Severe Boron Deficiency limiting Grain Legumes in the Inner Terai of Nepal

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Abstract

Lentils, chickpeas, and pigeon peas are important grain legumes grown in Nepal. They provide staple components of Nepalese diets by supplying vegetable protein, vitamins, and minerals that are otherwise only available in sparse amounts. Their cultivation also improves soil health through additions of fixed nitrogen and organic matter and by breaking cereal pest and disease cycles. However, their yields are generally low and unstable due to an array of biotic and abiotic constraints. Symptoms suggestive of micronutrient limitations had been observed prior to the 1990s, but it was difficult to differentiate these symptoms from those caused by other factors. For example, the poor podding of chickpea was usually attributed to Botrytis grey mould disease, until it was shown that boron (B) deficiency had a similar effect.

In order to establish if any, and if so which, micronutrients were limiting chickpea, an omission trial was conducted in the field at the Research Station of the Grain Legume Research Programme, Rampur, Chitwan District, inner Terai in the 1993/94 season. In the absence of applied B, there was no yield as no pods formed, in comparison to a yield of 300 kg ha^{-1} in the full nutrient treatment. There was yellowing of younger leaves and typical 'little leaf' symptoms when B was omitted. Molybdenum (Mo) was also shown to be deficient for chickpeas at this site. Subsequent field trials established that the optimum rate of B to apply to the soil to correct the deficiency at this location was 0.5 kg ha^{-1}. Further studies at this location confirmed positive responses of lentils and pigeonpea in terms of yield to B application. In lentil variety ILL-4605, absence of B reduced yield to 6% of the treatment with B; in the pigeonpea variety, Bageswari, the reduction was to 10% of the treatment with B. Lentils also responded to zinc. For all of these legumes, there were large genotypic differences in response to B, with exotic germplasm (e.g., lentil genotypes from the International Centre for Agricultural Research in Dry Areas [ICARDA] and chickpea and pigeonpea genotypes from the International Crop Research Institute for the Semi-Arid Tropics [ICRISAT]) being more susceptible to B deficiency than locally-derived ones. This indicates evolution of local genotypes to B-deficient conditions and scope for genetic enhancement to alleviate B deficiency.

In the 1997/98 and 1998/99 seasons, on-farm trials were conducted to evaluate the extent of B deficiency in the inner Terai. Chickpea and mustard, crops known to be particularly susceptible to B deficiency, were used as test crops. Yield responses were widespread but variable, with responses of up to 560% in chickpea and 360% in mustard crops being recorded. Responsiveness decreased with increasing soil organic matter content. A critical concentration range of 15-20 ppm B was found for the shoot tips of chickpeas.

The large responses to B obtained in these grain legumes, and appearance of symptoms likely to be due to B-deficiency in these and other crops in the central and eastern Terai, indicate a need for systematic

assessment of the spatial and temporal distribution of the extent of limitation of grain legumes by micronutrient deficiencies (primarily B, but also other micronutrients) in Nepal. Optimum methods of diagnosis of micronutrient deficiencies and remedial measures most appropriate for small-holder farmers need to be evaluated on-farm and widely disseminated if the potential levels of yield of grain legumes are to be achieved.

Introduction

Lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.), and pigeonpea (*Cajanus cajan* L. Millsp.) are important grain legumes in Nepal (Johansen et al. 2000). They contribute to the staple diet of Nepalese people by providing vegetable protein, vitamins, and minerals that are otherwise only available in sparse amounts. Being legumes, their cultivation improves soil health through additions of fixed nitrogen and organic matter and by breaking cereal pest and disease cycles. However, their yields are generally low and unstable due to a range of biotic and abiotic constraints (Johansen et al. 2000). Among the abiotic constraints, micronutrient deficiencies appear to be important but these effects have only been quantified recently. Symptoms suggestive of micronutrient deficiencies had been observed prior to the 1990s, but it was difficult to specify which particular micronutrients were involved and, indeed, to differentiate these symptoms from those caused by other factors. For example, the poor podding of chickpeas was usually attributed to *Botrytis* grey mould (BGM) disease, until it was shown that boron (B) deficiency had a similar effect (Srivastava et al. 1997). Soil analyses conducted by Khatri-Chhetri (1982) indicated wide occurrence of micronutrient deficiencies in the Chitwan Valley in the inner Terai: a region of acid soils.

In this paper we summarise earlier attempts to diagnose micronutrient deficiencies, firstly in chickpeas, and to determine the extent of limitation in the major grain legumes grown in the inner Terai, namely chickpeas, lentils, and pigeonpeas. Appropriate means of diagnosing micronutrient limitations across regions, and methods of alleviating them suitable for adoption by resource-poor farmers, are then proposed.

Materials and Methods

Chickpea

To diagnose which nutrient elements were deficient for chickpeas, an omission experiment was carried out at the field station of the Grain Legumes Research Programme (GLRP), Nepal Agricultural Research Council (NARC), Rampur, Chitwan, during the 1994/95 season. Treatments consisted of the following: 1) control with no nutrients added; 2) complete supply of all nutrients likely to be deficient: phosphorous (P), potassium (K), sulphur (S), boron (B), zinc (Zn), molybdenum (Mo), copper (Cu), manganese (Mn), iron (Fe); 3) complete minus B; 4) complete minus Zn; 5) complete minus Mo; 6) complete minus Fe; 7) complete minus Cu + Mn; 8) complete minus K + S; and 9) complete + lime. Cultural details are given in Srivastava et al. (1997).

In 1995/96, a field experiment with four levels of B (0, 0.5, 1.0 and 3.0 kg ha^{-1}) and three levels of Mo (0, 0.15 and 0.3 kg ha^{-1}) was conducted at the Rampur station. Details are given in Srivastava et al. (1997). The chickpea cultivar Kalika was used in both seasons' trials. In 1994/95, there was no rainfall during the winter growing period and the crop suffered terminal drought stress. However, in 1995/96, there was adequate soil moisture throughout the growing season, as stored soil moisture from the monsoon rains was supplemented by light winter rainfall. In both experiments, *Botrytis* grey mould (BGM) was controlled by spraying fungicide and pod borer (*Helicoverpa armigera*) by insecticide.

In the 1997/98 and 1998/99 seasons, on-farm trials were held in Chitwan and Nawalparasi Districts of the Chitwan Valley, inner Terai, to determine the spatial distribution of B responses of chickpeas. In 1997/98, seven on-farm trials, designed by researchers but managed by farmers, were conducted as indicated in Table 1. Five treatments (0, 0.5, 1.0 B, 0.5 B + 0.15 Mo, and 1.0 B + 0.15 Mo kg ha^{-1}) were tested in plots of 3 x 3.6m in a randomised complete block design replicated three times at all seven locations. Boron was applied as boric acid and Mo as sodium molybdate mixed with some dry soil and then broadcast over the plots. Triple superphosphate (TSP) at 40 kg ha^{-1} P_2O_5 and muriate of potash (MP) at 20 kg ha^{-1} K_2O were then broadcast and all fertilisers mixed into the top 10 cm of soil with a spade. Seeds of the Sita variety of chickpea were sown at a spacing of 30 x 15 cm and the crops grown rainfed. The crops were weeded as necessary. Dates of sowing, application of insecticide (Endocel) for pod borer control, and harvest are indicated in Table 1. Tip samples of chickpeas, comprising the apical 6-8 cm with 4-5 pinnules, were taken from each replication of the 0, 0.5 and 1.0 kg ha^{-1} B treatments in the field of Khagendra Pathak at 117 days after sowing (DAS). The samples were oven dried at 72°C to constant weight and analysed for B by optical emission spectrometry (Zarcinas and Cartwright 1983) at Analabs, Ashoknagar, Hyderabad, India. The net area of each plot harvested for yield was $7.2m^2$.

Table 1: Site details and timing of operations of successful [1] on-farm trials with chickpeas in 1997/98, Chitwan Valley, Nepal

Farmer	Village	District	Site elevation	Date of sowing (1997)	Sprays endocel (DAS[2])	Date of harvest (DAS[2])
Vishnu Hare Ghimi re	Shukranagar	Chitwan	upland	20 Nov	112, 129	144
Chandra Singh Chaudhary	Shukranagar	Chitwan	lowland	5 Nov	78, 124, 141	154
Khagendra Pathak	Rajahar	Nawalparasi	upland	6 Nov	119, 131	155
Dhruba Lamichhane	Rajahar	Nawalparasi	lowland	30 Nov	107, 121	139

[1] Three of the seven on -farm trials failed. [2] DAS = days after sowing

In 1998/99, seven researcher-designed, farmer-managed on-farm trials were conducted in different farmers' fields (all upland) in the village of Rajahar, Nawalparasi District (Table 2). Both chickpea (var. Kalika) and mustard (*Brassica campestris* L. var. Vikas) were used as test crops, as mustard is known to be particularly sensitive to B deficiency. Two levels of B, 0 and 1.0 kg ha^{-1}, were tested in paired plots of 3 x 3.6m replicated either three or four times (Table 2). Boric acid, TSP, and MP were applied to chickpeas and mustard as described for chickpeas in the previous season, but 60 kg ha^{-1} N as urea was also applied to mustard. The crops were grown rainfed (but there was no rainfall during the growing period) and weeded as required. Details of sowing time, chemical application, and harvest are given in Table 2. The net plot area at harvest for both crops was $6.48m^2$, except for the crops of Bhawani Pathak and Dandapani Pathak who harvested their entire plots of mustard.

Lentil

A field experiment with four levels of B (0, 0.5, 1.0, 2.0 kg ha^{-1}) as boric acid and three levels of Zn (0, 2.0, 4.0 kg ha^{-1}) as zinc chloride, in factorial combination, was conducted at the

Table 2: Timing of operations of successful [1] on-farm trials with chickpeas and mustard in 1998/99, Rajahar, Nawalparasi District, Nepal						
Farmer	**Replications per farm**	**Date of sowing (1998)**	**Sprays to chickpea (DAS[2,3])**	**Sprays to mustard (DAS[2,4])**	**Harvest of chickpea (DAS[2])**	**Harvest of mustard (DAS[2])**
Khagendra Pathak	3	23 Oct	95, 113	75	129	98
Ramakant Pathak	3	11 Nov	73, 81	73	107	98
Bhawani Pathak	3	13 Nov	75, 81	75	121	89
Narayan Dutta Lamichane	4	13 Nov	75, 93	75	116	91
Dandapani Pathak	4	6 Nov	68, 86	68	136	97

[1] Two of the seven on -farm trials failed ; [2] DAS = days after sowing ; [3] Bavistin and Endosulphan ; [4] Endosulphan

GLRP Rampur station in 1997/98. Lentil variety ILL-4605 was grown. Cultural details are given in Srivastava et al. (1999). At 107 DAS, apical shoot samples, including the top five pinnules, were sampled from selected treatments and oven dried at 70 °C to constant weight. The plant samples were analysed for B by optical emission spectrometry (Zarcinas and Cartwright 1983).

Pigeonpea

A field experiment was conducted at the GLRP Station, Rampur, in 1998/99. The experiment was conducted using a split plot design replicated four times with three pigeonpea varieties in main plots and three B treatments in sub-plots. The pigeonpea varieties were: ICPL 87 (short duration), Rampur Rahar 1 (RR1: medium duration), and Bageswari (long duration). The B treatments were: no B applied, 1 kg B ha^{-1} soil applied; and 1 kg B ha^{-1} soil application with an additional foliar application of 0.03% B solution at flowering time of each variety.

The experimental field was harrowed twice and levelled by tractor-drawn implements. Basal fertiliser was applied to all experimental plots as 40 kg ha^{-1} P_2O_5 and 20 kg ha^{-1} K_2O, as TSP and MP respectively. Soil applied B, as borax, was mixed with friable soil and evenly broadcast over designated plots. After hand broadcasting of TSP and MP on all plots, all fertilisers were mixed in the soil to a depth of 10 cm with a spade. The plot size was 3 x 4m and plant spacings were 50 x 40 cm for ICPL 87 and RR1 and 75 x 40 cm for Bageswari. Seed was sown in furrows 50 cm apart, for ICPL 87 and RR1, and 75 cm apart for Bageswari. Two seeds were sown per hill at 40 cm spacing on 25 July 1998, and seedlings were later thinned to one plant per hill. The plots were hand weeded at 46 DAS. A spray of 0.03% B, to give an additional 1.0 kg B ha^{-1} above the basal soil application, was applied to ICPL 87 and RR1 at 90 DAS and Bageswari at 189 DAS. At these times, Thiodan was also sprayed to protect against pod-boring insects. Plants were harvested at maturity of ICPL 87 (after several flushes of flowers and pods); RR1 at 165 DAS (16 January 1999); and Bageswari at 251 DAS (2 April 1999). The two central rows were taken for grain-yield determination. Where plants were missing from these inner rows, mainly as a result of waterlogging at the seedling stage, plants from border rows were used so that an even number of plants was sampled per plot.

Results and Discussion

In treatments omitting B at the GLRP Rampur station, chickpea plants showed B deficiency symptoms of 'little leaf' and tip yellowing from about 30 DAS and persisting to maturity. Absence of B also induced flower drop and, subsequently, poor podding (Srivastava et al. 1997). There were positive responses of chickpea to B in terms of yield increases in both seasons at Rampur, with maximum yield being reached in 1995/96 at 0.5 kg ha^{-1} B (Figure 1). There was also a significant Mo response in the first year, but no Mo response in the second year (Srivastava et al. 1997). Boron response appeared stronger in the first year than in the second year. Plants were limited by terminal drought stress in the first year, perhaps exacerbating the effect of B deficiency in limiting pod set. Other factors may also have been involved such as possible differences in availability of soil B between the experimental sites of different years (soil B levels not measured in 1995/96), and suppressive effects of other nutrients on B in the omission experiment (Srivastava et al. 1997). With the application of 3 kg ha^{-1} B, there was greater flower drop than at lower B application rates, suggesting a narrow range of B sufficiency and the danger of B toxicity at rates of 3 kg ha^{-1} B or above.

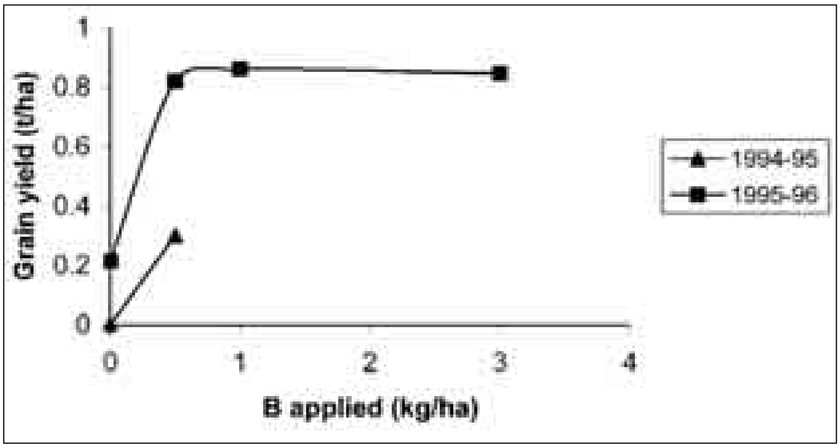

Figure 1: **Effect of application of B on grain yield of chickpeas in 1994/95 and 1995/96, Rampur, Chitwan Valley, Nepal**. Notes: in 1994/95 least significant difference (LSD) between treatments = 0.016 t/ha; in 1995/96 LSD for all treatments = 0.22 t/ha (mean value of 0, 0.15 and 0.3 kg ha^{-1} Mo treatments, as there was no significant effect of Mo treatment). Data derived from Srivastava et al. (1997).

In plots without applied B, there was clear development of B deficiency symptoms in lentils in 1997/98, from about 30 DAS (Srivastava et al. 1999). Chlorosis developed from the shoot tips, with a progressive reduction in sizes of younger leaves ('little leaf' symptoms). Eventually terminal buds died and the number of lateral branches increased, resulting in stunting of plants. These symptoms were most severe in introduced varieties, mainly 'ILL' introductions from ICARDA. Such symptoms were much less severe in local landraces of lentils such as Simul, Sindur, and Simrik (Srivastava et al. 1999). A similar effect was observed in chickpeas at the Rampur station, introduced chickpea lines, such as those from ICRISAT, had more severe B deficiency symptoms than 'local' varieties. Thus local varieties of chickpeas and lentils appear to have adapted to situations of low available soil B. According to the manifestations of symptoms, it appears that there are substantial genotypic differences in susceptibility to B deficiency, and thus scope for genetic improvement in ability to acquire B.

There was a more than ten-fold response of lentil grain yield to B, but with maximum yield reached at 0.5 kg ha^{-1} B (Figure 2). Grain yield also responded significantly to Zn application from 0 to 2 kg ha^{-1}, however, at the higher B rates there was a decrease in yield with increasing Zn from 2 to 4 kg ha^{-1} (Figure 2).

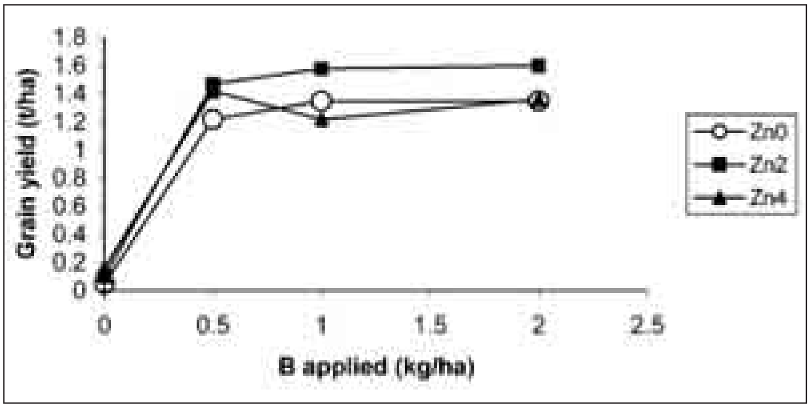

Figure 2: **Effect of application of B and Zn on grain yield of the lentil cultivar ILL-4605 in 1997/98, Rampur, Chitwan Valley, Nepal**. Note: Zn0, Zn2 and Zn4 = 0, 2 and 4 kg/ha Zn, respectively, LSD for all treatments = 0.20 t/ha. Data derived from Srivastava et al. (1999)

Significant responses in grain yield to B application were found in all genotypes of pigeonpea tested, and there was an additional response to foliar application of B (Table 3). However, even with maximum B application, yields were well below the potential yields of these varieties. This was because of poor physiological adaptation to this environment (ICPL 87), loss of plant stands through early waterlogging, and insect damage to flowers and pods. Nevertheless, despite the other constraints affecting yield, a clear response to B was apparent. The further increase in yield with foliar B application suggests that the optimum B level may not have been reached, and higher rates and frequencies of B application might

Table 3: **Grain yield (kg ha^{-1}) of three varieties of pigeonpea at three levels of B application, Rampur, Nepal, 1998 -99**

Variety	B level (kg ha^{-1})			
	0	1 (soil applied)	1 (soil) + foliar spray	Mean
ICPL 87	0	87	151	79
RR1	84	319	417	273
Bageswari	34	270	324	209
Mean	*39*	*225*	*297*	
	SE	LSD (0.05)		
Variety**	19.6	73.4		
Boron**	9.5	48.8		
Variety x boron**	23.8	68.1 across boron levels		
		28.2 across varieties		
CV (%) (main plot)	36.2			
CV (%) (sub-plot)	17.6			
** Significant at P < 0.01				

have increased yield levels beyond those presently obtained. In Bageswari, pod numbers per plant were also recorded and these increased with B treatment to the extent of 87, 140, and 154 pods/plant. Thus the impact of B deficiency on pod numbers is consistent with its general effect in legumes in causing flower and pod abortion.

At all B levels, RR1 had higher yields, consistent with its better adaptation to the Rampur environment, with respect to environmental factors in general and perhaps also with its ability to acquire B from B-deficient soil. Without B application, ICPL 87 could not form any pods. Despite low yield levels, these results show that B deficiency is limiting pigeonpea yield at this location. Further studies are needed to determine optimum rates, timing, and methods of B application to achieve maximum yields in different pigeonpea varieties. Further studies are also needed to understand the extent of genotypic differences in the response of pigeonpea crops to B.

In the on-farm trials with chickpeas in 1997/98, a significant response to B was only recorded in the fields of Khagendra Pathak (Table 4). There were indications of a B response in the fields of the other three farmers who harvested the trials but these did not reach statistical significance. However, it was noted that Vishnu Hari Ghimire applied compost and manure to the crop preceding the chickpea crop, and this may have contributed to the high yields but lack of B response. The chickpea yields of the other two farmers were low, indicating that other constraints to chickpea growth, such as excessive soil moisture for much of the growing period, may have masked any B response. No response to Mo was recorded in these trials (Table 4).

Large positive responses of both chickpea and mustard to B application were found in farmers' fields in 1998/99 (Table 5). The extent of the response was slightly larger in mustard, a crop known to be sensitive to B deficiency. It is clear that B deficiency is widespread across this region. The variation in response between farmers' fields may have been influenced by previous applications of organic manure, a common practice in the region. Soils with more organic matter appeared to be less prone to B deficiency, although a comprehensive analysis of this aspect was not undertaken in the present studies.

Initial soil analyses did not indicate any clear relationship between hot water soluble soil B levels and response of legumes to B application, although all soil analyses carried out indicated levels well below the reported critical level of 0.5 mg kg^{-1} B (Tandon 1993). The possibility of using plant tissue analysis to diagnose B deficiency was assessed. Figure 3 indicates the relationship between relative grain yield and B concentration in shoot tips of chickpeas at the vegetative stage. The B concentration at 80-100% yield is in the range of 15-20 ppm B, which can be considered to be the critical range of B concentration. This value corresponds with the critical value of 20 ppm B found in chickpea shoots at the pre-flowering stage by Sakal et al. (1990). Thus it appears possible to diagnose deficient, marginal, and adequate B concentrations in chickpeas. In lentils, the B concentration in shoot tips from plots without B was 5.7 ± 0.3 ppm. In plots with 0.5 kg ha^{-1} B but no Zn it was 26.7 ± 3.5 ppm and in plots with 0.5 kg ha^{-1} B and 2 kg ha^{-1} Zn it was 23.7 ± 1.2 ppm. Thus the critical value for B in shoot tips of the lentil cultivar ILL-4605 lies within the range 5-25 ppm. More detailed studies than provided by these preliminary analyses would be required to define critical B values of lentils definitively (Srivastava et al. 1999).

Table 4: Effect of B and Mo application on grain yield (kg ha^{-1}) of chickpeas in farmers' fields in the Chitwan Valley, 1997/98 season				
Treatment	Farmer			
	Khagendra Pathak	Vishnu Hari Ghimire	Chandra Singh Chaudhary	Dhruba Lamichhane
Control	213	842	244	556
O.5 B	1,080	1,213	383	700
1.0 B	1,192	1,190	283	519
0.5 B + 0.15 Mo	1,148	1,289	297	517
1.0 B + 0.15 Mo	1,144	935	287	703
Significance [1]	P < 0.01	ns	ns	ns
LSD (0.05) [2]	189	-	-	-
CV (%) [3]	10.5	22.3	21.3	21.3

[1] Statistical significance of difference s between means , ns = not significant ; [2] least significant difference at P = 0.05; [3] coefficient of variation

Table 5: Effect of B application on grain yields (kg ha^{-1}) of chickpeas and mustard in farmers' fields in the Chitwan Valley, 1998/99 season						
Farmer	Chickpea			Mustard		
	B applied (kg ha^{-1})		*% increase due to B[1]*	B applied (kg ha^{-1})		*% increase due to B[1]*
	0	1.0		0	1.0	
Khagendra Pathak	287	849	*196**	285	664	*133***
Ramakant Pathak	448	702	*57**	99	257	*160**
Bhawani Pathak	441	615	*39 ns*	46	181	*293 ns*
Narayan Dutta Lamichane	525	1,168	*122***	60	276	*361***
Dandapane Pathak	764	1,364	*79**	201	443	*120***

[1] Statistical significance of difference , ns = not significant; * = P < 0.05; ** = P < 0.01

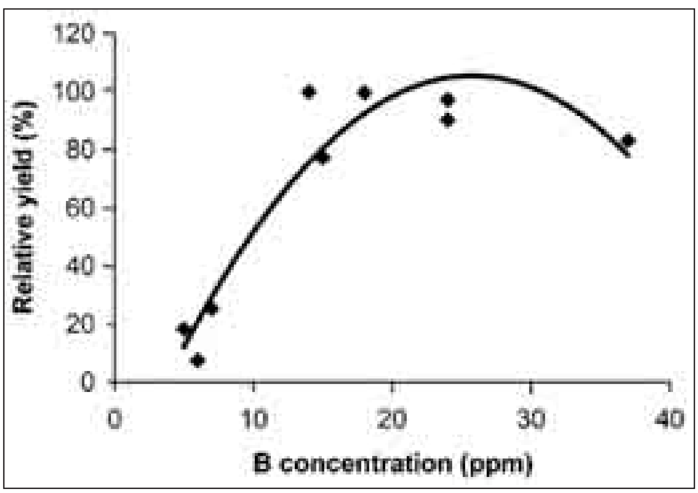

Figure 3: **Relationship between relative grain yield and B concentration in shoot tips of chickpeas grown at different B application rates in the field of Khagendra Pathak, Rajahar, Nawalparasi District, Chitwan Valley, Nepal, 1997/98**

To date, we have sporadic information, in time and space, that B is a major limitation to grain legumes, as well as other crops such as *Brassica* spp., in the Chitwan Valley. A systematic spatial analysis through geographic information system (GIS) analysis is required to determine the extent of B deficiency across Nepal. Plotting of soil extractable B levels is not enough, as we are unsure of the critical soil B levels as they apply to each crop and under different soil and climatic conditions. Relationships between extent of B response and the various soil and climatic factors that could affect B response need to be examined. Soil factors would, of course, not only include soil B level, but also other factors such as pH, soil organic matter, cation exchange capacity, and so on. To achieve this, more on-farm B response data are needed across representative soil and climatic zones. Outputs of such an exercise would allow mapping of probability and likely extent of a response to B for a range of crops (Bell 2004). This would then provide a basis for planning and implementing corrective measures.

Other micronutrients, such as Mo and Zn, also limit yield of grain legumes in Nepal, and thus the proposed GIS study should be comprehensive for all micronutrients suspected to be limiting. Further, the interaction between B and other nutrients, and various soil and climatic factors, needs better understanding in order to interpret the likelihood of B response.

Once the likelihood of B response is known, there are various options for overcoming the deficiency which are suitable for implementation by resource-poor farmers. These are primarily soil and foliar applications (Murphy and Walsh 1972) with the B application rates required (e.g., 0.5 kg ha^{-1} B for chickpeas and lentils, but perhaps more for pigeonpeas) being low enough not to commit resource-poor farmers to substantial investments in B fertiliser. The large genotypic differences in B response apparent in chickpeas and lentils at least suggest that breeding of B-efficient varieties is a longer-term option.

To extend this knowledge to farmers, a series of farmer-managed evaluations of B response is advocated. It is suggested that these be conducted in plots at an operational scale (similar plot sizes to those the farmer normally cultivates) divided between a B application treatment and no B application. This would have the effect of allowing farmers to assess for themselves whether B application is efficacious and demonstrate the effect to neighbouring farmers. It would also provide further feedback on B responsiveness for risk analysis of B response and on constraints to adopting methods of alleviating B deficiency.

If the value of grain legume cultivation is to be realised in Nepal, it is necessary to address systematically the micronutrient constraints to these legumes, which in the case of B at least can be severe. Increased legume cultivation is required to improve both soil health and human health. Adequate uptake of micronutrients by legumes is needed, not only to meet plant requirements but also to meet the consumption requirements of humans and their domestic animals (Welch and Graham 2002).

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