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Effect of Growth Regulators on Nodulation and Nitrogen Fixation in Groundnut (*Arachis Hypogaea* L.)

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ABSTRACT

To study the effect of growth regulators *viz*., Indole-3-Acetic Acid (IAA), Kinetin, and Homobrassinolide (HBL) on nodulation and nitrogen fixation in groundnut. The present investigation was under taken with IAA, Kinetin @ 10 ppm and HBL @ 3.0 µM at 30 DAS. Nitrate reductase activity (5.67 ìM NO_2^- g^{-1} hr^{-1}), leghaemoglobin content (1.93 mg per fresh weight of nodules), leaf nitrogen content (3.48 %), soil nitrogen content (0.052 %), number and fresh weight of nodules (68.96 and 0.57 g) respectively were increased in the treatment pre soaking of seeds before sowing with kinetin @ 10 ppm followed by foliar spray of kinetin @ 10 ppm (T_4) at 30 DAS which is on par with the treatment, pre soaking of seeds before sowing with HBL @ 3.0 µM followed by foliar spray of HBL @ 3.0 µM at 30 DAS (T_{12}).

Key words: *Groundnut, Indole Acetic Acid, Kinetin, Homo brassinolide, Nitrogen fixation.*

Groundnut oil is edible oil which is used for manufacturing of vegetable oil, cosmetics, lubricants, olein, stearin and their salts. Kernels are consumed as raw, roasted or sweetened. Oil cake is a rich source of valuable organic manure and cattle feed. The cake contains about 7-8 per cent N, 1.5 per cent P_2O_5 and 1.5 per cent K_2O. Groundnut crop has the ability to fix atmospheric nitrogen through the root nodules thereby building up the soil fertility. In India, groundnut is cultivated in an area of 4.7 m ha^{-1} with a production of 4.69 million tonnes and the productivity of 995 kg ha^{-1} during 2014-15. In Andhra Pradesh, groundnut area is 1.34 m ha^{-1} with a production and productivity of 1.12 million tonnes and 829 kg ha^{-1} respectively (Indiastat, 2014-15). Nitrogen is essential constituent of many compounds of plant, such as chlorophyll, nucleotides, nucleic acids, proteins, alkaloids, enzymes, hormones, vitamins etc. The interaction between roots of leguminous plants and bacteria leads to the formation of the root nodule, in which bacteria fix atmospheric nitrogen.

Growth regulators mainly auxins, gibberellins, cytokinins and brassinosteroids play an important role in increasing the nodule organogenesis, development and stimulation of nitrate reductase activity, which contribute increase in yield of legume crops.

'Brassinosteroids' is the first steroidal plant growth regulator was isolated from *Brassica napus*. The growth promoting substance discovered was named as "Brassinolide" (Grove *et al.,* 1979). Pre soaking and foliar application of Brassenosteroids (BRs) @ 3.0 microM increased the nodulation and nitrogenase activity and yield components in Groundnut (Vardhini *et al*., 1999). Chemically, kinetin is 6-furfurylaminopurine, a plant hormone in the class of cytokinins (Skoog *et al*., 1965). Pre soaking and foliar application of Kinetin caused increase in leghaemoglobin content and nodule bacteroid region over the control (Singh *et al.* 1993). The most important member of the auxin family is Indole-3-Acetic Acid (IAA), a native auxin in plants. The possible involvement of auxins in nodule formation was first reported by Thimann in 1936. Thimann reported that the nodules of *Pisum sativum* contained auxin and that the auxin content increased during root nodule development.

MATERIAL AND METHODS

The field experiment was conducted at "F" block of Agricultural College Farm, Bapatla, on a sandy soil in *Kharif* season, 2015. The experiment was laid out in a Randomized Block Design with three replications. The crop was fertilized with 30:40:50 kg ha^{-1}. Nitrogen was applied in two equal

splits of half as basal dose and the remaining half at 30 DAS. Phosphorus and Potassium were applied as the basal dose at the time of sowing. Pre soaking of seeds before sowing with IAA, Kinetin @ 10 ppm and HBL @ 3.0 µM followed by foliar sprays of IAA, Kinetin @ 10 ppm and HBL @ 3.0 µM, water spray serve as control. The observations on growth parameters like plant height, number of branches, total dry matter accumulation, were recorded at 15 days interval starting from 15 Days after sowing (DAS). The observations on biochemical parameters like nitrate reductase activity, leghaemoglobin content, nitrogen content of leaf were recorded at 25 and 45 DAS, and number and fresh weight of nodules at 60 DAS, finally nitrogen content of soil at the time of harvest.

RESULTS AND DISCUSSION

The data related to effect of growth regulators on nodulation and nitrogen fixation in groundnut was presented in Table 1.

Nitrate reductase activity (ìM NO_2^- g^{-1} hr^{-1})

The observations at 25 DAS were not significant. At 45 DAS, pre soaking of seeds before sowing with kinetin followed by foliar spray of kinetin @ 10 ppm (T_4) showed higher NR activity. At 45 DAS, low NRA was recorded in control (T_{13}-3.25 ìM NO_2^- g^{-1} hr^{-1}) and pre soaking of seeds before sowing with IAA followed by foliar spray of IAA @ 10 ppm (T_8-3.64 ìM NO_2^- g^{-1} hr^{-1}). High NRA value recorded was 5.67 ìM NO_2^- g^{-1} hr^{-1} with pre soaking of seeds before sowing with kinetin followed by foliar spray of kinetin @ 10 ppm (T_4) which is on par with the pre soaking of seeds before sowing with HBL @ 3.0 µM followed by foliar spray with HBL @ 3.0 µM (T_{12}-5.33 ìM NO_2^- g^{-1} hr^{-1}). All the treatments are significantly high over the control (T_{13}) and on par with each other.

The increase in NRA by kinetin might be due to the positive effect of cytokinin in nodulation in Medicago truncatula (Gonzalez-Rizzo *et al.*, 2006, Murray *et al*., 2007 and Tirichine *et al*., 2007). This is supported by the findings of Maibangsa *et* al. (2000) stated that application of BRs and salicylic acid increased the nitrate reductase activity with increased assimilation and biomass accumulation. The application of cytokinins stimulated nitrate induced nitrate reductase activity in the dark (Rao et al., 1984).

Leghaemoglobin content (mg per fresh weight of nodules)

The observations at 25 DAS were not significant. At 45 DAS, pre soaking of seeds before sowing with kinetin followed by foliar spray of kinetin @ 10 ppm (T_4) showed higher leghaemoglobin content. At 45 DAS, low leghaemoglobin content was recorded in control (T_{13}-1.52 mg) and pre soaking of seeds before sowing followed by foliar spray of IAA @ 10 ppm (T_8-1.57 mg). High leghaemoglobin content value recorded was 1.86 mg per fresh wt of nodules with pre soaking of seeds before sowing with kinetin followed by foliar spray of kinetin @ 10 ppm (T_4), which is on par with the pre soaking of seeds before sowing with HBL @ 3.0 µM followed by foliar spray with HBL @ 3.0 µM (T_{12}-1.84 mg). All the treatments are significantly high over the control (T_{13}) and on par with each other.

The increase in leghaemoglobin content by kinetin might be due to the positive effect of cytokinin in nodulation (Gonzalez-Rizzo *et al.*, 2006, Murray *et al*., 2007 and Tirichine *et al*., 2007). Kinetin caused increase in leghaemoglobin content and nodule bacteroid region over the control in chickpea (Singh, 1993). Dayal and Bharti (1991) and Garg *et al*., (1995) also observed kinetin-induced increase in nodule dry weight as well as in the nitrogenase activity in chickpea.

Number and fresh weight of nodules (g^{-1} plant)

At 60 DAS, among all the treatments, the treatment, pre soaking of seeds before sowing with kinetin followed by foliar spray of kinetin @ 10 ppm (T_4) showed higher number and fresh weight of nodules (68.9 & 0.57 g^{-1} plant) respectively, which is on par with pre soaking of seeds before sowing with HBL @ 3.0 µM followed by foliar spray with HBL @ 3.0 µM (T_{12}-58.94 & 0.51 g^{-1} plant). All the other treatments are significantly high over control (T_{13}-40.14 & 0.40 g^{-1} plant) and on par with each other.

The observed increase in nodule weight per plant in kinetin treatment indicate the distinct role of cytokinin in nodule morphogenesis (Syono *et al.* 1976, Hirsch & Fang 1994). Hardy (1977) observed that delay of nodule senescence is one of the factor(s) that contribute significantly to nitrogen fixation in legume. Dayal and Bharti (1991) and Garg *et al*., (1995) also observed kinetin-induced

Table 1. Effect of growth regulators on nodulation and nitrogen fixation in groundnut.

Treatments (pre soaking/foliar spray)	Nitrate Reductase activity		Leghaemoglobin content		Leaf nitrogen (%)		Number and fresh weight of nodules		Soil Nitrogen (%)	
	25 DAS	45 DAS	25 DAS	45 DAS	25 DAS	45 DAS	60DAS	60 DAS	Initial	Final
T_1: Pre soaking with Kinetin @ 10 ppm	1.37	4.76	1.81	1.75	2.50	3.13	53.38	0.44	0.015	0.052
T_2: Pre soaking with IAA @ 10 ppm	1.15	4.19	1.43	1.68	2.07	2.98	41.83	0.41	0.015	0.037
T_3: Pre soaking with HBL @ 3.0 µM	1.24	4.49	1.38	1.79	2.38	3.27	56.33	0.46	0.015	0.045
T_4: Pre soaking with Kinetin @ 10 ppm + Foliar spray of kinetin @ 10 ppm at 30 DAS	1.30	5.67	1.48	1.86	2.42	3.48	68.96	0.57	0.015	0.034
	1.28	4.20	1.42	1.70	2.31	3.00	44.46	0.42	0.015	0.041
T_5: Pre soaking with Kinetin @ 10 ppm + Foliar spray of IAA @ 10 ppm at 30 DAS	1.25	4.83	1.40	1.82	2.12	3.31	58.41	0.49	0.015	0.032
T_6: Pre soaking with Kinetin @ 10 ppm + Foliar spray of HBL @ 3.0 µM at 30 DAS	1.21	4.26	1.33	1.72	2.46	3.03	52.01	0.42	0.015	0.043
T_7: Pre soaking with IAA @ 10 ppm + Foliar spray of kinetin @ 10 ppm at 30 DAS	1.17	3.64	1.41	1.57	2.15	2.89	41.32	0.41	0.015	0.033
T_8: Pre soaking with IAA @ 10 ppm + Foliar spray of IAA @ 10 ppm at 30 DAS	1.29	4.32	1.21	1.73	2.02	3.11	52.10	0.43	0.015	0.046
T_9: Pre soaking with IAA @ 10 ppm + Foliar spray of HBL @ 3.0 µM at 30 DAS	1.26	5.17	1.35	1.83	2.36	3.34	56.94	0.47	0.015	0.031
T_{10}: Pre soaking with HBL @ 3.0 µM + Foliar spray of kinetin @ 10 ppm at 30 DAS	1.29	4.37	1.36	1.74	2.19	3.12	55.20	0.44	0.015	0.035
T_{11}: Pre soaking with HBL @ 3.0 µM + Foliar spray of IAA @ 10 ppm at 30 DAS	1.32	5.33	1.55	1.84	2.42	3.42	58.94	0.51	0.015	0.049
T_{12}: Pre soaking with HBL @ 3.0 µM + Foliar spray of HBL @ 3.0 µM at 30 DAS	1.13	3.25	0.98	1.52	2.01	2.70	40.14	0.40	0.015	0.026
T_{13}: Control (water spray)										
SEm ±	0.04	0.05	0.06	0.03	0.16	0.11	3.19	0.03		0.002
CD	NS	0.13	NS	0.08	NS	0.33	9.33	0.08		NS
CV (%)	5.72	1.67	6.98	2.71	12.46	2.29	10.58	11.14		12.94

increase in nodule dry weight as well as in the nitrogenase activity in chickpea. Previous work of Bano (1986), Zarrin *et al*., (1998) demonstrated the positive role of cytokinin in nodulationin soybean. Dayal *et al.* (1991) reported that kinetin caused to increase nitrogenase enzyme activity in chickpea root nodules as a growth-inducing hormone and this increased the haemoglobin amount and finally lead to increase in nodule weight.

Leaf nitrogen content (%)

The effect of different treatments on leaf nitrogen content of groundnut was found to be non significant at 25 DAS. All the pre soaked and spray treatments resulted in an increase in leaf nitrogen content (%) at 45 DAS. At 45 DAS, among all the treatments, the treatment, pre soaking of seeds before sowing with kinetin followed by foliar spray of kinetin @ 10 ppm (T_4) showed higher leaf nitrogen content (3.48 %) which is on par with pre soaking of seeds before sowing with HBL @ 3.0 µM followed by foliar spray with HBL @ 3.0 µM (T_{12}-3.42 %). Less leaf nitrogen content recorded for the control (T_{13}-2.7 %). All the other treatments are significantly high over control (T_{13}) and on par with each other.

Kinetin is a growth-promoting hormone, increases the chlorophyll content in leaves and delayed the senescence process. Hardy (1977) observed that delay of nodule senescence is one of the factor(s) that contribute significantly to nitrogen fixation in legumes. Rao *et al.* (1984) reported that stimulatory effect of kinetin is to increase the efficiency of nitrogen fixation in maize. Fazel *et al.* (2012) reported that foliar spray of cytokinin @ 10 ppm increased plant dry matter and thus increase the amount of nitrogen fixation through the stimulation of growth and delay in senescence of leaves**.**

Nitrogen content of soil (%)

The effect of different treatments on nitrogen content of soil in groundnut was found to be non significant at harvest. But the pre soaked and spray treatments resulted in an increase in nitrogen content of soil (%) over control (T_{13}) and the increase was not significant.

Kinetin hormone increases the nitrogen fixation. It seems, cytokinin increased plant dry matter and thus increases the amount of nitrogen fixation through the stimulation of growth and delay in senescence of leaves. This is supported by the findings of Hardy (1977) observed that delay of nodule senescence is one of the factor(s) that contribute significantly to nitrogen fixation in legumes. Rao *et al*. (1984) reported that stimulatory effect of kinetin is to increase the efficiency of nitrogen fixation in maize.

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