Molybdenum Response of Chickpea in the High Barind Tract (HBT) of Bangladesh and in Eastern India

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Abstract

Chickpea (Cicer arietinum L.) grown on residual soil moisture after the rice harvest is a promising crop for the High Barind Tract (HBT) of Bangladesh, an uplifted, slightly undulating area in northwestern Bangladesh where the soils have an acid surface horizon (pH 4.5-5.5 at 0–10 cm) but are neutral to alkaline with depth (pH >6 below 20 cm). Major constraints to chickpea cultivation are initial and terminal drought stress, pod borer (Helicoverpa armigera), and nutrient deficiency. Application of phosphorous (P) fertiliser can increase chickpea yields, but even when this is done symptoms of reddening, yellowing, and necrosis of older leaves are widespread across the HBT. Nodulation is generally sparse but responses to Rhizobium inoculation have been inconsistent. To determine which elements could be limiting to chickpea, a small-plot field experiment was conducted in the southern HBT in the 2001/02 season. A subtractive design was used in which the absence of either sulphur (S), boron (B), zinc (Zn), or molybdenum (Mo) was compared to a complete nutrient control. Only Mo was found to be limiting, giving a grain yield response of 73%.

In 2002/03, multilocational trials in farmers' fields were conducted to test the effect of soil application with 500g Mo ha^{-1} and Rhizobium inoculation. Despite frequent rain during the reproductive phase, causing unprecedented infestation by Botrytis grey mould and exacerbating damage by pod borer, grain yield responses to Mo application alone were 173% in the northern HBT, 61% in the central-north HBT, and 58% in the southern HBT. There was a further slight, non-significant response to Rhizobium inoculation.

There are no compound fertilisers containing Mo available in Bangladesh, and it is impractical to broadcast the small amount of Mo required evenly (<500g Mo ha^{-1}). Previous studies indicated that sufficient Mo and Rhizobium could be added via seed priming – soaking the seed in water overnight prior to sowing. In the 2003/04 season, the effect of adding Mo alone or Mo with Rhizobium via seed priming was compared with surface application of Mo in multilocational trials in farmers' fields. Adding Mo alone to the priming water did not significantly improve yield over the control, but there were significant responses to adding both Mo and Rhizobium in priming, of the same order as the response obtained when Mo was applied directly to the soil.

The response of chickpea to Mo, applied either through priming or broadcast on the soil surface, and with inoculation of Rhizobium through priming, was evaluated in on-farm trials conducted on rice-fallow lands with acid soils in eastern India in 2003/04. In 29 trials with chickpea cv. ICCV 2, the mean yield increase *over a control without Mo (mean yield 869 kg ha^{-1}) was 21.6% when Mo was applied through seed priming water and 20.3% when Mo was applied to the soil. In 19 trials with chickpea cv. KAK 2, the mean yield increase over a control without Mo (mean yield 784 kg ha^{-1}) was 16.8% when Mo was applied through seed priming water and 24.6% when Mo was applied to the soil.*

The results suggest that the severe nitrogen (N) deficiency of chickpea crops commonly observed in the HBT and in eastern India is caused by inadequate levels of Mo and Rhizobium in the soil. This problem can be alleviated effectively by a simple low-cost technology within the scope of resource-poor farmers by adding these entities in the seed priming process. To confirm this technology and its acceptability to farmers, it is intended to carry out a large number of farmer-implemented, operational-scale evaluations in the 2004/05 season.

Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume in the Middle East and South Asia. It normally grows on receding soil moisture on the alkaline soils of these regions; the crop has evolved on and is adapted to such soils (Saxena and Singh 1987). In Bangladesh, it is increasingly being grown on residual soil moisture after rainy season rice in the High Barind Tract (HBT) in the northwest of the country (Musa et al. 2002). The soils in this region are formed on alkaline alluvium, but the surface soils have acidified in the humid, tropical climate (pH 5.0-6.0 at 0-20 cm but pH 6.0-7.5 below 20 cm) (Brammer 1996). Chickpea yields are usually <1t ha^{-1} even though the yield potential for the region, obtained in some plots under favourable circumstances, is >2t ha^{-1}. The major constraints to chickpea cultivation in the region are drought stress and pod borer (*Helicoverpa armigera*), but in many locations vegetative growth is stunted with yellowing and reddening of older branches and their eventual necrosis. These symptoms resemble nitrogen (N) and/or phosphorous (P) deficiency (Smith et al. 1983). Experiments using inoculation of *Rhizobium* have been carried out at various times, but responses have been negligible and inconsistent (e.g., Kumar Rao et al. 2001). Although growth and yield responses to P application can be found (e.g., Ali 2000), addition of P fertiliser does not necessarily alleviate the symptoms.

It is possible to alleviate drought effects on chickpea through seed priming (Musa et al. 2001) and use of varieties with a duration providing a better match to the period of soil moisture availability. It is also possible to minimise pod borer damage through an integrated pest management (IPM) approach (Johansen and Musa 2004; Musa and Johansen 2003b). However, to increase yields and stability of yield, it is necessary to diagnose and address the suspected nutrient limitations. Besides P deficiency, sulphur (S) deficiency is increasingly recognised as a limitation to the yields of many crops in Bangladesh, especially those of oilseeds and pulses (Brammer 1996). Boron (B) deficiency is reported to be widespread in many crops, including chickpea crops, in northern Bangladesh where surface soils are acid (Ahmed and Hossain 1997). Boron deficiency also severely limits chickpea yields in the acid soils of the Terai in Nepal (located just to the north of Bangladesh); and chickpea also responds to Mo on these soils (Srivastava et al. 1997). In the predominantly alkaline soils of Bangladesh (unlike in the HBT with its acid surface soil), zinc (Zn) deficiency is common (Brammer 1996), and this may be a problem for roots feeding on deeper soil horizons in the HBT.

There are large areas of rice-fallow land in adjacent parts of India and Nepal with acid soils but otherwise apparently suitable for chickpea cultivation, but they are rarely used for chickpea or other legume cultivation (Subbarao et al. 2001). Diagnosis of the nutrient constraints to chickpea cultivation in the HBT of Bangladesh may also have relevance to these areas. A programme was introduced to first diagnose the nutrient limitations, and second develop methods for alleviating any deficiencies detected that are relevant for the resource-poor farmers of the region who grow chickpeas.

Materials and Methods

Diagnostic trial, 2001-02

An experiment was conducted using a subtractive experimental design with the following treatments.

1. Control: no seed treatment or addition of minor elements
2. Seed-treated control: seed priming (soaking of seeds in water for eight hours overnight prior to sowing), inoculation with *Rhizobium*
3. Full nutrient control (all): Treatment 2 with the following elements added (kg ha^{-1}): 1.0 B + 0.5 Mo + 5 Zn + 20 S – reagent grade salts were used.
4. Treatment 2 with Mo + Zn + S; i.e., all minus B
5. Treatment 2 with B + Zn + S; i.e., all minus Mo
6. Treatment 2 with B + Mo + S; i.e., all minus Zn
7. Treatment 2 with B + Mo + Zn; i.e., all minus S

An experiment was carried out in a farmer's field at Chabbishnagar Village, Godagari Upazilla, Rajshahi District, HBT (southern part), Bangladesh. Soil samples were taken at depths of 0–10, 0–15, and 15-30 cm, with one composite sample per replication. They were analysed by the Soil Resources Development Institute (SRDI), Shyampur, for pH, organic carbon, total N, and available P. A complete randomised block design was used with four replications. The plot size was 1.8 x 2.0m, with six rows of chickpea variety BARI chola 5 sown 30 cm apart and 2m long. The land was ploughed and levelled on 16 November 2001 and fertilisers added and seeds sown on 17 November 2001. Furrows were opened to about 6-8 cm depth and fertiliser added evenly throughout the furrows of a plot, according to treatment. Test fertilisers were mixed with river sand to aid even distribution. Phosphorous was also added to each plot in the furrows at 20 kg P ha^{-1}, as triple superphosphate (TSP). Furrows were then partially filled with soil, to a depth of 4-5 cm, in order to prevent direct contact of the seeds with fertiliser and hence possible nutrient toxicity, and seeds placed at 5 cm intervals within a row. The furrows were immediately covered and spray irrigation applied to the soil surface through a hose. This was considered necessary to ensure even germination. Previous attempts to carry out the experiment in the preceding two seasons had had to be abandoned due to uneven emergence caused by rapid drying of the soil surface once furrows were opened. Careful planting in rows in small plots was considered necessary in order to reduce variability to the extent that treatment differences could be detected.

The crop was grown rainfed, with only 16 mm of rainfall falling on the crop during January/February 2002. Plots were thinned to give a within-row spacing equivalent to 10 cm at 20-30 days after sowing. No weeding was necessary. Sprays of *Helicoverpa* nuclear polyhedrosis virus (HNPV) were given to the crop on 5 and 23 January 2002 to control pod borer (*Helicoverpa armigera*) (Musa and Johansen 2003b). This proved effective as there was negligible pod damage. Vegetative growth was ranked in each plot on 2 February 2002. The plot with the least growth was designated '1' and that with most '5'. A plot intermediate between these was designated '3'; a plot intermediate between '1' and '3' was designated '2'; and one between '3' and '5' was designated '4'. All other plots were ranked in relation to these reference plots. Due to the small plot size, plant samples were not cut to calibrate rank with dry mass; thus only rankings were analysed. Plots were harvested at maturity on 22 March 2002. The central two rows of each plot were harvested to avoid border effects, grain was separated from above-ground residues, and all samples were sun-dried. Four adjacent plots along one edge of the experiment nearest a footpath had been damaged by passing humans and grazing animals. These included two full nutrient control plots (Treatment 3) and one each of 'minus B' (Treatment 4) and 'minus Zn' (Treatment 6). It was therefore considered appropriate, and necessary, to exclude these plots from the statistical analysis of the experiment. A missing plot technique was applied prior to analysis of variance, according to Steel and Torrie (1980).

Multilocational Mo experiments

In order to determine how widespread Mo deficiency is across the HBT, and whether or not a *Rhizobium* response can be found in the presence of Mo, on-farm trials were carried out in the northern (Porsha), central (Gomostapur), and central-southern (Amnura) regions of HBT in 2002/03. Treatments were applied as follows.

1. Control: recommended agronomic practices for chickpea (Musa and Johansen 2003a), including seed priming, 20 kg P ha^{-1} as TSP, hand broadcasting, cross-ways ploughing, rainfed, IPM for pod borer and so on
2. As for control but Mo added as sodium molybdate, mixed with river sand, at a rate of 500g Mo ha^{-1}
3. As for control but with Mo added and *Rhizobium* inoculation with inoculum from the Bangladesh Institute of Nuclear Agriculture (BINA), including lime pelleting after coating seed with sticker and inoculum

The experiment was laid out in a randomised block design in farmers' fields with five dispersed replications each at three locations with acid surface soils: a) Naogaon District, Porsha Upazilla – farm of Shamsul Huq, Gupinathpur village (Nithpur); b) Chapai Nawabganj District, Gomostapur Upazilla – farm of Md. Ashraf Ali, Borodadpur village ; and c) Chapai Nawabganj District, Nawabganj Sadar Upazilla, Amnura – farm of A. Mannan, Sukhandighi village. The unit plot size was 10 x 10m. The three treatment plots of a replication were placed near each other in the same field. The chickpea variety BARI chola 5 was sown as follows: Porsha – 25 November 2002; Gomostapur – 26 November; Amnura – 27 November.

Soil samples were taken at depths of 0-10, 10-20, and 20-30 cm at each location, one set from the approximate location of each replication (i.e., 5 replications of samples per site). They were analysed by SRDI, Shyampur, for pH, organic carbon, total N, and available P. Sub-samples of the 0-10 and 10-20 cm samples were sent to ICRISAT for estimation of the most probable number (MPN) of chickpea rhizobia (Toomsan et al. 1983) and analysis of soil Mo after extraction with ammonium bicarbonate-diethylenetriamine-pentaacetic acid (AB-DTPA) and measurement using an inductively-coupled plasma (ICP) emission spectrometer according to the method of Sims (1996). Plant symptoms and incidence of pests and diseases were noted during crop growth. Nodulation was scored, according to the visual ranking scale of Rupela (1990), on 8 February at Amnura and 9 February at Porsha and Gomostapur. Five plants per plot were dug up and ranked. Plots were harvested as follows: Porsha – 5 April 2003; Gomostapur – 3 April; Amnura – 6 April. At harvest, 5 x $1m^2$ quadrats were cut from each plot and plant number and grain and residue yields estimated after air drying. Some seed samples from treatments with and without Mo (Treatment 2) were ground and sent to Cornell University, USA, for Mo analysis by inductively coupled atomic emission spectroscopy (Gupta 1998).

Mo x priming experiments

To determine if Mo could be applied in the seed priming process, and whether a response to *Rhizobium* could be obtained in the presence of Mo, on-farm trials with the following treatments were established at three locations in the HBT in the 2003/04 season.

1. Control: recommended agronomic practices for the HBT (Musa and Johansen 2003a), i.e., seed priming, 20 kg P ha^{-1} as TSP, hand broadcasting, cross-ways ploughing, rainfed, IPM for pod borer, and so on
2. As for control but Mo added as sodium molybdate at the rate of 500g Mo ha^{-1}, mixed with river sand and spread on the soil surface prior to ploughing
3. As for control but Mo added to the priming water at the rate of 0.5g sodium molybdate l^{-1} priming solution, ensuring that all seeds were covered with the priming solution but that most of the priming water was absorbed by the seeds after 8 hours
4. As for control but with both Mo and *Rhizobium* (BINA inoculum) added in the priming solution (*Rhizobium* inoculum at 4g l^{-1} priming solution) (Kumar Rao et al. in prep)

The experiment was laid out in a randomised block design in farmers' fields with five dispersed replications each at three locations with acid surface soils: a) Naogaon District, Porsha Upazilla, Shaharandpur Village; b) Chapai Nawabganj District, Gomostapur Upazilla, Borodadpur Village ;and c) Rajshahi District, Tanor Upazila, Mundamallah Village . Unit plot size was 7.07 x 7.07m ($50m^2$). The four treatment plots of a replication were placed next to each other in the same field. The chickpea variety BARI chola 5 was sown as follows: Porsha – 30 November 2002; Gomostapur – 29 November; Tanor – 1 December. The crops were grown rainfed following the practices recommended by Musa and Johansen (2003a).

Soil sampling and analysis, nodulation estimation (5 February 2004 at all locations), harvesting (Porsha and Gomostapur – 29 March 2004; Tanor – 28 March 2004) were carried out as described for the previous season. However, two replications were discarded from the Porsha site, due to irrigation spillage from adjacent fields, and one from Gomostapur due to poor establishment.

Mo x priming experiments Eastern India, 2003/04

Seventy-two on-farm trials with the following three treatments were conducted in selected rice-fallows of Orissa, Chattisgarh, Jharkhand, eastern part of Madhya Pradesh, and West Bengal states of India during the 2003/04 post-rainy season with the objective of knowing the response of chickpea crops to Mo applied through seed priming in comparison to Mo applied to soil. The three treatments were as follows.

1. Primed chickpea seed + *Rhizobium* but without Mo (control) (4g of peat-based *Rhizobium* culture were mixed with one litre of water used to prime one kg chickpea seed – seed priming comprised of soaking the seed in water for four to six hours, followed by air drying to facilitate seed handling for sowing)
2. Primed chickpea seed + *Rhizobium* + Mo (Mo was added as sodium molybdate at a rate of 0.5g l^{-1} of priming water per kg of chickpea seed)
3. Primed chickpea seed + *Rhizobium* but with Mo added to soil at a rate of 500g Mo ha^{-1} as sodium molybdate – 12.2g of sodium molybdate was mixed with river sand or fine soil and applied evenly to a 10 x 10m plot and mixed with soil before sowing.

The three treatments were placed close to one another in a given farmer's field. A composite soil sample was taken from a depth of 0–15 cm from the control treatment for chemical analysis. A basal dressing of 150 kg single superphosphate ha^{-1} (= 24 kg P_2O_5 ha^{-1}) was given uniformly by broadcasting it to all the treatments. Two chickpea varieties, namely, ICCV 2 and KAK 2, both cream-coloured Kabuli varieties, were used for these trials: however, only one variety was used in a given farmer's field. The seed was sown following rainy season rice in November/December 2003, either by dropping it in a furrow behind the plough then covering it during the next pass of the plough or broadcasting followed by cross-ways ploughing and laddering. The crop was grown rainfed and farmer managed. The crop was sampled for nodulation near flowering time. For this, three to five plants in each treatment were uprooted carefully and assigned a nodulation ranking according to Rupela (1990). At crop maturity in March 2004, three to four quadrats of 1 m^2 per treatment were harvested and sun dried. Observations on the plant stand, grain yield and stover yield were recorded. The yield data were analysed using REML (restricted or residual maximum likelihood) with a linear fixed model – GENSTAT version 7.1.0.198); overall treatment means of each genotype are presented with a standard error of deviation. Chickpea grain samples were ground, digested with a tri-acid mixture sulphuric, nitric, and hyperchloric acid (H_2SO_4 + HNO_3 + $HClO_4$) and the digest analysed for Mo by inductively-coupled plasma (ICP) emission spectrometer (Benton Jones and Case 1990).

Results

Diagnostic trial, 2001/02

The soil at the Chabbishnagar location was typical of that across the HBT (Brammer 1996) with acid soil at the surface, pH increasing with depth, and low organic carbon and total N levels decreasing with depth (Table 1).

From the end of January 2002, there was a lighter green colour and, in early February, less estimated vegetative growth in plots to which Mo was not added (viz. Treatments 1, 2, and 5) (Table 2). The oldest branches of plants in plots with Mo had much less red and yellow colouration than those in plots without Mo. At harvest, grain yield was significantly less in plots without Mo (Table 2). Compared to the 'minus Mo' treatment, yield was 73% higher in the full nutrient control due to addition of Mo alone. There was a similar effect on yield of total aerial biomass (grain yield + residue yield), although there was a late recovery of growth in the non-nutrient controls (Treatments 1 and 2) (Table 2). Omission of B, Zn, or S did not cause any reduction in growth or grain yield (Table 2).

Location in HBT	Soil depth (cm)	pH (1:2 soil: water)	Organic C (%)	Total N (%)	Available P (Olsen's μg g^{-1})
2001/02 season					
Chabbishnagar - south	0-10	4.9 ± 0.05	1.6 ± 0.03	0.07 ± 0.005	5.3 ± 1.35
	0-15	5.4 ± 0.05	1.2 ± 0.10	0.07 ± 0.005	4.7 ± 0.71
	15-30	6.2 ± 0.21	0.7 ± 0.08	0.04 ± 0.010	2.6 ± 0.54
2002/03 season					
Porsha – north	0-10	5.3 ± 0.51	2.7 ± 0.17	0.12 ± 0.035	3.3 ± 0.88
	10-20	5.9 ± 0.42	1.7 ± 0.28	0.09 ± 0.031	1.7 ± 0.53
	20-30	6.3 ± 0.34	0.9 ± 0.07	0.08 ± 0.004	1.0 ± 0.33
Gomostapur – central-north	0-10	5.8 ± 0.38	2.3 ± 0.33	0.12 ± 0.007	1.1 ± 0.61
	10-20	6.5 ± 0.51	1.6 ± 0.18	0.07 ± 0.028	1.4 ± 0.38
	20-30	7.5 ± 0.30	1.0 ± 0.09	0.07 ± 0.011	1.3 ± 0.42
Amnura – central-south	0-10	5.9 ± 0.41	2.1 ± 0.21	0.09 ± 0.026	2.0 ± 1.57
	10-20	6.7 ± 0.36	1.4 ± 0.07	0.07 ± 0.019	2.4 ± 1.45
	20-30	7.3 ± 0.23	0.8 ± 0.07	0.05 ± 0.011	1.5 ± 0.81
2003/04 season					
Shaharandpur – north	0-10	4.6 ± 0.07	1.6 ± 0.07	0.09 ± 0.004	1.9 ± 0.21
	10-20	5.2 ± 0.26	1.0 ± 0.08	0.06 ± 0.000	1.5 ± 0.14
	20-30	6.3 ± 0.23	0.6 ± 0.16	0.04 ± 0.009	1.2 ± 0.18
Gomostapur – central-north	0-10	5.3 ± 0.21	1.3 ± 0.13	0.08 ± 0.008	2.2 ± 0.98
	10-20	6.1 ± 0.26	0.9 ± 0.14	0.05 ± 0.001	1.5 ± 0.49
	20-30	6.8 ± 0.26	0.5 ± 0.07	0.03 ± 0.005	1.3 ± 0.28
Mundamullah – south	0-10	4.8 ± 0.40	1.4 ± 0.20	0.12 ± 0.015	3.1 ± 0.98
	10-20	5.4 ± 0.76	0.8 ± 0.21	0.07 ± 0.015	2.5 ±0.64
	20-30	6.2 ± 0.49	0.5 ± 0.08	0.05 ± 0.008	2.8 ± 1.10

Table 1: **Soil analyses mean ± standard deviation at locations of on-farm experiments in the High Barind Tract, 2001/02 to 2003/04 seasons (n = 4 in 2001-02 and n = 5 subsequently)**

Multilocation Mo experiments, HBT 2002/03

Soil surface pH was lowest at Porsha, but at all locations pH increased with depth (Table 1). Organic carbon, total N, and available P declined with soil depth and were generally at low levels (Table 1). The concentration of Mo in the soil was below the detectable limit of 0.02 ppm at all locations. There were most native rhizobia, of the type that could nodulate chickpea, at Amnura, and least at Porsha (Table 3). By early February 2003, treatment differences were apparent at all locations, with more yellowing/reddening and less growth vigour in the control treatment. At Porsha and Gomostapur, treatments with Mo were a bright, dark green, consistent with nitrogen adequacy. At Porsha only, there appeared to be a further response to addition of *Rhizobium*. Nodulation was poor in the control treatment at all locations and consistent with the N deficiency symptoms apparent (Table 3). Addition of Mo alone caused a significant increase in nodulation over the control. Addition of *Rhizobium* caused a further nodulation response only at Porsha, consistent with the lowest *Rhizobium* population found here (Table 3). It therefore seems that rhizobia able to nodulate and form N-fixing nodules in chickpea were present at all sites, but that addition of Mo was required for proper functioning of nodules.

Table 2: Effect of different nutrient treatments on eye -estimated vegetative growth at 77 days after sowing, and grain and residue yield at harvest (125 days after sowing), Chabbishnagar, High Barind Tract, Bangladesh, 2001/ 02 post-rainy season			
Treatment	**Growth ranking [1]**	**Grain yield ($g\ m^{-2}$)**	**Aerial biomass ($g\ m^{-2}$)**
1. Zero control	2.1	117	288
2. Seed treated control	2.0	105	249
3. Full nutrient control	3.4	163	334
4. All –B	3.5	164	328
5. All –Mo	2.1	94	211
6. All –Zn	3.8	140	329
7. All –S	4.0	152	327
Standard error of difference between means	*±0.63*	*±16.6*	*±26.8*
Significance of difference	*P<0.05*	*P<0.005*	*P<0.001*

[1] Eye estimated growth ranking where 1 is least growth and 5 is most growth.

Table 3: Estimation of chickpea rhizobial population and mean nodulation ratings [1] in on-farm molybdenum response trials conducted during the 2002/03 rabi season, HBT, Bangladesh (MPN g^{-1} dry wt. soil; mean and range)			
Source of sample	**Location**		
	Porsha	**Gomostapur**	**Amnura**
Rhizobial population (MPN g^{-1} dry wt soil [2])			
0-10 cm soil	4×10^1 ($0 – 1.5 \times 10^2$)	4.99×10^6 ($0 – 2.45 \times 10^7$)	4.99×10^3 ($1.5 \times 10^2 – 2.47 \times 10^5$)
10-20 cm soil	5×10^1 ($0 – 1.5 \times 10^2$)	1.2×10^4 (1.5×10^2 - 4.36×10^4)	4.94×10^5 ($1.5 \times 10^2 – 2.45 \times 10^6$)
Nodulation score			
Control	1.0	1.4	1.1
+ Mo	2.3	2.9	2.0
+ Mo + *Rhizobium*	3.5	3.0	2.3
SE (±) [3]	*0.33*	*0.34*	*0.37*
Significance	*P<0.005*	*P<0.005*	*P<0.05*

[1] According to the 1 -5 scale of Rupela (1990) where 1 = minimal nodulation and 5 = abundant nodulation;
[2] MPN = most probable number; [3] Standard error of difference between two sample means.

Unusually excessive rain in March and April 2003 resulted in an unprecedented severe infestation of *Botrytis* grey mould (BGM) and promoted pod borer damage, thereby generally lowering yields. This effect of excess moisture was most severe at Gomostapur. Nevertheless, responses of grain yield and total aerial biomass to Mo were apparent at all locations, although the variability at Amnura prevented the response reaching significance there (Table 4). The overall Mo response across sites was significant (Table 4). Only at Gomostapur was there an additional response to application of *Rhizobium*.

Addition of Mo to the soil markedly increased seed concentration of Mo at Porsha from 1.35 mg g^{-1} in the control to 3.57 mg g^{-1} with Mo. Respective values for Amnura were 0.64 and

Table 4: **Effect of application of Mo and *Rhizobium* on grain and total aerial biomass yield of chickpea in the HBT in the 2002/03 season**

Treatment	Location			
	Porsha	Gomostapur	Amnura	All locations
Grain yield (t ha^{-1})				
Control	0.37	0.33	0.70	0.47
+ Mo	1.01	0.54	1.10	0.88
+ Mo + *Rhizobium*	1.12	0.70	1.13	0.98
SE (±)[1]	*0.156*	*0.033*	*0.197*	*0.152*
Significance	*P<0.005*	*P<0.005*	*n.s.*	*P<0.001*
Aerial biomass yield (t ha^{-1})				
Control	1.02	1.03	1.68	1.25
+ Mo	2.28	1.35	2.63	2.09
+ Mo + *Rhizobium*	2.61	1.70	2.49	2.27
SE (±)[1]	*0.314*	*0.093*	*0.473*	*0.336*
Significance	*P<0.005*	*P<0.005*	*n.s.*	*P<0.001*

[1] Standard error of difference between two sample means

0.86 mg g^{-1} and for Gomostapur 0.67 and 0.53 mg g^{-1}. The pod damage due to rainfall and BGM infestation at the reproductive stage may have contributed to such variability and lack of response at Amnura and Gomostapur. A separate study on the effects of Mo fertilisation on accumulation of Mo in vegetative tissue and seed was carried out at Amnura in this season, and the results are being reported separately.

Mo x priming experiments HBT, 2003/04

Trends of decreasing acidity, organic carbon, total N, and available P with soil depth were apparent at each trial location, as in previous seasons (Table 1). Soil Mo concentrations were below the detectable limit of 0.02 ppm. Rhizobial numbers in 2003/04 soil samples are currently under assessment. Nodulation was poor at all locations in control treatments and only significantly improved in the presence of Mo and *Rhizobium* at Gomostapur and Mundamullah (Table 5). In contrast to the previous season, there was no rain during the chickpea growing period in 2003/04 and crops suffered terminal drought stress, with consequent yield reduction. Only at Gomostapur was grain and aerial biomass yield significantly improved by addition of Mo to the soil (Table 6). Addition of Mo and *Rhizobium* in the priming water had a similar effect, but addition of Mo alone to the priming water did not significantly increase yield above the control. These trends were similar at the other locations but statistical significance was not reached (Table 6), although the overall response across sites was significant.

Mo x priming experiments Eastern India, 2003/04

A total of 245 soil samples at depths of 0-15 cm, representing farmers' fields having rice-fallows in Chattisgarh, Madhya Pradesh; Orissa, West Bengal; and Jharkhand were collected and analysed for pH and Mo at the ICRISAT Centre, Patancheru, Andhra Pradesh, India. So far about 90 of these samples have been analysed for B, S, available P (Olsen's P), Zn, and organic carbon. The results indicate that the soils are mostly acidic with mean pH ranging

Table 5: Mean nodulation ratings[1] in on-farm Mo response trials conducted during the 2003/04 rabi season, HBT, Bangladesh.			
Source of sample	**Location**		
	Shaharandpur	**Gomostapur**	**Mundamullah**
Control	1.2	1.1	1.7
Soil applied Mo	1.3	1.6	1.7
Mo with priming	1.1	1.9	2.1
Mo + *Rhizobium* with priming	1.7	2.3	2.8
SE (±)[2]	*0.29*	*0.40*	*0.33*
Significance	*n.s.*	*P<0.10*	*P<0.05*

[1] According to the 1-5 scale of Rupela (1990) where 1 = minimal nodulation and 5 = abundant nodulation
[2] Standard error of difference between two sample means.

Table 6: Effect of adding Mo and *Rhizobium* in the priming solution on grain and total aerial biomass yield of chickpea at three locations in the HBT in the 2003/04 season				
Treatment	**Location**			
	Shahrandpur	**Gomostapur**	**Mundamullah**	**All locations**
Grain yield (t ha^{-1})				
Control	0.62	0.32	0.67	0.54
Soil applied Mo	0.89	0.68	0.96	0.84
Mo with priming	0.66	0.48	0.93	0.69
Mo + *Rhizobium* with priming	0.93	0.61	0.92	0.82
SE (±)[1]	*0.180*	*0.090*	*0.218*	*0.126*
Significance	*n.s.*	*P<0.01*	*n.s.*	*P<0.01*
Aerial biomass yield (t ha^{-1})				
Control	1.42	0.74	1.30	1.15
Soil applied Mo	2.07	1.37	1.91	1.78
Mo with priming	1.57	1.01	1.76	1.45
Mo + *Rhizobium* with priming	2.06	1.25	1.74	1.68
SE (±)[1]	*0.388*	*0.172*	*0.366*	*0.210*
Significance	*n.s.*	*P<0.05*	*n.s.*	*P<0.001*

[1] Standard error of difference between two sample means

5.4-7.5. The Mo content of the rice-fallows was either nil or less than 0.04 ppm (Table 7). Most of the soils were deficient in B, S, and available P (Olsen's P, data not presented). The soil analysis for native populations of chickpea *Rhizobium* is in process.

A total of 72 on-farm trials spread over the five states of India mentioned above were sown. In many of these trials the chickpea plants were light green in the control plots, i.e., without Mo, but greener in the plots that received Mo either through seed priming or soil application. Sixty-nine out of 72 trials were sampled at flowering time and scored for nodulation ranking

Table 7: **Mean of soil pH and Mo levels at 0 -15 cm in rice fallows in eastern India, some fields of which were used for Mo response trials with chickpea during the 2003/04 season**

State	District	No. of farmers fields	pH	Mo (ppm)
Madhya Pradesh	Satna	60	6.78	0.021
Madhya Pradesh	Dindori	41	6.66	0.008
Madhya Pradesh	Mandla	34	6.68	0.038
Jharkhand	Latehar	8	6.46	0.036
West Bengal	Purulia	39	6.12	0.029
West Bengal	Malda	2	5.79	0.033
West Bengal	Darjeling	1	4.32	0.052
Chattisgarh	Bastar	11	6.02	0.024
Orissa	Sundergarh	6	5.69	0.001
Orissa	Kandamal	5	5.93	0.002
Orissa	Mayurbhanj	23	6.54	0.002

on a scale of 1 to 5 (Rupela 1990). The nodulation was lowest in the 'control' plot. Mo application through seed priming resulted in a 58% increase in chickpea nodulation compared to the control, while soil application of Mo resulted in a 31% increase in nodulation over the control (Table 8). These results suggest that Mo application was essential for proper nodule development and functioning.

At maturity, yield data were collected from only 48 trials (29 trials having cv. ICCV 2 and 19 trials having cv. KAK 2) as the remaining trials were damaged either by theft by human beings and damage by animals or by drought stress. Molybdenum application through seed priming increased grain yield of ICCV 2 by 21.6% (1057 kg ha^{-1} vs 869 kg ha^{-1}), while soil application of Mo resulted in 20.3% increase (1045 kg ha^{-1} vs 869 kg ha^{-1}) (Table 9). Molybdenum application either through seed priming or soil application increased stover yield by 15%. In the case of cv. KAK 2, a bold seeded Kabuli chickpea variety, Mo application through seed priming increased grain yield by 16.8% (916 kg ha^{-1} vs 784 kg ha^{-1}), while soil application of Mo increased the yield by 24.6% (977 kg ha^{-1} vs 784 kg ha^{-1}). Stover yield of KAK 2 was also increased by Mo application – 20.2% through seed priming and 28.5% through soil application (Table 9).

Discussion

In the diagnostic trial, alleviation of the yellow and red coloration in the oldest branches in treatments with Mo added is consistent with the role of Mo in nitrogen fixation (Srivastava 1997). At this location, the pH of the soil was strongly acidic near the surface and increased with depth (Table 1), which would indicate unavailability of Mo to plants in the surface soil at least (Lindsay 1972). There was no response to addition of either B, Zn, or S and thus Mo deficiency appears to be the major nutrient limitation to growth and yield of chickpea crops at this location.

The soil at this location is typical of that across the HBT, at least in terms of pH changes with soil depth (Brammer 1996). It could thus be expected that there would be a Mo limitation across the entire region and, therefore, multilocational trials to measure Mo response across the HBT were initiated in the 2002/03 season. It was realised that Mo

Table 8: **Mean nodulation ratings [1] of chickpea grown in rice fallows in on-farm Mo response trials conducted during the 2003/04 season, eastern India**

State	Village	T 1[2]	T 2	T 3	SE (±)[3]	Significance[4]
West Bengal	Gobradhi	0.3	1.3	0.4	0.16	P<0.05
West Bengal	Jahajpur	1.5	2.6	2.4	0.28	P<0.05
West Bengal	M. Sahar	0.8	1.2	1.2	0.14	ns
West Bengal	Parkidi	0.9	1.6	0.9	0.18	P<0.05
Jharkhand	Sirish	2.3	2.6	2.3	0.23	ns
Madhya Pradesh	Bhodhgundi	1.5	1.9	1.6	0.17	ns
Madhya Pradesh	Divlaha	0	0.7	1.3	0.24	P<0.05
Madhya Pradesh	Kanpur	0.7	1.3	1.3	0.19	ns
Madhya Pradesh	Mudukhua	2.3	2.3	1.7	0.77	ns
Madhya Pradesh	Patni	1	2.7	1.7	0.19	P<0.01
Madhya Pradesh	Padariya	1.6	1.8	2.4	0.20	P<0.05
Madhya Pradesh	Rohania	1.7	2	2	1.26	ns
Madhya Pradesh	Singarpur	1.1	1.5	1.3	0.13	ns
Madhya Pradesh	Umariya	0	0.7	1.3	0.24	P<0.05
Chattishagarh	Chivurgaon	0	2	1	0.33	P<0.05
Chattishagarh	Kirigoli	0	1.1	0.7	0.11	P<0.01
Chattishagarh	Kondagav	0	1.2	0.7	0.22	P<0.01
Orissa	Asana	1.2	1.3	1.3	0.09	ns
Orissa	Kanchikana	0.6	2.8	1.2	0.31	P<0.01
Orissa	Thakurpalli	1.6	1.4	1.4	0.51	ns
	Mean	0.9	1.7	1.4		

[1] According to the 1-5 scale of Rupela (1990) where 1= minimal nodulation and 5= abundant nodulation.
[2] T1 = Control (seed primed with *Rhizobium*); T2 = Seed primed with *Rhizobium* + Mo;
T3 = Seed primed with *Rhizobium* but Mo applied to soil ; [3] Standard error of difference between means
[4] P = probability; ns = not significantly different

Table 9: **Effect of Mo application through seed priming and soil application on grain and stover yields of chickpea cvs. ICCV 2 and KAK 2 in farmers' fields of eastern India following rice, post-rainy season 2003/04**

Treatment	Chickpea cultivar			
	ICCV 2[1]		KAK 2[2]	
	Grain yield ($t\ ha^{-1}$)	Stover yield ($t\ ha^{-1}$)	Grain yield ($t\ ha^{-1}$)	Stover yield ($t\ ha^{-1}$)
Control (no Mo)	0.87	1.71	0.78	1.57
Mo applied by seed priming	1.06	1.97	0.92	1.89
Mo applied to soil	1.05	1.96	0.98	2.02
SE (±)[3]	*0.036*	*0.069*	*0.050*	*0.129*
Significance	*P<0.01*	*P<0.01*	*P<0.01*	*P<0.01*

[1] Mean of 29 on-farm trials spread over Orissa, West Bengal, Jharkhand, Madhya Pradesh and Chattisgarh states of India ; [2] Mean of 19 on-farm trials spread over Madhya Pradesh, Orissa and Chattisgarh; [3] Standard error of difference between two sample means

deficiency may have been limiting any response to *Rhizobium* inoculation, and so a treatment with *Rhizobium* inoculation was included. The 2002/03 results confirm that Mo deficiency is widespread across the HBT, and that it occurs when pH of the surface soil is in the range pH 5-6, even though sub-surface soil pH tends towards neutral. Although addition of *Rhizobium* in the presence of Mo further improved nodulation, it only significantly improved crop growth and grain yield at one location, Gomostapur. Addition of Mo alone significantly improved nodulation (Table 3), and this indicates that its presence is necessary for nodule development.

In the on-farm experiments initially conducted, Mo had been applied through a carrier, inert river sand, due to the small amounts of Mo required to improve functioning of the nitrogen-fixing symbiosis. This is impractical for resource-poor farmers to implement over large areas. In other countries where Mo deficiency is common, macronutrient fertilisers are fortified by adding appropriate amounts of Mo (e.g., molybdenised superphosphate in Australia). No such compound fertilisers are available in Bangladesh. Another common method of applying Mo is through a seed dressing, such as inoculation of *Rhizobium* on to seed (Murphy and Walsh 1972), but the experience of resource-poor farmers in South Asia adopting seed-dressing techniques has usually not been successful (e.g., failure to adopt *Rhizobium* inoculation technologies even when good responses can be demonstrated – Rupela et al. 1994). Other methods of applying Mo include soil or foliar fertilisation of the seed crop to load the seed with sufficient Mo for the next generation; again, not practical for resource-poor rural communities.

Recent studies in Nepal have shown that sufficient Mo can be loaded into chickpea seed through soaking seed prior to sowing in a solution with Mo (Johnson 2004). A concentration of 0.5g sodium molybdate l^{-1} of water was found optimum, adding sufficient Mo to the seed to elicit a Mo response but not enough to induce Mo toxicity. During 1998/99 to 2001/02, a successful programme of seed priming of chickpeas was carried out in the HBT (Musa et al. 2001). This involved soaking the seed overnight for eight hours prior to sowing in order to increase seedling vigour in rapidly drying seedbeds. Mean yield increases due to priming were in the range of 20-50% over the four-year period (Musa et al. 2001) and farmers readily adopted this simple but effective technology (Saha 2002). It was therefore considered worthwhile to try combining Mo application with the seed-priming process. Further, Kumar Rao et al. (2001) had shown that when *Rhizobium* was added in the priming solution its effectiveness was the same as that of seed inoculation by traditional methods. Thus there was the possibility of combining Mo and *Rhizobium* application with the priming process already adopted which was tested in the HBT in the 2003/04 season.

In the HBT, adding Mo to the priming solution was not as effective as soil application of Mo, but adding both Mo and *Rhizobium* in priming was as effective (Table 6). The best nodulation was also obtained in the primed treatment with both Mo and *Rhizobium* (Table 5). It therefore appears necessary to add both Mo and *Rhizobium* inoculum to overcome the problem of inadequate N fixation in the HBT which is caused by both Mo deficiency and low numbers of native rhizobia that are infective and effective for chickpea crops.

In general, the analysed rice-fallow soils of eastern India were of low fertility with pH in the acidic to neutral range (about 5 to 7). Our recent studies indicate that most of the rice-fallow soils were either devoid of chickpea rhizobia or if present they were in low numbers. Further, the soils were deficient not only in Mo but also in other nutrient elements such as available P, B, and S. Molybdenum application resulted in a significant increase in chickpea nodulation, growth, and final yields, and it created a positive impact on farmers. Therefore it is imperative to recommend *Rhizobium* inoculation and application of Mo for effective symbiosis and nitrogen fixation of chickpeas particularly in rice-fallows of eastern India. We also need to know the extent of limitation of chickpea growth and yield as a result of other nutrient deficiencies such as B, S, and others. If they play a critical role in legume growth, then we need to consider ways of supplementing these specific nutrients as well.

Addition of requisite amounts of Mo and *Rhizobium* to the priming solution shows promise as a technique that is feasible for use by resource-poor farmers to overcome Mo, and ultimately N, deficiencies in chickpeas. Widespread farmer-managed evaluation of the technique is now required in potential chickpea-growing areas with acid soil in South Asia. This would require production of packets of the requisite quantities of Mo salt and *Rhizobium* inoculum to be added to a specified volume of water as the seed-priming solution. After training in quality control, preparation of such packets could be developed as a village-level enterprise, contributing to local income generation. Packets and the recipe to follow could then be distributed to farmers. Such a programme is intended for Bangladesh, eastern India, and Nepal in the 2004/05 chickpea season.

Despite the promise of pursuing on-farm evaluation of the technique, some further back-up research is needed. For soil application of Mo, a rate of 500g Mo ha^{-1} was used, although optimum rates could be much less (e.g., 50-200g Mo ha^{-1}; Murphy and Walsh 1972). To add Mo through seed priming, only around 10g Mo ha^{-1} is required. However, further work is needed to establish optimum concentrations in the priming solutions to meet the Mo requirements of the plant but to avoid Mo toxicity to the germinating seed. There may be varietal differences in this regard. Also, this technique of applying Mo, and *Rhizobium* if required, in the priming solution needs to be evaluated for other crop species that are grown on acid soils. Efforts are also required to establish protocols for quality control of Mo and *Rhizobium* supply and use, so that farmers will have confidence in the technology.

Further work is also required to establish the extrapolation zone of the technology, the regions prone to Mo deficiency in South Asia. Primarily, this is defined by soil acidity (Cox and Kamprath 1972; Johansen et al. 1997) and thus comprehensive geographic information system (GIS) maps indicating the soil reaction characteristics of the region are needed. Measures of soil Mo have not previously proved very useful for prediction of Mo response, due to the dependence of Mo availability to plants on soil pH. Critical values of Mo in chickpea tissue have yet to be established, as reports of Mo responses in chickpeas are rare as they are normally cultivated on neutral to alkaline soils. Actual growth response to Mo obtained in the field also needs to be plotted on GIS to obtain a comprehensive picture of Mo deficiency across the region; and thus scope will be provided for its correction through priming technology.

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