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THE ROLE OF MOLYBDENUM ON GROWTH AND NODULATION WITH COMBINATION OF *RHIZOBIUM* IN *VIGNA RADIATA* (L.)

LALITA, ASHOK KUMAR AND SHALU MALIK
Department of Botany, C.C.S. University, Meerut-250004, India

The applications of molybdenum by combination of *Rhizobium* were studied on the growth of *Vigna radiata* (L). The Vigna seeds were grown in different concentrations (0, 3, 6 and 9ppm) of molybdenum in natural conditions. The results showed that molybdenum was able to increase the root, shoot fresh and dry biomass, nodule number and protein content gradually increase from lower to higher concentration after molybdenum treatment with rhizobium.

Keywords: *Rhizobium*, Growth, *Vigna radiata* (L.)

Since the past few decades green revolution has improved the productivity of daily used food crops. Foods that are rich in protein and reasonable in price are a demand for the poor, living in developing countries. In this regard, pulses were found more versatile and appealing in providing protein rich diets, long time storage and low prices. The exclusive and usual feature of *Vigna radiata* (L.) is the root nodules that contain aerobic bacteria grouped as rhizobia which fix atmospheric nitrogen in the root and thus raise soil fertility (Ashraf *et al.* 2003). *Vigna radiata* (L.) is one of the most significant conventional protein rich pulse crops grown in India. Normally pulses are grown in soils with low fertility or with applications of low quantities of organic and inorganic plant nutrients, which has resulted in decay of soil health and poor crop yield (Kumpawat 2010). The low production of *Vigna radiata* (L.) may be due to nutritional deficiency in soil and imbalanced external fertilization besides waterlogging (Pramod Kumar *et al.* 2013). The productivity of *Vigna radiata* (L.) can be enhances by inoculation with Rhizobium culture (Awomi *et al.* 2012). Molybdenum is a trace element necessary for growth of nearly all biological organisms including plants (Graham and Stangoulis 2005). Molybdenum is a constituent of enzyme nitrogenase and nitrate reductase, which regulate the reduction of inorganic nitrate and help in fixing N_2 to NH_3 (Hale *et al.* 2001). Thus, molybdenum is an essential component to nitrogen fixation by legumes. Use of molybdenum promoted nodulation and biological nitrogen fixation, thus increasing the legume yield (Brikics *et al.* 2004). Molybdenum is also crucial for absorption and translocation of iron in plants (Awomi *et al.* 2012). So, it is required to examine the effects of different concentrations of molybdenum and assess their best combination in terms of enhancing N fixation and productivity of *Vigna radiata* (L.).

MATERIALS AND METHODS

A field experiment was conducted in the field of Department of Botany C.C.S. University Campus Meerut in U.P. India during the summer season of 2014. To determine the effect of Rhizobium and molybdenum on *Vigna radiata* (L.), the studies were carried out in natural conditions. Healthy seeds of Vigna radiata (L.) were selected and washed with distilled water. Molybdenum trioxide (MoO_3) solution of 3, 6 and 9ppm were prepared in distilled water, and tap water was used as a control. Experimental field designed in five plots of equal size(1x1m), four plots for treatment and one plot for control. The treatments comprised of *Rhizobium* alone in one plot and three levels of Molybdenum (0, 3, 6 and 9ppm) along with common application of *Rhizobium* (0.31 gm./plot in powder form with 1 ml sugar sticker)in three plots. *Rhizobium* culture in powder form was procured from IARI, New Delhi.

Three treatment of molybdenum (3, 6 and 9ppm) as molybdenum trioxide and *Rhizobium*

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were applied in the plots one day before sowing on moist soil. Fifty healthy seeds of *Vigna radiata* (L.) were sown in each plot. The seed germination percentage was calculated after counting the difference between germinated (coming out of the soil) and non-germinated seeds (remaining inside, non emergent). Five plants were selected randomly from each plot and tagged for recording plant height, fresh weight/ plant, dry weight/ plant, number of nodules/ plant. Nodules were detached from the plant roots with the help of forceps. Fresh weight of nodules was measured immediately and followed by dry weight after drying them at 60ºC for 24 h to obtain dry weight. Bradford (1976) method was used to determine the total protein content of nodules. The proline content of fresh leaves was estimated as devised by Sadasivam and Manickam (1996).

RESULT AND DISCUSSION

Seed germination

The maximum seed germination was observed at 6ppm Mo+Rhizobium application (Table-1). Higher concentrations of molybdenum decreased the seed germination. Molybdenum is an essential minor chemical element that is required at a minimum level by plants for metabolism (Kaiser *et al*. 2005, Mendel 2005, 2007). As a heavy metal, high concentrations will damage plant cells. Seed germination is the beginning of a physiological process which needs water imbibition (Wierzbicka and Obidzinska1988). Seed germination of the crop occurred normally but the toxic effect was more pronounced in the roots, may be due to the seed coat, which can act as protector for the embryo but cannot completely guard the whole seed. Early seed germination may be possible with early reserve breakdown and mobilization causing metabolic repair of damage during the treatment and that change in germination events, that is, possible early activation or de novo synthesis of cell wall degrading enzymes (Hisashi and Maciaa 2005).

Root and shoot length

Plant height was affected by the application of molybdenum and Rhizobium applications. The maximum plant height was obtained in case where 9ppm Mo+Rhizobium were used as treatment and minimum plant height was achieved in control (Table-2). All the growth parameters increased with increasing levels of molybdenum. Molybdenum at 10 kg/ha increased the vegetative growth of blackgram compared to control (Singh et al. 2006). Application of molybdenum produced the tallest plants with number of nodules and dry weight per plant (Awomi *et al*. 2012). Leguminous plants are very sensitive to molybdenum deficiency, but excess also impair growth, decrease the biomass and deteriorates the quality of yield (Nautiyal and Chatterjee 2004). During different growth processes in legumes molybdenum is involve in a number of different enzymatic processes (Vieira *et al*. 1998). For example, molybdenum is constitutes of nitrogenase enzyme and Rhizobium bacterium also needs molybdenum during the nitrogen fixation process (Vieira *et al*. 1998). Therefore molybdenum has positive effect on growth, productivity and nitrogen content of root and nodule forming in legume crops (Togay *et al*. 2008). This could be attributed to increase the metabolic pools required for the synthesis of saccharides along with the enhanced photosynthetic apparatus (Abd El-Samad *et al*. 2005). With regard to other aspects of plant nutrition, molybdenum enzymes are involved in nitrogen metabolism and in improving quality of ascorbic acid and soluble sugar (Chen and Nian 2004).

Fresh and Dry matter Production

The effect of molybdenum and Rhizobium inoculant on root and shoot fresh and dry weight of *Vigna radiata* (L) was observed (Table 3). The highest root and shoot fresh and dry weight were recorded in 9ppm Mo+Rhizobium, which was higher than other treatments. The lowest fresh and dry weight of root and shoot was recorded in control. Rhizobium inoculation increased fresh and dry weight of root and shoot over control. Rhizobium inoculation significantly increased shoot dry weight of chickpea compared to control (Eusuf Zai *et al*.1999). Molybdenum is an indispensable element in the process of nitrogen fixation and plant metabolism, because of its effect on the enzyme nitrogenase and nitrate reductase activities (Pollock *et al.* 2002). Molybdenum application affects positively on wheat growth and total N-yield in non-inoculated or inoculated plants with *Azospirillum*. The stimulatory effect of molybdenum application was associated with the increased Mg++ content, which was accomplished with significant elevation of nitrate reductase activity (El-Samad *et al*. 2005). Mo might have enhanced the activity of enzyme nitrogenase, thereby increasing the N supply to plants through the process of biological N fixation, resulting in better growth and increased yield (Biswas *et al*. 2009).

Nodulation

There was highly significant nodulation response of *Vigna radiata* (L.) plants treated with molybdenum and *Rhizobium*. Maximum number of nodules per plant (25.6) was found in case where 6ppm Mo+Rhizobiumwas used (Table 4). Above this level the number of nodule remarkably decreased. Minimum number of nodules per plant (22) was found in control. There was a constructive increase in number of nodules per plant with the increase in the molybdenum level and seed inoculation with *Rhizobium* because of the synergistic effect of both on nodule formation (Hale et al.2001). Mo+*Rhizobium* treated plants at 6ppm concentration show greater increase in values of nodules fresh and dry weight of nodules per plant as compared to other treatments and control (Table 4). Molybdenum is a important component of *Rhizobium*-legume symbiosis and has a key role in nitrogenase enzyme complex catalysing the N-fixation (Marschner 1995). The increase in concentration may be related to early nodulation, which can encourage root development. Molybdenum helps root nodules bacteria to fix atmospheric nitrogen (Compo *et al*. 2000). Molybdenum application can play a vital role in increasing the nitrogen fixation and gave better nodulation in mungbean (Bhuiyan *et al*. 2008). Molybdenum highly significantly affected the number of nodules/ plant and enhanced the number of nodules in chickpea observed as the formation of nodules increase the nitrogen content of plant linearly increased (Khan *et al*. 2014).

Protein content

The minimum protein contents were found in control (Table 5). The protein content increased with the increasing concentration of molybdenum. Molybdenum treatment increased the protein contents might be due to more nitrogen availability to nodules (Liu 2002). Molybdenum application along with seed inoculation increased the nitrogen and phosphorus availability which improves the protein synthesis because it is an essential part of phosphorus containing amino acids (Tahir *et al.* 2011). Where the plant has insufficient molybdenum the nitrates accumulate in the leaves and the plant cannot use them to make proteins. The result is that the plant becomes stunted, with symptoms similar to those of nitrogen deficiency. Molybdenum enhanced absorption of a large amount of K+ from the soil to the root. This could be linked to the higher accumulation of proteins which can enhances the root fresh and dry weights (El-samad *et al*. 2005).

Proline content

Proline, an amino acid, is essential for primary metabolism (Tan *et al*. 2011). Higher plants which are tolerant to water deficits and salinity stress usually accumulate higher levels of proline to cope with the harsh environments (Dinakar *et al*. 2009). The proline content was increase with the increasing concentration of molybdenum (Table 5). The *Rhizobium* treated plant decrease the proline content as compared to molybdenum treated plant. In control plant proline content was found maximum. Proline acts as a signaling molecule and can help plants in recovering from stress conditions (Szabados and Savoure 2009). More proline is produced in plant tissues encountering heavy metal stress to maintain the plant's ability to tolerate stress conditions (Nazarbeygi *et al*. 2011). Rapid catabolism of proline can provide relief to

Table 1:Seed germination percentage of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of molybdenum.

Treatment	5 DOS	10 DOS	15 DOS
Control	66	68	68
Rhizobium	64	70	72
Rhizobium+3ppm Mo	76	78	78
Rhizobium+6ppm Mo	82	86	86
Rhizobium+9ppm Mo	80	82	82

Table 2: Root length and shoot length of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of molybdenum

Treatment	Plant height (cm)	Root length (cm)	Shoot length (cm)
Control	47.6	7.6	40
Rhizobium	49.4	8.2	41.2
Rhizobium+3ppm Mo	57.6	9.2	48.4
Rhizobium+6ppm Mo	50.6	9.5	51
Rhizobium+9ppm Mo	64	9.7	54.4

Table 3:Root and shoot fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of molybdenum(5 replicates/treatment).

Treatment	Root		Shoot	
	Fresh wt. (gm)	Dry wt. (gm)	Fresh wt. (gm)	Dry wt. (gm)
Control	0.7556	0.335	10.7228	2.3594
Rhizobium	0.7702	0.2628	10.9088	2.6432
Rhizobium+3ppm Mo	0.8016	0.2728	13.8852	3.4942
Rhizobium+6ppm Mo	0.8134	0.2814	17.8754	4.3284
Rhizobium+9ppm Mo	0.96	0.2844	19.6438	4.4298

Table 4: Nodule number, volume, fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of molybdenum.

Treatment	Nodule number	Volume	Fresh wt. (gm)	Dry wt. (gm)
Control	22	1.3	0.0818	0.0122
Rhizobium	25.2	1.4	0.2014	0.01608
Rhizobium+3ppm Mo	23.4	1.7	0.1384	0.0252
Rhizobium+6ppm Mo	25.6	2.9	0.2064	0.0256
Rhizobium+9ppm Mo	23	0.9	0.0412	0.0174

Table 5: Protein and proline content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of molybdenum.

Treatment	Protein mg/ g fresh wt.	Proline µM/g tissue
Control	2.8929	0.009538
Rhizobium	3.3067	0.002008
Rhizobium+3ppm Mo	3.5257	0.002551
Rhizobium+6ppm Mo	3.6004	0.003511
Rhizobium+9ppm Mo	3.6659	0.006526

stress which may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress-induced damages (Hare *et al*.1998). Moreover, it can protect enzymes by scavenging reactive oxygen species (ROS) (Gajewska and Sklodowska 2005).

Conclusion

Molybdenum plays a vital role in increasing the nitrogen fixation process with *Rhizobium* and is also responsible for the formation of nodular tissues and enhancing the N_2 fixation. The results of this study showed that, the addition of molybdenum led to increase in *Vigna radiata* (L.) growth and nodulation. Molybdenum increased plant height, fresh and dry weight of shoot and root and protein content of crop.

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