The Andhra Agric. J 68 (1): 59-68, 2021

Studies on Impact of Kinetin and Nitrogenous Compounds on Delaying of Nodule Senescence in Blackgram (*Vigna mungo* (L.) Hepper).

Ch Nanibabu, B Sreekanth, K Jayalalitha and R D Sandeep

Department of Crop Physiology, Agricultural College, Bapatla. A. P.

ABSTRACT

A field experiment was carried out to assess the impact of kinetin and nitrogenous compounds on delaying of nodule senescence in blackgram at Agricultural College, Bapatla during *kharif*, 2018. Treatments consisting of three different concentrations of kinetin (10^{-2}, 10^{-3} and 10^{-4} M), potassium nitrate (5 and 10 g L^{-1}) and urea (10 and 20 g L^{-1}) along with untreated control and water application were imposed as foliar treatments on blackgram *cv* PU-31 in a randomized block design, replicated thrice. Treatments were imposed at three different times of reproductive growth *viz.*, 10 days before 50% flowering, 50% flowering and 10 days after 50% flowering in blackgram. Results revealed that foliar application of kinetin at lower concentration (10^{-4} M) and higher concentrations of nitrogen sources *i.e.*, potassium nitrate (1%) and urea (2%) decreased the nodule senescence most effectively in blackgram.

Keywords: *Blackgram, kinetin, nodule senescence, potassium nitrate, and urea.*

Blackgram or urad bean (*Vigna mungo* (L.) Hepper) is the fourth important pulse crop in India, mainly cultivated in Andhra Pradesh, Uttar Pradesh, Bihar, Madhya Pradesh and West Bengal during both *Kharif* and *Rabi* seasons. India is the world's largest producer as well as consumer of blackgram, producing about 2.83 M tons with an area of 4.47 M ha with an average productivity of 632 kg ha^{-1}. In Andhra Pradesh, the crop is grown in an area of 0.50 M. ha with a production of 0.32 M. tons and a productivity of 658 kg ha^{-1} (Datanet India, 2018). It has special significance in A.P as it fits well in rice fallow pulse cropping system particularly in Krishna – Godavari and North Coastal Zones.

Nitrogen is an important essential nutrient for plants. It increases growth and development of all living tissues and protein content in pulses (Rahman *et al.*, 2007). Puppo *et al.* (2005) reported that delayed nodule senescence may have a positive effect on the yield of legumes, which was a key aim of legume improvement programme.

Nodule senescence coincides with pod filling in many legumes (Lawn and Brun, 1974). Nodule senescence is a biochemical and physiological event with rapid decline in biological nitrogen fixation (BNF), that requires transcription of new genes (Alesandrini *et al.*, 2003; Thomas *et al.*, 2003). Stress-induced senescence in the lower leaf ranks of plants leads to nodule senescence in soybean (Marquez-Garcia *et al.*, 2015).

The present investigation was undertaken to assess the impact of kinetin and nitrogenous compounds in delaying nodule senescence in blackgram.

MATERIAL AND METHODS

A field experiment was conducted at the Field No. 28 of Northern Block of Agricultural College Farm, Bapatla, during *kharif* 2018, with blackgram *cv* PU-31. Randomized block design was adopted with three replications and there were nine foliar application treatments viz., kinetin at 10^{-2}, 10^{-3} and 10^{-4} M, potassium nitrate at 5 and 10 g L^{-1}, urea at 10 and 20 g L^{-1}, and two controls of no foliar application and foliar application of water. Certified seeds of blackgram *cv* PU-31 were used to grow the crop and the seeds were treated with blackgram specific rhizobial culture (Bio-Fertilizer Unit, Agricultural Research Station, Amaravathi, Guntur Dt.) before sowing. A spacing of 30 cm x 10 cm was adopted with a plot size of 4 x 5 m for each treatment. Recommended package of practices was followed for the management of crop during the growth period. Treatments were imposed at three different points of reproductive growth period of blackgram *cv* PU-31 *viz., at* 10 days before 50% flowering, at 50% flowering and at 10 days after 50% flowering. Root nodules were carefully isolated from the plants of all the treatments and the following parameters were studied at the Crop Physiology Laboratory at various stages *i.e.,* 10 days before 50% flowering, 50% flowering and 10 days after 50% flowering.

Leghemoglobin content

Leghaemoglobin content of nodules was estimated as per the method of Appleby and Bergersen (1980). Leghaemoglobin content was calculated and expressed as µg g^{-1} FWT, by using an extinction coefficient of leghaemoglobin *i.e.,* 23.4 mM^{-1} cm^{-1}.

Ascorbate peroxidase (APX) activity

Ascorbate peroxidase activity in nodules was estimated by following the method of Nakano and Asada (1981). The extinction coefficient of 2.8 mM^{-1} cm^{-1} for reduced ascorbate was used in calculating the enzyme activity that was expressed in terms of micromole of ascorbate per minute per gram fresh weight (µ mole min^{-1} g^{-1} FWT).

Soluble nitrogen content

Soluble nitrogen content in nodules was estimated by "micro Kjeldahl" method as given by Thimmaiah (1999).

$$\text{Nitrogen content (mg g}^{-1}\text{)} = \frac{\text{(ml Hcl in sample)-(ml Hcl in blank) x normality of acid x 14.01}}{\text{weight of sample (g)}}$$

RESULTS AND DISSCUSSION

Leghemoglobin content (µg g^{-1} FWT)

Leghemoglobin content of blackgram root nodules was significantly affected by the different treatments imposed at 10 days before flowering (Table 1). Foliar application of urea @ 10 g L^{-1} and foliar application of potassium nitrate @ 10 g L^{-1} were on par with each other with respect to leghemoglobin content of root nodules (313.162 and 346.700 µg g^{-1} FWT, respectively) at 10 days before 50% flowering, while showing significantly higher values than the foliar application of kinetin @ 10^{-2} M, 10^{-4} M. Foliar application of kinetin @ 10^{-3} M recorded significantly higher leghemoglobin content (292.635 µg g^{-1} FWT) than the remaining kinetin treatments. Though potassium nitrate @ 5 g L^{-1} showed highest nodule leghemoglobin content (361.809 µg g^{-1} FWT) at 10 days before 50% flowering, it didn't differ significantly from potassium nitrate @ 10 g L^{-1} (346.700 µg g^{-1} FWT). Foliar application of urea @ 10 and 20 g L^{-1} didn't differ significantly in the nodule leghemoglobin content. Among the treatments, highest leghemoglobin content of nodules was recorded with potassium nitrate @ 5 g L^{-1} (361.809 µg g^{-1} FWT),

while it was lowest with untreated control (145.760 µg g^{-1} FWT). In blackgram with treatments applied at 10 days before flowering all the exogenous chemicals significantly enhanced the nodular leghemoglobin over untreated control and this effect was more pronounced with the foliar application of nitrogen, either in potassium nitrate or urea form, than the application of cytokinins (kinetin), within the adopted concentrations. In the urea treatments, lower concentrations resulted in higher root nodular leghemoglobin content, while the opposite was true with potassium nitrate with the adopted concentrations in the current experiment on blackgram at 10 days before flowering.

Significant differences were observed among the treatments with respect to leghemoglobin content of nodules at 50% flowering (Table 2). Foliar application of water didn't affect the nodular leghemoglobin content in any significant manner when compared to the untreated control. From the results it was evident that increasing concentrations of potassium nitrate increased the nodule leghemoglobin content significantly, but the same didn't occur with kinetin and urea, whose increasing concentrations resulted in decreased nodule leghemoglobin content in blackgram at 50% flowering. Among the kinetin treatments, all the adopted concentrations for foliar application (10^{-2}, 10^{-3} and 10^{-4} M) differed significantly from each other. Foliar application of potassium nitrate at 1% recorded significantly higher nodule leghemoglobin content (300.907 µg g^{-1} FWT) than that of potassium nitrate at 0.5%. Urea at 1% showed significantly highest leghemoglobin content (385.641 µg g^{-1} FWT) than all the other treatments including urea at 2% (153.829 µg g^{-1} FWT). It should be pointed out that the leghemoglobin content increased by more than 2-fold between urea @ 20 g L^{-1} and urea @ 10 g L^{-1} at this stage. Lowest leghemoglobin content of nodules was seen with untreated control (63.562 µg g^{-1} FWT). With the foliar application of water as the baseline, since the water is the medium of application for all treatments, lower concentrations of kinetin and urea were more effective in increasing nodular leghemoglobin, while higher concentration was more effective in case of potassium nitrate for the same at 50% flowering stage in blackgram.

Significant differences were observed in the leghemoglobin content of blackgram root nodules with treatments applied at 10 days after flowering (Table 3). Foliar application of water has significantly increased the nodule leghemoglobin content (132.650 µg g^{-1} FWT) when compared to the untreated control (82.502 µg g^{-1} FWT). Among the kinetin treatments, kinetin @ 10^{-2} M (214.701 µg g^{-1} FWT) and kinetin @ 10^{-3} M (169.573 µg g^{-1} FWT) which were on par with each other, showed significantly higher leghemoglobin content than the kinetin @ 10^{-4} M. Foliar application of potassium nitrate at 0.5% 310.427 µg g^{-1} FWT) recorded significantly higher nodule leghemoglobin content than that of potassium nitrate at 1%. Foliar application of urea @ 10 g L^{-1} recorded significantly higher value (459.487 µg g^{-1} FWT) than that of urea at @ 20 g L^{-1}. Among the treatments, highest nodule leghemoglobin content was recorded with urea @ 10 g L^{-1} $^{459.487}$ µg g^{-1} FWT), while the lowest was with untreated control 82.502 µg g^{-1} FWT). In the current experiment with blackgram, higher concentrations of kinetin and lower concentrations of nitrogen sources as foliar treatments resulted in higher nodular leghemoglobin content at 10 days after flowering and the enhancement effect was more with nitrogen sources. It is of interest that with foliar N treatments applied at 10 days after flowering, enhancement effect was more than 2-fold and 3-fold in potassium nitrate and urea, respectively.

Increase in leghemoglobin content of rhizobial root nodules with foliar application of kinetin was

earlier observed in greengram (Dey and Srivastava, 2006) and in chickpea (Jain *et al.*, 2008). Application of kinetin directly to the root system in pea resulted in similar positive effect on nodule leghemoglobin content (Nandwal *et al.*, 1981). Though actual leghemoglobin content was not measured, observations of the increased volume of pink bacteroid tissue with exogenous kinetin were reported in soybean (Ali and Bano, 1999), and in chickpea (Dayal and Bharti, 1991 and Ali and Bano, 2008). These reports support the current results of enhanced nodular leghemoglobin content with kinetin in blackgram. The observations of Jain *et al.* (2008) in chickpea idicated that enhancement effect on leghemoglobin content was higher with lower concentrations of kinetin than those with higher concentrations and agree with the findings in the current experiment with blackgram (10 days before flowering and 50% flowering), though the observations at 10 days after flowering opposed.

In many legumes, nitrate induced reduction in leghemoglobin content of root nodules was recorded. This nitrate induced reduction in leghemoglobin content was attributed to oxidation of the heme Fe, formation of a complex with NO, or degradation of the globin or heme group (Becana *et al.*, 1991., Becana and Klucas, 1992). But, in the current experiment in blackgram with foliar application of potassium nitrate, leghemoglobin content of nodules significantly increased and this increase was more than 2 times with lower concentration (5 g L^{-1}) than the higher concentration (10 g L^{-1}) at 10 days after flowering. Results of promotion of nodular leghemoglobin content were not reported in any legume with foliar nitrate supply, till date.

Aggarwal *et al.*(2015) reported increased root nodule leghaemoglobin content with foliar application of 2% urea in chickpea, and the current results in blackgram agree with their findings. However, they reported the more effectiveness of foliar urea when applied at both vegetative and flower initiation stages, while in the current experiment the results appear to be more effective at later stages of reproductive phase, than earlier stages.

Soluble nitrogen content (mg g^{-1} FWT)

Soluble nitrogen content differed significantly with treatments applied at 10 days before 50% flowering (Table 1). Further the treatments *viz.*, kinetin @ 10^{-3} M, urea @ 10 g L^{-1} and urea @ 20 g L^{-1} were on par with each other with respect to nodule soluble nitrogen content (2.522, 2.242 and 2.242 mg g^{-1} FWT, respectively) at 10 days before 50% flowering, while showing significantly higher values than the untreated control, foliar application of water, kinetin @ 10^{-2} M, kinetin @ 10^{-4} M, potassium nitrate @ 5 g L^{-1} and potassium nitrate @ 10 g L^{-1}. There were no significant differences between both the controls. Among the kinetin treatments, 10^{-3} M recorded significantly higher nodule soluble nitrogen content (2.522 mg g^{-1} FWT) than 10^{-2} M and 10^{-4} M. Though potassium nitrate at 1% showed higher nodule soluble nitrogen content at 10 days before 50% flowering stage, it didn't differ significantly from potassium nitrate at 0.5%. Similar parity was seen between the treatments of urea @ 10 g L^{-1} and 20 g L^{-1}. The results also showed that highest nodule soluble nitrogen content was recorded with kinetin @ 10^{-3} M (2.522 mg g^{-1} FWT), while it was lowest with foliar application of water 1.588 mg g^{-1} FWT). In comparison, kinetin and urea treatments when applied at 10 days before 50% flowering increased nodular soluble nitrogen, while potassium nitrate showed no effect.

Significant differences were observed in soluble nitrogen content of blackgram nodules with the treatments imposed at 50% flowering as seen from

the results presented in the table 2. Foliar application of water, kinetin @ 10^{-4} M and urea @ 10 g L^{-1} were on par with each other with respect to nodule soluble nitrogen content (1.774, 1.681 and 1.868 mg g^{-1} FWT, respectively) at 50% flowering, while showing significantly higher values than the untreated control, kinetin @ 10^{-3} M and urea @ 20 g L^{-1}. Foliar application of water significantly increased the nodule soluble nitrogen content (1.774 mg g^{-1} FWT) compared to untreated control 1.214 mg g^{-1} FWT). Among the kinetin treatments kinetin @ 10^{-2} M recorded significantly higher nodule soluble nitrogen content (2.802 mg g^{-1} FWT) than the kinetin @10^{-3} M and kinetin @ 10^{-4} M. Though potassium nitrate at 1% showed higher nodule soluble nitrogen content at 10 days before flowering stage, it didn't differ significantly from potassium nitrate at 0.5%. Foliar application of urea at 1% recorded significantly higher value (1.868 mg g^{-1} FWT) than that of urea at 2% (1.214 mg g^{-1} FWT). Among the treatments highest nodule soluble nitrogen content was recorded with kinetin @ 10^{-2} M (2.802 mg g^{-1} FWT), while it was lowest with untreated control. When water as medium of application was taken into consideration, kinetin at highest concentration (10^{-2} M) and potassium nitrate at both 0.5 and 1% concentration significantly enhanced the nodule soluble nitrogen, while other treatments had no effect of significance.

Significant differences were observed among the treatments with respect to soluble nitrogen content of blackgram root nodules at 10 days after 50% flowering (Table 3). There were no significant differences in the nodule soluble nitrogen content of the foliar application treatments of water and kinetin @ 10^{-3} M. Kinetin @ 10^{-4} M, potassium nitrate @ 5 g L^{-1} and urea @ 20 g L^{-1} were on par with each other with respect to nodule soluble nitrogen content (1.868, 1.774 and 2.054 mg g^{-1} FWT, respectively) at 10 days after 50% flowering, while showing significantly higher values than the foliar application of water and kinetin @ 10^{-3} M. Among the kinetin treatments, kinetin @ 10^{-4} M recorded significantly higher nodule soluble nitrogen content (1.868 mg g^{-1} FWT) than the kinetin @10^{-2} M and kinetin @ 10^{-3} M. Foliar application of potassium nitrate at 1% (2.428 mg g^{-1} FWT) recorded significantly higher nodule soluble nitrogen content than that of potassium nitrate at 0.5%. Foliar application of urea @ 20 g L^{-1} recorded significantly higher nodular soluble nitrogen (2.054 mg g^{-1} FWT) than that of urea @ 10 g L^{-1}. Among the treatments highest nodule soluble nitrogen content was recorded with potassium nitrate @ 10 g L^{-1} (2.428 mg g^{-1} FWT), while it was lowest with untreated control 0.467 mg g^{-1} FWT). At 10 days after flowering, all the external agents enhanced the soluble nitrogen content of nodules and this effect was more with higher concentration of nitrogen sources particularly potassium nitrate.

The current observations agree with the results reported by Nandwal *et al.* (1981), who reported the increased nitrogen contents of root nodules of pea with kinetin application that was concentration dependent.

As with leghemoglobin and nitrogenase, nitrate studies in legumes were mostly undertaken with supply of nitrate to the root system and it was reported that nitrates caused a decrease in soluble protein of nodules in pea (Escuredo *et al.*, 1996) along with leghemoglobin. In current experiment, potassium nitrate when applied either at 50% flowering or at 10 days after 50% flowering enhanced the nodule soluble nitrogen, while decreasing it at 10 days before 50% flowering in blackgram. These results are first of its kind in blackgram, if not in all the legumes.

The current results also show that in blackgram foliar application of urea significantly increased the nodular soluble nitrogen content during the reproductive stage, though not found to be effective

when applied at 50% flowering. Foliar applied urea is hydrolysed by urease into ammonia, which is assimilated by plant amino acid synthetases and these resultant amino acids will probably be moved into active sinks (Witte *et al.*, 2002). The current results showed that at the adopted times of application in the current experiment with blackgram, nodules belong to the active sinks.

Ascorbate peroxidase activity of nodules (µ mole $min^{-1}g^{-1}$ FWT)

Significant differences were observed in nodule Ascorbate peroxidase activity of blackgram with the treatments imposed at 10 days before 50% flowering (Table 1). Kinetin @ 10^{-2} and 10^{-3} M, potassium nitrate @ 5 and 10 g L^{-1} and urea @ 20 g L^{-1} were on par with each other with respect to nodule APX activity at 10 days before 50% flowering, while showing significantly higher values than the untreated control, foliar application of water and kinetin @ 10^{-4} M. There were no significant differences between both the controls regarding nodule APX activity. Though potassium nitrate at 1% showed higher nodule APX at 10 days before flowering stage, it didn't differ significantly from potassium nitrate at 0.5%. Foliar application of urea at 1% and 2% didn't differ significantly in the nodule APX activity. Increasing concentrations of kinetin increased the nodule ascorbate peroxidase activity compared to controls. In contrast, increasing concentrations of urea decreasesd the nodule APX activity. Among the treatments highest nodule ascorbate peroxidase activity was recorded with urea @ 10 g L^{-1} (17.810 µ mole min^{-1} g^{-1} FWT), while it was lowest with foliar application of water (9.690 µ mole min^{-1} g^{-1} FWT). At 10 days before 50% flowering in blackgram, all external agents applied resulted in increased nodule APX activity, which was comparatively more with nitrogen sources than with kinetin. While all the nitrogen sources as foliar application increased nodule APX significantly, kinetin was significant only at higher concentration of 10^{-2} M.

Significant differences were observed in APX activity of blackgram root nodules with treatments imposed at 50% flowering (Table 2). Untreated control, foliar application of water, potassium nitrate @ 5 and 10 g L^{-1} didn't differ significantly with respect to nodule APX activity (16.595, 15.024, 15.976 and 16.881 µ mole min^{-1} g^{-1} FWT, respectiely) at 50% flowering, while showing significantly higher values than the kinetin @ 10^{-2} M, kinetin @ 10^{-3} M, urea @ 10 g L^{-1} and urea @ 20 g L^{-1} (13.548, 10.524, 11.405 and 10.333 µ mole min^{-1} g^{-1} FWT, respectively). Nodule APX activity didn't differ between both the controls at 50% flowering. Among the kinetin treatments, 10^{-4} M concentration recorded significantly higher nodule APX activity (18.643 µ mole min^{-1} g^{-1} FWT) than @ 10^{-2} and 10^{-3} M concentration. Though potassium nitrate at 1% showed higher nodule soluble nitrogen content at 50% flowering stage, it didn't differ significantly from potassium nitrate at 0.5%. Foliar application of urea at 1% and 2% didn't differ significantly in the nodule APX activity. Among the treatments, highest nodule APX activity was recorded with kinetin @ 10^{-4} M (18.643 µ mole min^{-1} g^{-1} FWT). When the medium of application *i.e.*, water is taken into consideration, foliar urea decreased the nodular APX significantly, while foliar nitrate application didn't affect it in any significant manner. In this respect, the effect of kinetin was without any significance at 10^{-2} M, with significant decrease at 10^{-3} M and with significant increase at 10^{-4} M.

Significant differences were observed among the treatments with respect to APX activity of blackgram nodules at 10 days after 50% flowering (Table 3). Foliar application of water, kinetin @ 10^{-2} and 10^{-3} M, potassium nitrate @ 10 g L^{-1} and urea @ 20 g L^{-1} were on par each other with respect to

Table 1. Biochemical constituents of blackgram nodules with treatments applied at 10 days before 50% flowering.

S. No.	Treatments	Leg-hemoglobin content	Soluble nitrogen content	Ascorbate peroxidase activity
		(µg g^{-1} FWT)	(mg g^{-1} FWT)	(µ mole min^{-1} g^{-1} FWT)
1	Untreated control	145.76	1.681	11
2	Foliar application of water	191.754	1.588	9.69
3	Foliar application of kinetin @ 10^{-2} M	228.067	1.961	14.857
4	Foliar application of kinetin @ 10^{-3} M	292.635	2.522	13.619
5	Foliar application of kinetin @10^{-4} M	243.419	1.868	11.452
6	Foliar application of potassium nitrate @ 10 g L^{-1}	346.7	1.775	15.667
7	Foliar application of potassium nitrate @ 5 g L^{-1}	361.809	1.588	14.81
8	Foliar application of urea @ 10 g L^{-1}	313.162	2.242	17.81
9	Foliar application of urea @ 20 g L^{-1}	293.81	2.242	15.452
	SEm±	22.64	0.16	1.02
	CD (0.05)	67.87	0.47	3.05
	CV (%)	14.6	13.95	12.77

Table 2. Biochemical constituents of blackgram nodules with treatments applied at 50% flowering.

S. No.	Treatments	Leg-hemoglobin content	Soluble nitrogen content	Ascorbate peroxidase activity
		(µg g^{-1} FWT)	(mg g^{-1} FWT)	(µ mole min^{-1} g^{-1} FWT)
1	Untreated control	63.562	1.214	16.595
2	Foliar application of water	72.85	1.774	15.024
3	Foliar application of kinetin @ 10^{-2} M	88.071	2.802	13.548
4	Foliar application of kinetin @ 10^{-3} M	114.872	1.401	10.524
5	Foliar application of kinetin @10^{-4} M	170.94	1.681	18.643
6	Foliar application of potassium nitrate @ 10 g L^{-1}	300.907	2.708	16.881
7	Foliar application of potassium nitrate @ 5 g L^{-1}	242.049	2.335	15.976
8	Foliar application of urea @ 10 g L^{-1}	385.641	1.868	11.405
9	Foliar application of urea @ 20 g L^{-1}	153.829	1.214	10.333
	SEm±	8.473	0.136	1.125
	CD (0.05)	25.402	0.408	3.373
	CV (%)	8.292	12.485	13.603

Table 3. Biochemical constituents of blackgram nodules with treatments applied at 10 days after 50% flowering.

S. No.	Treatments	Leg-hemoglobin content	Soluble nitrogen content	Ascorbate peroxidase activity
		($\mu g\ g^{-1}$ FWT)	($mg\ g^{-1}$ FWT)	(μ mole min^{-1} g^{-1} FWT)
1	Untreated control	82.502	0.467	8.857
2	Foliar application of water	132.65	0.84	10.667
3	Foliar application of kinetin @ 10^{-2} M	214.701	1.307	11.286
4	Foliar application of kinetin @ 10^{-3} M	169.573	1.12	10.952
5	Foliar application of kinetin @10^{-4} M	105.299	1.868	7.69
6	Foliar application of potassium nitrate @ 10 g L^{-1}	151.795	2.428	11.19
7	Foliar application of potassium nitrate @ 5 g L^{-1}	310.427	1.774	17.5
8	Foliar application of urea @ 10 g L^{-1}	459.487	1.407	7.285
9	Foliar application of urea @ 20 g L^{-1}	141.518	2.054	9.833
	SEm±	16.861	0.111	0.534
	CD (0.05)	50.55	0.334	1.601
	CV (%)	14.867	13.117	8.739

nodule APX activity (10.667, 11.286, 10.952, 11.190 and 9.833 µ mole min^{-1} g^{-1} FWT, respectively) at 10 days after flowering, while showing significantly higher values than the untreated control, kinetin @ 10^{-4} M and urea @ 10 g L^{-1}. Foliar application of water significantly increased the nodule APX activity compared to untreated control. Among the kinetin treatments kinetin @10^{-2} M showed significantly higher nodule APX activity (11.286 µ mole min^{-1} g^{-1} FWT) than kinetin @ 10^{-4} M, but on par with kinetin @ 10^{-3} M. Foliar application of potassium nitrate at 0.5% recorded significantly higher nodule APX activity (17.500 µ mole min^{-1} g^{-1} FWT) than at 1%. Foliar application of urea @ 20 g L^{-1} recorded higher nodule APX activity (9.833 µ mole min^{-1} g^{-1} FWT) than @ 10 g L^{-1}. Among the treatments highest nodule APX activity was recorded with potassium nitrate @ 0.5 g L^{-1} (17.500 µ mole min^{-1} g^{-1} FWT). In the current experiment with blackgram, when the adopted treatments were applied at 10 days after 50% flowering, potassium nitrate at lower concentrations significantly increased the nodule APX activity, while urea and kinetin at lower concentrations significantly decreased the same from control.

Increase in nodule APX activity with foliar application of kinetin and potassium nitrate might be due to their possible role in keeping leaves green and thereby the nodules in active state. Active nodules require optimal APX activities for oxidant scavenging as suggested by Swaraj *et al.* (1993). However, studies of this kind with foliar applicants were not found.

The current results were supported by the findings of Dalton *et al.* (1986), who reported the decreased APX activity of nodules in soybean with supply of fixed nitrogen *i.e.*, urea.

CONCLUSION

The present work was conducted to study the impact of foliar applied kinetin and nitrogen

compounds on inhibition of nodule senescence in blackgram *cv* PU-32. Results revealed that higher concentrations of kinetin and lower concentrations of nitrogen sources as foliar treatments resulted in higher nodular leghemoglobin content and the enhancement effect was more with nitrogen sources. With foliar N treatments at 10 days after flowering, enhancement effect was more than 2-fold and 3-fold in potassium nitrate and urea, respectively. Potassium nitrate when applied either at 50% flowering or at 10 days after flowering enhanced the nodule soluble nitrogen, while decreasing the same at 10 days before flowering in blackgram. Foliar application of urea significantly increased the nodular soluble nitrogen content during the reproductive stage, though not found to be effective when applied at 50% flowering. Increasing the concentrations of kinetin increased the nodular APX activity. Foliar application of kinetin @ 10^{-2} M significantly increased the nodular ascorbate peroxidase activity (APX) at 10 days before flowering and 10 days after flowering stages. In blackgram *cv* PU-32, foliar application of kinetin at lower concentration (10^{-4} M) and nitrogen sources (potassium nitrate and urea) at higher concentrations (1 and 2%, respectively) during reproductive stage, effectively decreased the nodule senescence.

LITERATURE CITED

Aggarwal N, Singh G, Ram H, Sharma P and Kaur J 2015 Irrigated chickpea's symbiotic efficiency, growth and productivity as affected by foliar application of urea. *International Journal of Agriculture Sciences*. 7(5): 516-519.

Alesandrini F, Mathis R, Van De Sype G, Herouart D and Puppo A 2003 Possible roles for a cysteine protease and hydrogen peroxide in soybean nodule development and senescence. *New Phytologist*. 158(1): 131-138.

Ali S and Bano A 1999 The role of growth regulators on nodule senescence and nitrogen fixation in soybean. *Pakistan Journal of Biological Sciences*. 2(4): 1305-1306.

Ali S and Bano A 2008 Leaf and nodule senescence in chickpea (*Cicerarietinum* L.) and the role of plant growth regulators. *Pakistan Journal of Botany*. 40(6): 2841-2492.

Appleby C A and Bergersen F L 1980 *Methods for Evaluating Biological Nitrogen Fixation*. John Wiley and Sons, New York. 315.

Becana M and Klucas R V 1992 Transition metals in legume root nodules: Iron-dependent free radical production increases during nodule senescence. *Proceedings of the National Academy of Sciences*. 89(19): 8958-8962.

Becana M, Salin M L, Ji L and Klucas R V 1991 Flavin-mediated reduction of ferric leghaemoglobin from soybean nodules. *Planta*. 183(4): 575-583.

Dalton D A, Sterling A, Russell F J, Hanus G A, Pascoe and Harold J Evans 1986 Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. *Proceedings of the National Academy of Sciences*. 83(11): 3811-3815.

Datanet India 2018 Selected State/Season-wise Area, Production and Productivity of UradinIndia. 21-08-2018. https://www.indiastat.com/table/agriculture/2/urad/19573/1131299/data.aspx

Dayal J and Bharti S 1991 Effects of kinetin, cycocel and colchicine on nitrogenous activity and ATP production in chickpea (*Cicer arietinum* (L.). in *New Trends in Plant*

Physiology. Proceedings of National Symposium on Growth and Differentiation in Plants (Eds.): New Delhi, India. Today and Tomorrow's Printers and Publishers 235-238 ISBN 81: 7019-375-3(India); 1-55528-217-2 (USA).

Dey B and Srivastava R 2006 Effect of kinetin on soluble protein, leghaemoglobin content and nitrogen metabolizing enzymes in *Vignaradiata*. (L.) Wilczek. *Indian Journal of Plant Research*. 19: 53-57.

Escuredo P R, Minchin F R, Gogorcena Y, Iturbe-Ormaetxe I, Klucas R V and Becana M 1996 Involvement of activated oxygen in nitrate-induced senescence of pea root nodules. *Plant Physiology*. 110(4): 1187-1195.

Jain R K, Jain A K and Gera V K 2008 Effect of growth regulators on leghaemoglobin biosynthesis in chickpea nodules. *Legume Research*. 31(4): 303-305.

Lawn R J and Brun W A 1974 Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulations. *Crop Science*. 14: 11–16.

Marquez-Garcia B, Shaw D, Cooper J M, Karpinska B, Quain M D, Makgopa E M, Kunert K and Foyer C H 2015 Redox markers for drought-induced nodule senescence, a process occurring after drought-induced senescence of the lowest leaves in soybean (*Glycine max*). *Annals of Botany*. 116(4): 497-510.

Nakano, Y and Asada, K 1981 Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22(5): 867-880.

Nandwal A S, Bharti S, Garg O P and Ram P C 1981 Effects of indole acetic acid and kinetin on nodulation and nitrogen fixation in pea (*Pisumsativum* L.). *Indian Journal of Plant Physiology*. 24: 47-52.

Puppo A, Groten K, Bastian F, Carzaniga R, Soussi M, Lucas M M, De Felipe M R, Harrison J, Vanacker H and Foyer C H 2005 Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural aging process. *New Phytologist*. 165(3): 683-701.

Rahman M A, Hoque A K M S, Rahman M N, Talukdar M B and Islam M S 2007 Effect of nitrogen and phosphorus on the growth and yield of french bean. *Journal of Soil and Nature*. 1(2): 30-34.

Swaraj K, Laura J S and Bishnoi N R 1993 Nitrate induced nodule senescence and changes in activities of enzymes scavenging H_2O_2 in clusterbean (*Cyamopsiste tragonaloba* Taub.). *Journal of Plant Physiology*. 141(2): 202-205.

Thimmaiah, S K 1999 *Standard methods of biochemical analysis.* Kalyani publishers. New Delhi. 82-85.

Thomas H, Ougham H J, Wagstaff C and Stead A D 2003 Defining senescence and death. *Journal of Experimental Botany*. 54(385): 1127-1132.

Witte C P, Tiller S A, Taylor M A and Davies H V 2002 Leaf urea metabolism in potato. Urease activity profile and patterns of recovery and distribution of ^{15}N after foliar urea application in wild-type and urease-antisense transgenics. *Plant Physiology*. 128(3): 1129-1136.

Received on 13.12.2020 and revised on 20.03.2021