments *Rhizobium* strain TAL 1148 (A) performed better as compared to TAL 620 (B) whether used alone or in combination with plant growth regulators.

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