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Response of chickpea (*Cicer arietinum*) to molybdenum in moderately-alkaline soil

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ABSTRACT

A pot experiment was conducted at ICAR-Indian Institute of Pulses Research, Kanpur during 2010–12 to assess the response of chickpea crop to molybdenum (Mo) in moderately-alkaline Inceptisol (pH 8.0-8.1). Soil application of Mo at 1 kg/ha increased the grain yield by 7.8–11.9% ($P< 0.05$). However, the Mo seed treatment (4 g/kg seed) had a marginal and mostly non-significant effect on growth and yield attributes of chickpea. The higher aboveground dry matter (10.0–19.3%), root weight (11.6–12.5%), nodule weight (7.1–12.1%), and pod number per plant (11.8–22.0%) were observed with soil application of Mo over control treatment. Notably, a negative interaction ($P<0.05$) between phosphorus and Mo was noticed for aboveground growth of chickpea.Thus, Mo was observed as a limiting nutrient for chickpea in moderately-alkaline soil and application of Mo at 1 kg/ha to soil may be recommended to harvest the potential productivity of chickpea.

Key words: Molybdenum, Nodulation, Phosphorus, Phosphate solubilising bacteria, Seed treatment

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop globally, grown in 14.6 mha area with average productivity of 1.01 t/ha (FAOSTAT 2017). Chickpea is extensively grown in rainfed areas (Maqbool *et al.* 2015) and nutrient deficiency is widespread in these areas (Venkatesh *et al.* 2013). Micronutrient deficiency causes significant yield loss in chickpea and application of deficient micronutrient are often recommended to maintain the desired yield level (Montenegro *et al.* 2010). Among the micronutrients, Mo deficiency affects crop growth and yield of grain legumes including chickpea (Ahlawat *et al.* 2007). Given the key constituent of nitrate-reductase, nitrogenase, xanthine-reductase, and SO_3-oxidase enzymes, Mo is involved in important functions like nitrogen metabolism, nitrogen-fixation, and transportation of sulphur-containing amino acids in legumes (Togay *et al.* 2008). Particular to chickpea, Mo deficiency causes deep chlorosis of old leaflets (Nautiyal and Chatterjee 2004), abnormality in the reproductive physiology like reduction in flower number and size, and many flowers fail to open or to mature (Ahlawat *et al.* 2007). The higher response of legumes to Mo application has been observed under well-drained and leached soils (Chakraborty 2009). In low *p*H soils, Mo is strongly bound in metal oxides and with *p*H increase MoO_4^- availability in soil increases (Bambara and Ndakidemi 2010). Additionally, several other factors influence the availability of Mo in soils, for instance, interaction with other nutrients, e.g. phosphorus (Dutta *et al.* 2011), soil moisture and microorganisms, and soil organic matter content (Jiang *et al.* 2015).

The studies on Mo nutrition are confined to acid to neutral soils only, and studies on crop response to Mo in alkaline soils are lacking. The possible influence of acid-forming amendments like phosphate solublizing bacteria (PSB) and fertilizer phosphorus on Mo nutrition in alkaline soil is still uncertain. A pot experiment was conducted to assess the response of chickpea to different methods of Mo application in moderately-alkaline soil. The objectives of the study were to assess the response of chickpea to different methods of Mo application in moderately-alkaline soil and envisage the relative efficacy of different Mo treatments with PSB and fertilizer phosphorus application.

MATERIALS AND METHODS

A pot experiment was conducted during the winter season of 2010–11 and 2011–12 in the nethouse of ICAR-Indian Institute of Pulses Research (ICAR-IIPR), Kanpur (26°27'N, 80°14'E and 152.4 m amsl) to study the effect of Mo application in chickpea. For the study, cultivated soil was collected from the main farm of the institute which had

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not been fertilized and in each pot (top diameter 21 cm, bottom diameter 15 cm, depth 30 cm) was filled with 10 kg soil. The experiment was conducted under natural condition. Chickpea cultivar DCP 92-3 was used for the study. The experimental soil of the first year (2010-2011) had *p*H 8.1, soil organic carbon 2.9 g/kg, and soil available nitrogen, phosphorus, and potassium were 183, 12.0, and 196 kg/ha, respectively. In the second year experiment (2011-2012), the soil had *p*H 8.0, soil organic carbon 3.1 g/kg, and 193, 11.7, and 207 kg/ha available nitrogen, phosphorus, and potassium, respectively.

The treatments comprised three levels of Mo treatment [without Mo application (control), soil application of Mo at 1 kg/ha and Mo seed treatment at 4 g/kg seed], two levels of fertilizer phosphorus rate (no application, P_2O_5 at 40 kg/ha) and two levels of phosphate solubilising bacteria (PSB) treatment (non-inoculated; and with inoculation of PSB). The sources of fertilizer Mo and phosphorus were ammonium molybdate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$ and diammonium phosphate $[(NH_4)_2HPO_4]$, respectively. The *Bacillus polymyxa* as PSB was applied at 20 g/kg seed. The pots were arranged in a factorial completely randomized design with six replications. All pots were fertilized with the basal dose of 20 kg nitrogen, and 40 kg K_2O per ha in

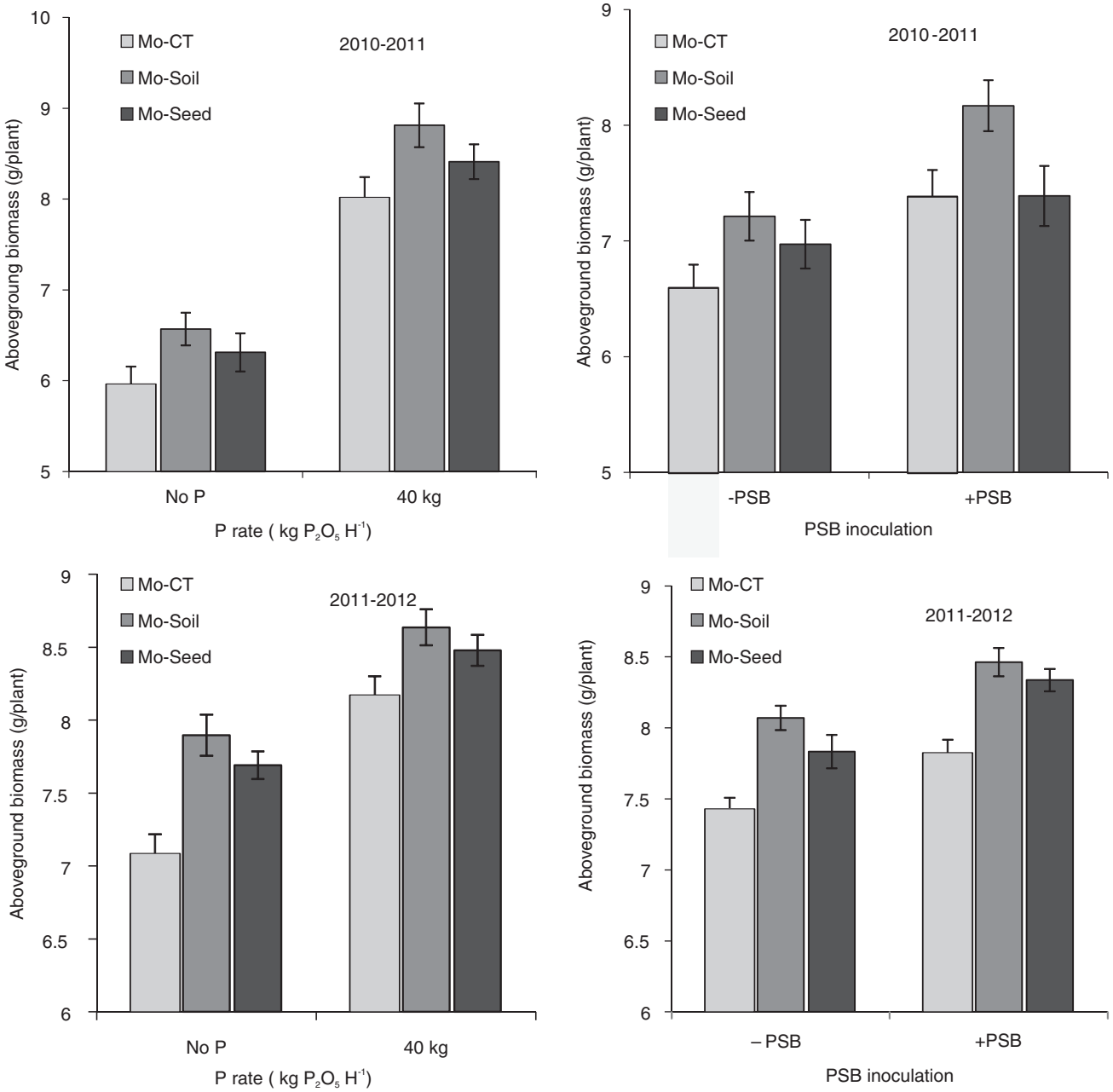

Fig 1 Effect of Mo treatments on aboveground dry biomass (g/plant) accumulation in chickpea at maturity under variable P rate and PSB inoculation level. Mo-CT: no Mo application (Mo control), Mo-Soil: Soil application of Mo at 1 kg/ha, Mo-Seed: Seed treatment of Mo at 4g/kg seed. -PSB: without PSB inoculation, +PSB: with PSB inoculation. The error bar represents the standard error of mean.

Table 1 Above- and belowground growth attributes of chickpea as influenced by different levels of phosphorus, molybdenum, and PSB inoculation during 2010-2011 and 2011-2012

Treatment	2010-2011								2011-2012							
	PH	NB	AGDW			NDW	RL	RW	PH	NB	AGDW			NDW	RL	RW
			60 DAS	90 DAS	Maturity						60 DAS	90 DAS	Maturity			
Fertilizer P rate (P)																
No P application	28.2	2.56	0.32	0.65	4.83	131	39.8	0.87	30.8	2.72	0.57	1.01	7.56	163	38.9	0.93
Fertilizer P (P_2O_5 at 40 kg/ha)	31.1	2.96	0.45	0.74	6.47	151	44.5	1.15	32.3	3.23	0.79	1.36	8.43	189	44.5	1.28
LSD (P = 0.05)	1.1	0.18	0.03	0.03	0.27	4.1	0.78	0.02	0.3	0.08	0.11	0.17	0.36	6.2	1.6	0.09
Molybdenum application (Mo)																
No Mo application	28.8	2.63	0.37	0.68	5.38	133	40.3	0.95	31.0	2.80	0.59	1.08	7.63	168	39.3	1.04
Soil application of Mo (1 kg/ha)	31.0	2.94	0.39	0.71	5.92	150	43.5	1.06	32.1	3.14	0.76	1.31	8.27	180	43.7	1.17
Mo Seed treatment (4 g/kg)	29.1	2.71	0.39	0.70	5.66	140	42.6	1.03	31.5	2.98	0.70	1.16	8.09	180	42.1	1.10
LSD (P = 0.05)	1.3	0.22	ns	ns	0.34	5.3	0.95	0.02	0.3	0.10	0.16	0.22	0.52	7.7	1.9	0.12
PSB inoculation (PSB)																
No inoculation	28.7	2.46	0.37	0.67	5.33	139	41.1	0.93	31.2	2.85	0.66	1.15	7.78	172	40.8	1.08
With inoculation	30.6	3.06	0.40	0.71	5.97	143	43.1	1.09	31.9	3.10	0.71	1.22	8.21	180	42.6	1.13
LSD (P = 0.05)	1.1	0.18	0.03	0.03	0.27	ns	0.78	0.02	0.3	0.08	ns	ns	0.36	6.2	1.6	0.09
Interactions																
P × Mo	ns	ns	ns	ns	*	ns	ns	**	ns	ns	*	*	*	ns	ns	*
P × PSB	ns	ns	ns	ns	**	ns	*	*	ns	ns	ns	ns	*	ns	ns	ns
Mo × PSB	ns	ns	ns	**	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P × Mo × PSB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

PH, Plant height at harvest (cm); NB, number of branches/plant; AGDW, above ground plant dry matter (g/plant); NDW, nodule dry weight/plant at 60 DAS (mg/plant); RL, root length (cm); RW, root dry weight (g/plant); ns, non-significant, * significant at P<0.05, ** significant at P<0.01.

the form of urea and muriate of potash, respectively. The nitrogen rate applied through urea was adjusted considering the nitrogen added through diammonium phosphate. Irrespective of treatment, to ensure optimum biological nitrogen-fixation, chickpea seeds were inoculated with *Rhizobium*. Five chickpea seeds were sown in each pot and three healthy seedlings were retained after emergence for plant biometric observations. The soil moisture in the pots was maintained close to field capacity (~ 12% w/w) by watering at regular intervals.

Periodical growth observations and plant dry weight were recorded at 60 and 90 days after sowing (DAS) and at maturity (135 days) by destructive plant sampling. For periodic growth observations (i.e. 60, 90, and 135 DAS), plants from one single pot of each treatment were used. This way, three replications (or pot represents a replication) were used for destructive plant sampling and remaining three replications were finally used to estimate the grain yield and yield-related attributes at harvest. For periodical growth observations, plants from one replication (one pot) were used for measurement of above and below ground biomass, root length, nodule weight at each sampling stages and single plant from each pot were designated as biological replication for statistical analysis of data. The plant samples were oven-dried at 65ºC for 48 h for estimation of dry weight. The observation on plant growth and yield attributes was recorded as per the standard methodology.

All data were statistically analyzed following ANOVA procedure using the online statistical program OPSTAT (Sheoran *et al.* 1998). Main effects of all the factors and their interactions were assessed following the principle of F statistics, and the mean of treatments was compared by the LSD values at P=0.05. The correlation values were determined using MS Excel 2007.

RESULTS AND DISCUSSION

Crop growth: In both the years, Mo application significantly (P<0.05) increased chickpea growth, and the effect of Mo was more prominent at the late growth stages, i.e. at 90 DAS, and harvest (Table 1). Among the Mo treatments, soil application of Mo at 1 kg/ha had the most prominent and significant (P<0.05) influence on all the growth and yield attributes of chickpea (Table 1). Molybdenum application increased the root weight (11.6–12.5%), root length (7.9–11.2%), number of branches (11.8–12.1%), and nodule dry weight (7.1–12.8%) over Mo control treatment. Significant P × Mo interaction (P<0.05) was observed for root weight and dry matter accumulation at crop maturity. Fig 1 shows that the effect of Mo is reduced with fertilizer P application.

Both the fertilizer P application and PSB inoculation treatments increased the crop growth and yield parameters of chickpea. Fertilizer P application (P_2O_5 at 40 kg/ha) increased the plant height (4.99-10.3%), number of branches/plant (15.6-18.8%), root length (11.8-14.4%), root biomass (32.1-37.6%) over no P application. Fertilizer P, Mo, and

Table 2 Grain yield and yield attributes of chickpea as influenced by different levels of phosphorus, molybdenum, and PSB inoculation during 2010-2011 and 2011-2012

Treatment	2010-2011				2011-2012			
	PPP	GPP	GY	HI	PPP	TGW	GY	HI
Fertilizer P rate (P)								
No P application	9.8	1.78	2.29	47.4	18.7	145.8	3.57	47.3
Fertilizer P (P_2O_5 at 40 kg/ha)	16.6	1.82	2.89	44.7	23.5	157.3	4.12	48.8
LSD (P = 0.05)	0.29	0.04	0.07	0.45	0.61	2.9	0.12	ns
Molybdenum application (Mo)								
No Mo application	11.7	1.78	2.44	45.4	20.0	148.4	3.70	48.5
Soil application of Mo (1 kg/ha)	14.3	1.84	2.73	46.1	22.4	155.1	3.99	48.3
Mo seed treatment (4 g/kg)	13.6	1.79	2.60	45.9	20.9	151.1	3.84	47.5
LSD (P = 0.05)	0.35	0.05	0.13	ns	0.75	3.5	0.18	ns
PSB inoculation (PSB)								
No inoculation	11.4	1.78	2.44	45.8	20.3	148.9	3.73	48.0
With inoculation	15.0	1.83	2.74	45.9	21.9	154.2	3.95	48.2
LSD (P = 0.05)	0.29	0.04	0.07	ns	0.61	2.9	0.12	ns
Interactions								
P × Mo	ns	ns	ns	ns	*	ns	*	ns
P × PSB	*	**	*	ns	ns	ns	*	ns
Mo × PSB	ns	ns	ns	ns	ns	ns	ns	ns
P × Mo × PSB	ns	**	ns	ns	ns	ns	ns	ns

PPP, Number of pods/plant; GPP, grains/pod; TGW, thousand grain weight (g); GY, grain yield (g/plant); HI, harvest index (%); ns, non-significant, * significant at P<0.05, ** significant at P<0.01.

PSB inoculation increased the nodule dry weight (Table 1) and the scale of increase in nodulation was found in the order of fertilizer P>Mo>PSB. Notably, the effect of fertilizer P and Mo application on chickpea nodulation was registered up to 15.6% and 9.9%, respectively.

Grain yield, yield attributes and correlations: The effect of Mo application was also prominent on yield attributes and grain yield of chickpea (Table 2). Molybdenum application at 1 kg/ha increased number of pods/plant (11.9–22.0%, P<0.05) over Mo control treatment. Likewise, the higher grains/pod were observed with soil application of Mo for the study year 2010–11. The effect of Mo application was also apparent on chickpea seed weight. The effect of Mo seed treatment had a marginal effect on yield attributes of chickpea and mostly had non-significant effect when compared with Mo control. The increased growth and yield attributes with soil application of Mo at 1 kg/ha resulted in 7.8–11.9% higher grain yield (grain weight/plant) over Mo control. The interactions fertilizer P × PSB was significant for grain yield in both the study year. The fertilizer P × Mo interaction was observed above the significant level for grain yield during 2011–12 only. Significant correlations between grain yield and growth and yield attributes of chickpea were also evident. In both the years, chickpea grain yield had significant correlation (P<0.05) with nodule dry weight, root length, root weight, pods/plant, grains/pod (Table 3).

Table 3 Pearson's correlation coefficient (r) between grain yield and plant growth and yield attributes in chickpea

Year	Parameter	Variable	Correlation coefficient (r)	p value
2010-11	Grain yield	Plant height	0.57	0.108
		Branches/plant	0.49	0.181
		Aboveground dry matter	0.76	0.018
		Nodule dry weight/plant	0.74	0.023
		Root length	0.93	0.000
		Root weight	0.88	0.002
		Pods/plant	0.81	0.008
		Grain weight/ pod	0.64	0.063
2010-11	Grain yield	Plant height	0.71	0.034
		Branches/plant	0.74	0.021
		Aboveground dry matter	0.96	0.000
		Nodule dry weight/plant	0.71	0.032
		Root length	0.69	0.040
		Root weight	0.67	0.048
		Pods/plant	0.91	0.001
		Thousand grain weight	0.62	0.075

Our results demonstrate that Mo may be a limiting plant nutrient for chickpea crop in moderately-alkaline soils (*p*H 8.0-8.1). In this study, no clear deficiency symptoms of Mo were observed during crop growth stages and the crop responded significantly to Mo application. This implies that the chickpea crop has a hidden hunger for Mo. Molybdenum has several important functions in legumes particularly in the biological nitrogen fixation, and thus reduced the accessibility of Mo may induce N deficiency, thereby limits the plant growth (Shil *et al.* 2007). Significant improvement in nodule dry weight was observed with soil application of Mo at 1 kg/ha that thus signifies the role of Mo in nodule development and biological nitrogen fixation. Our finding suggests that the efficacy of Mo seed treatment is marginal in alkaline soil; and the possible reason may be the low rate of Mo application (4 g/kg seed) as compared to soil application, where a higher rate of Mo (1 kg/ha) was added to the soil. On the contrary, a much stronger response of Mo seed treatment in soybean was reported from strongly acidic Brazilian soils (*p*H 4.64-5.20) (Campo *et al.* 2009). Overall the effect of Mo was not very strong in the study in alkaline soil, which indicates that native Mo and the soil availability of Mo might have good but not optimum for grain legumes like chickpea. The higher growth (above- and belowground biomass) of chickpea plant with soil application may be associated with higher biological N fixation resulting in improved nitrogen nutrition to the plant that in turn increased the shoot and root biomass accumulation (Roy *et al.* 2006). In both the years, the higher pods/plant with Mo application indicates that Mo might have an important role in seed setting as likewise mentioned by Ahlawat *et al.* (2007).

The grain legumes including chickpea release organic acids that lower the *p*H in rhizosphere being higher in alkaline soils (Hazra *et al.* 2018). Additionally, in the process of biological N fixation, grain legumes release protons (H^+) into the rhizosphere. As a result, the rhizospheric soil of legumes is more acidic than the bulk soil. In some cases, one unit drop of *p*H value in legume rhizosphere has also been reported. This may be the primary reason for low Mo availability in the rhizosphere and often an external application of Mo has shown good response on legumes even in alkaline soils. Results further demonstrate that both PSB and fertilizer phosphorus influenced Mo nutrition in chickpea. Notably, the relative response of chickpea crop to Mo was found higher when PSB and Mo were applied together (Fig 1). In both the years, the effect of Mo was lower on plant growth with application of P fertilizers. Our results were consistent with earlier findings of negative interaction of Mo × fertilizer P. Fundamentally, H_2PO_4 and MoO_4 ions the soil available forms of P and Mo, respectively both compete for the same soil exchangeable sites (Hodges 2010). In moderately-alkaline soils, the availability of P is also limited due to high rate of P sorption (Venkatesh *et al.* 2019a, Venkatesh *et al.* 2019b) and thus a higher response of fertilizer P was observed in the study.

Thus it is concluded that even in alkaline soil Mo application had a significant influence on chickpea crop

growth and development. Molybdenum application at 1 kg/ha improves the aboveground and belowground biomass accumulation, nodulation, and also pod setting. However, the effect of Mo seed treatment on chickpea growth and yield attributes was marginal. A detailed investigation is warranted on Mo availability in soil and its acquisition pattern in grain legumes as the element is identified as limiting in moderately-alkaline soil.

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