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A COMPARISON OF THE EFFECTS OF MICRONUTRIENT SEED PRIMING AND SOIL FERTILIZATION ON THE MINERAL NUTRITION OF CHICKPEA (*Cicer arietinum*), LENTIL (*Lens culinaris*), RICE (*Oryza sativa*) AND WHEAT (*Triticum aestivum*) IN NEPAL

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SUMMARY

Soil deficiencies of zinc (Zn) and boron (B) limit crop production in Nepal. Improving the micronutrient status of plants would increase yield and increase micronutrient content of the seeds, leading to better nutrition of the progeny crop and to improved human micronutrient nutrition. The primary micronutrient problem in grain legumes is B deficiency, while in rice (*Oryza sativa*), Zn deficiency is more important, and wheat (*Triticum aestivum*) suffers from both deficiencies. A series of field experiments was carried out over two seasons to compare soil fertilization and micronutrient seed priming as methods of improving Zn and B nutrition of each crop. Micronutrient treatments were evaluated for their effect on grain yield and grain micronutrient content. Soil B fertilization increased B content of the grain of lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), and wheat by a factor of two to five, while increasing the yield of chickpea only. Soil fertilization with Zn had no effect on yield of any crop, but resulted in a small increase in Zn in wheat grain. Sowing micronutrient-primed seeds had no effect on yield or micronutrient content of the progeny seeds in most cases. During the first season, the primed chickpea seeds failed to emerge at either site, causing complete yield loss, but this negative effect was not observed in the second season with similar priming treatments at nearby sites, and no effect of priming on yield was observed with any other crop in either season.

INTRODUCTION

There are two mutually reinforcing reasons to increase the micronutrient content of the grains of major food crops in South Asia: improvement of the agronomic productivity of the crop and improvement of the nutritional value of staple foods for humans. Agronomically important micronutrient deficiencies have been reported frequently in the rice-wheat cropping system. Zinc (Zn) deficiency is a common constraint for paddy rice (*Oryza sativa*) production across Asia (Lopes, 1980). For wheat (*Triticum aestivum*) and

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grain legumes such as chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*), which are often substituted into the cropping rotation in place of wheat, boron (B) deficiency has been a significant cause of yield loss (Rawson, 1996; Srivastava *et al*., 2000; Subedi *et al*., 1998). Since molybdenum (Mo) is required for nitrogenase, one of the enzymes involved in nitrogen fixation, it is has been suggested that some leguminous species growing in acid soils may be Mo-deficient (Mandal *et al*., 1998), even though plant Mo requirement is very low. Micronutrient deficiencies are also becoming increasingly apparent in human nutrition. It has been estimated that roughly 40 % of people throughout the world suffer from micronutrient malnutrition, most commonly from iron, zinc, iodine, or various vitamin deficiencies (Welch *et al*., 1997). Zinc deficiency has been shown to cause dermatitis, poor wound healing, impaired immunity and growth failure in humans (van Campen, 1991). Zinc improves human immune function (Shankar and Prasad, 1998), cognitive development and resistance to some infectious diseases, including diarrhoea (Fuchs, 1998; Black, 1998). Supplying the vegetative parts of crops with sufficient micronutrients during critical growth phases may be adequate to solve the immediate agronomic need, but to improve human nutrition, it is necessary to enrich the edible parts of the plants. Evidence suggests that sowing seeds enriched in micronutrients is also agronomically beneficial (Marcar and Graham, 1986; Rerkasem *et al*., 1990; Welch, 1986).

There are three main methods of adding micronutrients to crops: soil fertilization, foliar sprays and seed treatment. Each method has the potential to affect plant micronutrient nutrition both in the treated plant directly and in the progeny plants through enrichment of the seeds by micronutrient treatment of the parent. Foliar applications of micronutrient sprays have been effective towards both goals (Wilhelm *et al*., 1988; Savithri *et al*., 1999), but this method is too expensive to be widely practised by resource-poor farmers in some regions because of the amount of fertilizer, equipment and labour required for repeated spraying. Likewise, the difficulty in obtaining high quality micronutrient fertilizers and spreading them evenly on the soil can be prohibitive. Treating seeds with micronutrients potentially provides a simple inexpensive method for improving micronutrient plant nutrition. Farmers in South Asia have responded favourably to seed priming, a simple technology of soaking seeds overnight in water prior to sowing (Harris, 1997; Harris *et al*., 1999). Seed priming in water has been shown to decrease time between sowing and emergence and to improve seedling vigour (Harris, 1996; Parera and Cantliffe, 1994). Priming seeds in solutions of macro or micronutrients has been shown to improve yield of rice (Peeran and Natanasabapathy, 1980), wheat (Khalid and Malik, 1982; Marcar and Graham, 1986; Wilhelm *et al*., 1988) and forage legumes (Sherrell, 1984), but the potential to damage the seed and inhibit germination by priming at high nutrient concentrations has also been reported (Roberts, 1948).

The first objective of this research was to identify a safe procedure for micronutrient seed priming of rice, wheat, chickpea and lentil with Zn, B and Mo. The second objective was to compare the effects of this method of micronutrient application vs. soil fertilization on plant growth and yield and on the micronutrient content of the progeny seeds.

MATERIALS AND METHODS

Micronutrient priming method development

Micronutrient seed priming experiments were done in the laboratory to determine optimal priming solution concentrations before testing the procedure in the field. The laboratory seed priming germination tests were done with three legumes: chickpea, lentil and cowpea (*Vigna unguiculata* v. Prakash), using solutions at three concentrations each of Zn, B and Mo. There were three replications of each solution/species combination, plus three replications of an unprimed control of each species. After an initial experiment to determine the appropriate concentration range and soaking times for the different species (data not reported), the priming solution concentrations chosen for the germination test were: 0.0008 M, 0.004 M and 0.02 M zinc sulphate ($ZnSO_4 \cdot 7H_2O$); 0.0016 M, 0.008 M and 0.04 M boric acid (H_3BO_3); 0.00052 M, 0.0026 M and 0.013 M sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$). Each treatment involved weighing approximately 3 g of seed into a plastic cup, adding 15 ml of the appropriate priming solution (sufficient to submerge the seeds), and allowing the seed/solution mixture to sit covered with plastic for 12 h. The soaking time was followed by rinsing three times in water to remove excess salts from the seed coat. For each replicate observation, ten seeds from each priming treatment were then placed for a germination test in a Petri dish containing No. 2 filter paper (Advantec MFS, Inc., approximately equivalent to Whatman No. 1) that had been thoroughly moistened with water. The Petri dishes were placed in the dark, and germination was checked once a day for 6 d. The filter paper in the Petri dishes was changed once after the first 24 h (in case toxic amounts of micronutrients, which might inhibit seedling growth after germination, were leached from the seed coat into the paper during the first day). At each germination check, the total number of germinated seeds was recorded. Germinated seedlings were measured by stretching out each seedling to its full root-shoot length on a ruler and categorized by length into 5 groups (0–2, 2–3, 3–5, 5–7, > 7 cm).

The concentration of B, Zn and Mo in the chickpea and lentil seeds following priming in micronutrient solutions was measured after dry ashing 0.8 g ground samples at 450 °C for 12 h, followed by a second 2 h ashing at 450 °C with 0.25 ml 30 % hydrogen peroxide, followed by dissolution in 0.25 ml 6 M hydrochloric acid and dilution to 10 ml with 5 % (v/v) nitric acid. The solutions were analysed by inductively coupled plasma (ICP) atomic emission spectroscopy (using a Spectro Ciros^{CCD} ICP instrument, Kleve, Germany), using standards that varied in concentration from 0.5 to 5 $\mu g\,ml^{-1}$ for Mo and from 1 to 10 $\mu g\,ml^{-1}$ for B and Zn.

Field experiments

Location and cropping sequence. All the experimental sites were in the Chitwan district of Nepal, southwest of Kathmandu and bordering India, at an approximate elevation of 190 m asl. Sites labelled 'R' were on the campus of the Institute of Agriculture and Animal Science (IAAS) in Rampur (27° 37′N, 84° 25′E). Sites labelled 'T' and 'M' were on farmers' fields in Tandi and Mangalpur, respectively, villages that are located

Table 1. Soil characteristics in field experiment sites.

Site	pH†	Organic matter‡	Available nutrients¶						
			NO_3-N	P	K	Zn	B	Mn	Cu
		%	mg kg^{-1}, oven dry weight basis						
R1	5.1	2.1	8.0	1.4	52	0.3 ± 0.1	0.10 ± 0.03	14.8 ± 0.9	0.53 ± 0.08
R2	5.3	2.4	5.2	1.2	32	0.25 ± 0.08	0.109 ± 0.008	5.7 ± 1	0.71 ± 0.09
R3	5.4	2.3	0	1.3	36	0.23 ± 0.03	0.081 ± 0.006	6.7 ± 1	0.67 ± 0.07
R4	nd	nd	nd	nd	nd	0.7 ± 0.2§	0.05 ± 0.01	nd	nd
T1	7.3	2.1	25	7.1	26	1.6 ± 0.5	0.10 ± 0.01	8.2 ± 1	6.1 ± 0.3
T2, T3	5.5	2.0	0	2.8	32	1.2 ± 0.1	0.09 ± 0.01	41 ± 3	1.6 ± 0.1
M	6.6	2.7	28	0.8	39	0.29 ± 0.08	0.081 ± 0.007	3.3 ± 2	1.3 ± 0.2

† In water at 1:1 (by volume) soil:water ratio.
‡ Converted from loss on ignition (%) according to formula: (0.7 × LOI) − 0.23 = OM (Storer, 1984).
¶ NO_3-N, P, K, Ca, Mg, Al were extracted with 10% sodium acetate in 3% acetic acid (pH 4.8) (Morgan's solution). Zn, Mn, Fe, Cu, and Ni were extracted with DTPA-TEA, except where noted. B and S were extracted with hot 0.01 M $CaCl_2$. Where measurements were replicated, one standard deviation is noted.
§ Measured by DTPA-ammonium bicarbonate extraction, which generally extracts twice as much Zn as the DTPA-TEA extraction.
nd: No data.

within 20 km of Rampur. Different numbers following the same letter in a site label indicate that the two experiment sites were on separate but adjacent plots. Table 1 provides soil characteristics for each site. The first winter season was October 2001 to April 2002, the second season summer was July to October 2002, and the second season winter was October 2002 to April 2003. The following crops were grown at each site during the given season: R1 and T1 had chickpea and lentil during the first winter; R2 and T3 had lentil and R3 and T2 had chickpea during the second winter; R4 had rice during the second season summer and wheat during the second season winter; M had lentil during the second winter.

Chickpea and lentil. All chickpea and lentil experiments were designed as single-factor experiments laid out in randomized complete blocks, with micronutrient treatments as levels. The number of blocks and number of treatments varied by year and by space available in the farmers' fields. Treatment details are provided in Table 2. All R sites had four blocks; sites T1, T2 and M had three blocks; site T3 had two blocks.

Treatment plot size was 4 × 3.2 m or 12.8 m^2 at all sites (with harvested area 8.9 m^2 for chickpea and 10.4 m^2 for lentil). Chickpea was sown at a rate of 47 kg ha^{-1} into eight rows/plot, at 40-cm row-to-row and 10-cm plant-to-plant spacing. Lentil was sown at a rate of 30 kg ha^{-1} into 16 rows/plot, at 20-cm row-to-row spacing and continuous plant-to-plant spacing. Macronutrient fertilizer application rates were 20 kg N, 17 kg P and 17 kg K ha^{-1} for all plots, applied basally as a mixture of urea, diammonium phosphate and muriate of potash, all obtained from the local market. Experiments were hand-weeded and treated with pesticides as needed. The most important pest problems were pod borer (*Helicoverpa armigera*) in chickpea at all sites and fungal disease in lentil at site M. In the first year, all the seeds were treated with the fungicide Vitavax 200® (carboxin and thiram, Uniroyal Chemical, USA) prior to sowing; in the second

Table 2. Summary details of field experiment treatments.

Crop	Treatment label	Treatment details†	Sites at which treatment was included
Chick-pea and lentil	Control	No micronutrient treatment	R1, R2, R3; T1, T2; T3, M
	H_2O soak	Water primed control (no micronutrient treatment)	R2, R3; T2, T3; M
	B soak	Seed primed in 0.008 M H_3BO_3	R1, R2, R3; T2, T3; M
	Zn soak	Seed primed in 0.004 M $ZnSO_4{\cdot}7H_2O$	R1, R2, R3; T2, T3; M
	B+Zn soak	Seed primed in mixture Zn/B solution: first year: 0.004 M H_3BO_3 and 0.002 M $ZnSO_4{\cdot}7H_2O$ second year: 0.008 M H_3BO_3 and 0.004 M $ZnSO_4{\cdot}7H_2O$	R1, R2, R3; T1, T2, T3; M
	B fertilization	Untreated seed sowed in soil fertilized with 1.5 kg B ha^{-1}‡	R2, R3; T2, T3; M
	Zn fertilization	Untreated seed sowed in soil fertilized with 5.7 kg Zn ha^{-1}‡	R2, R3; T2, T3; M
	B+Zn fertilization	Untreated seed sowed in soil fertilized with 1.5 kg B and 5.7 kg Zn ha^{-1}	R1, R2, R3; T1, T2, T3; M
Rice and wheat	Soil: control	No micronutrient fertilizer applied to soil	R4
	Soil: B fertilization	1.1 kg B ha^{-1}, applied to soil 20 months prior to sowing rice§	R4
	Soil: Package fert	Package of micronutrient fertilizers, applied to soil 20 months prior to sowing rice§: 1.1 kg B, 5.7 kg Zn, 0.4 kg Mo, 1.5 kg Cu, 8 kg Mn ha^{-1}	R4
	Seed: Control	Rice: Unprimed seed Wheat: Water-primed seed	R4
	Seed: B soak	Seed primed in 0.008 M H_3BO_3	R4
	Seed: Zn soak	Seed primed in 0.004 M $ZnSO_4{\cdot}7H_2O$	R4

† All plots received the recommended application of NPK to the soil immediately prior to sowing. Seed priming was done by submerging the seeds in the priming solution the night before sowing, for the following amounts of time: lentil, wheat: 12 h; chickpea: 12 h (first year), 8 h (second year); rice: 36 h.

‡ B and Zn fertilizers for chickpea and lentil were applied as laboratory reagents boric acid (H_3BO_3, FW 61.83, ~17% B by weight) and zinc sulphate ($ZnSO_4{\cdot}7\ H_2O$, FW 287.54, ~ 23% Zn by weight) respectively.

§ Micronutrient fertilizers were applied to the rice/wheat site as borax at 10 kg borax ha^{-1} (11 % B by weight), zinc sulphate ($ZnSO_4{\cdot}7\ H_2O$, FW 287.54, 23% Zn by weight), sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$, FW 241.95, 40% Mo by weight), copper sulphate ($CuSO_4{\cdot}5H_2O$, FW 249.68, 25.5% Cu by weight), and manganese sulphate ($MnSO_4{\cdot}H_2O$, FW 169.01, 32.5 % Mn by weight). Borax was purchased as a fertilizer in the local market; all others were laboratory reagents.

year, only the lentil seeds sowed at site R2 received the fungicide. When used with unprimed seeds, the powdered fungicide was mixed with dry seeds 2–3 days before sowing at a rate of 3 g fungicide kg^{-1} seed dry weight. For primed seeds, the fungicide was mixed with seeds after priming and air-drying, immediately before sowing, at the same rate of 3 g kg^{-1} dry seed weight (the amount of fungicide was calculated based on their seed dry weight rather than the post-priming weight). Nutrient analyses of seeds and chaff were done by ICP analysis after dry ashing as described above.

All data from chickpea and lentil experiments were statistically analysed using SPSS® software to compute analysis of variance (ANOVA). Each year at each site

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was analysed as a separate experiment due to changes in experimental design between sites and between years at the same site.

Rice and wheat. The purpose of the rice and wheat experiments was to compare the effects of residual soil-applied micronutrient fertilizer with seed priming in micronutrient solutions on growth, yield and seed micronutrient content. The rice and wheat experiments were conducted in two consecutive seasons on the same site (R4). The experimental design was identical for the two crops: a split-plot experiment with five replications, with residual soil-applied micronutrients as the main plot treatments (three factors) and micronutrient seed priming treatments as the three subplots within each main plot (see Table 2 for soil and seed treatment details). The main plot size was 5×3 m, or 15 m^2, with a subplot harvested area of 3.8 m^2 for rice and 2.8 m^2 for wheat.

The micronutrient soil treatments were applied in November 2000, about 20 months prior to the beginning of the current rice study in July 2002, and three seasons of crops had been grown on the site without reapplication of micronutrients during that 20-month period (winter 2000/01 wheat or legumes, summer 2001 rice, winter 2001/02 wheat or legumes). Soil tests confirmed that B and Zn levels were still elevated in the plots to which they had been added as fertilizers nearly two years earlier compared with control plots, although the difference was small in the case of B (data not shown). NPK fertilizers were applied uniformly in every plot at recommended rates for each crop (for rice, 100 kg N, 13 kg P, and 25 kg K ha^{-1}, with N applied in three splits: 50 kg ha^{-1} incorporated into the soil at time of planting, 25 kg ha^{-1} broadcast at midtillering and again at panicle initiation stages; for wheat, 100 kg N, 22 kg P, and 42 kg K ha^{-1}, all incorporated at time of planting). Micronutrient fertilizers were not reapplied. Micronutrient treatment details are provided in Table 2.

Both crops were weeded and treated with pesticides as needed. The most severe pest problem in either crop was an attack of rice blast (*Pyricularia oryzae*), after which the last time-split of N fertilizer was eliminated in an attempt to control the disease. Rice grain yields are reported on a 14 % moisture content basis. Leaf tissue from transplanted rice seedlings and harvested seeds from each plot of each crop were dry ashed and analysed by ICP for nutrient content as described above.

All data from the rice and wheat experiments were statistically analysed as a split-plot experiment using the PROC GLM procedure of the SAS® program to obtain the analysis of variance (ANOVA) tables and to study which treatment differences were significantly different by Tukey's honest significant difference test.

Soil sampling and analysis

Soil samples were collected (as composite samples from the surface 0.15 m) from each control plot (with no micronutrient treatment) of each legume experiment at the time of harvest. Soil samples were collected from the control plots of the rice-wheat experiment periodically throughout both seasons and analysed on the day of collection, while still wet. They were then dried, and the moisture content was calculated and used to correct the soil weight and extracting solution volume so that the analytical

results could be expressed in terms of oven-dried soil weight. There were no significant differences over time in the Zn and B contents of the soil, so an average of several different sampling times is listed in Table 1.

For the legume experiments, Zn, manganese (Mn), iron (Fe), copper (Cu) and nickel (Ni) were extracted with the diethylenetriamine-pentaacetic acid-triethanolamine (DTPA-TEA, pH 7.3) method (Lindsay and Norvell, 1978). Nutrient concentrations were determined in extracted solutions by ICP analysis. For the rice-wheat experiment, Zn was extracted with the DTPA-ammonium bicarbonate (DTPA-AB) procedure (Soltanpour and Schwab, 1977). The DTPA-AB solutions were analysed for Zn by atomic absorption (AA) spectroscopy (using a Solar Unicam 929 Atomic Absorption Spectrometer) after acidification to 10 % (v/v) nitric acid to prevent clogging of the nebulizer.

Hot water extractions were done to measure the amount of 'plant-available' B in the soil. A practical modification of the procedure of Jeffrey and McCallum (1988) and Mahler *et al*. (1984) was utilized to extract B from low-B soils. Leakproof centrifuge tubes with screw caps were used to heat soil and extracting solutions. The centrifuge tubes were placed in a large (4 l) beaker of boiling water for 14 min, followed by immediate filtration through Whatman No. 42 filter paper while the solution was still hot. After filtration, the samples were analysed for B by ICP.

RESULTS

Micronutrient priming method development

Priming at the low and medium concentrations of each nutrient increased the rate of germination of the relatively large chickpea and cowpea seeds compared to an unprimed control, but not of the small lentil seeds (Figure 1a). After 5 d, all species had similar germination (approximately 100 %) in all solutions, except for the reduction in germination of chickpea in the most concentrated Mo solution and cowpea in the most concentrated B solution (Figure 1b). Chickpea and lentil seedling lengths were inhibited by the highest concentration of each nutrient, even though in most cases, germination was not inhibited at the same concentration (Figure 1c). Cowpea seedling growth was inhibited by the highest concentration of B and Zn, and by the two highest concentrations of Mo (Figure 1c).

Effect of micronutrient priming on nutrient content of primed seed

Priming chickpea seeds in solutions of optimal concentration of micronutrients (as determined by the method development experiments) resulted in an increase in Zn content from about 40–60 (unprimed) to 500–800 mg kg^{-1} (Zn-primed), an increase in B content from about 10 to 80–100 mg kg^{-1} (B-primed) and an increase in Mo content from about 3 to 300 mg kg^{-1} (Mo-primed) (Table 3). Priming lentil seeds had similar results (Table 3). There was no difference in Zn or B content of seeds primed for 8 h compared to those primed for 12 h in solutions with the same nutrient concentration. However, increasing the concentration of B and Zn in the priming solution increased

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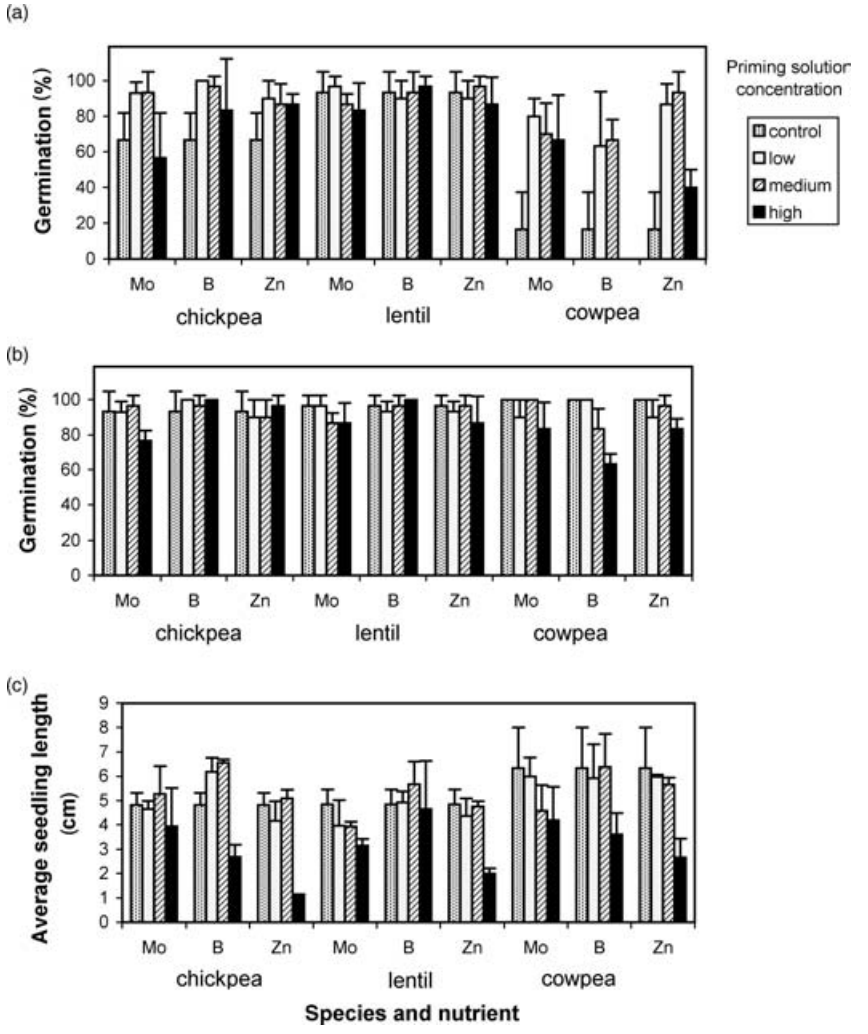

Figure 1. Germination of seeds after 1 d (a) and 5 d (b) and average seedling length after 5 d (c) of chickpea, lentil, and cowpea following priming in micronutrient solutions of different concentrations, compared to an unprimed control. Each bar is the average of three replicates of 10 seeds each. Error bars indicate standard deviation. There was only one control treatment (three replicates) for each species, so it was repeated with each micronutrient for comparison. Priming solution concentrations were : Mo: 0.00052 M, 0.0026 M and 0.013 M sodium molybdate; B: 0.0016 M, 0.008 M and 0.04 M boric acid; Zn: 0.0008 M, 0.004 M and 0.02 M zinc sulphate.

the amount of nutrient taken into both chickpea and lentil seeds (Table 3). Priming rice and wheat seeds in solutions of the same micronutrient concentration that were optimal for chickpea and lentil resulted in an increase in the Zn and B content of the primed seed (Table 4).

Based on a seed rate of 45 kg ha^{-1}, the amounts of B, Zn and Mo added to chickpea through priming were about 4 g ha^{-1}, 32 g ha^{-1} and 13 g ha^{-1} respectively. For lentil at a seed rate of 30 kg ha^{-1}, the amounts of B and Zn added through priming were

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Table 3. Content of Zn, B, and Mo in chickpea and lentil seeds from seed priming experiments. Each value is the mean of three analytical replicates.

		Seed content (mg kg^{-1})					
		Chickpea			Lentil		
Treatment category	Treatment	Zn	B	Mo	Zn	B	Mo
		mg kg^{-1}					
Seed source	(purchased)	40	9	3	50	6	2
Priming†	water	60	10	4	50	6	2
	B	60	100	3	50	100	2
	Zn	700	7	3	630	5	2
	½(B + Zn)‡	400	50	2	400	50	2
	B + Zn	800	80	3	660	100	2
	B, 12 h	40	100	3			
	Zn, 12 h	500	8	2			
	Mo	60	4	300			

† Priming times were 8 h for chickpea and 12 h for lentil, unless otherwise noted. Priming solutions were 0.004 M $ZnSO_4 \cdot 7H_2O$ (for Zn), 0.008 M H_3BO_3(for B), 0.0026 M $Na_2MoO_4 \cdot 2H_2O$ (for Mo), unless otherwise noted.
‡ For both chickpea and lentil, solution concentration was 0.002 M $ZnSO_4 \cdot 7H_2O$ and 0.004 M H_3BO_3. For chickpea, priming time was 12 h rather than 8 h.

Table 4. Content of Zn and B in rice and wheat seeds primed in micronutrient solutions. Each value is the mean of three analytical replicates.

	Seed content (mg kg^{-1})			
	Rice		Wheat	
Priming treatment†	Zn	B	Zn	B
	mg kg^{-1}			
Unprimed	25	1.5	35	0.9
Water	–	–	35	0.7
B	25	12	35	20
Zn	170	1.5	350	2

† Priming times were 36 h for rice and 12 h for wheat. Priming solutions were 0.004 M $ZnSO_4 \cdot 7H_2O$ (for Zn) and 0.008 M H_3BO_3 (for B).

about 2.8 and 18 g ha^{-1} respectively. Micronutrients added through priming for both species were substantially less than the fertilizer recommendations of 1 kg B, 5.7 kg Zn and 1 kg Mo ha^{-1}.

Effect of micronutrient treatments on grain yield

Chickpea. In the first season, there was no plant emergence from any of the micronutrient seed priming treatments at either site (Sites R1 and T1, Figure 2), and, consequently, no grain yield. During the second season, the same seed priming treatment did not have a negative effect on seedling emergence, and no effect on grain yield (Sites R3 and T2, Figure 2). Soil fertilization with a combination of Zn and B resulted in significant yield increases compared to the control at sites R1 and

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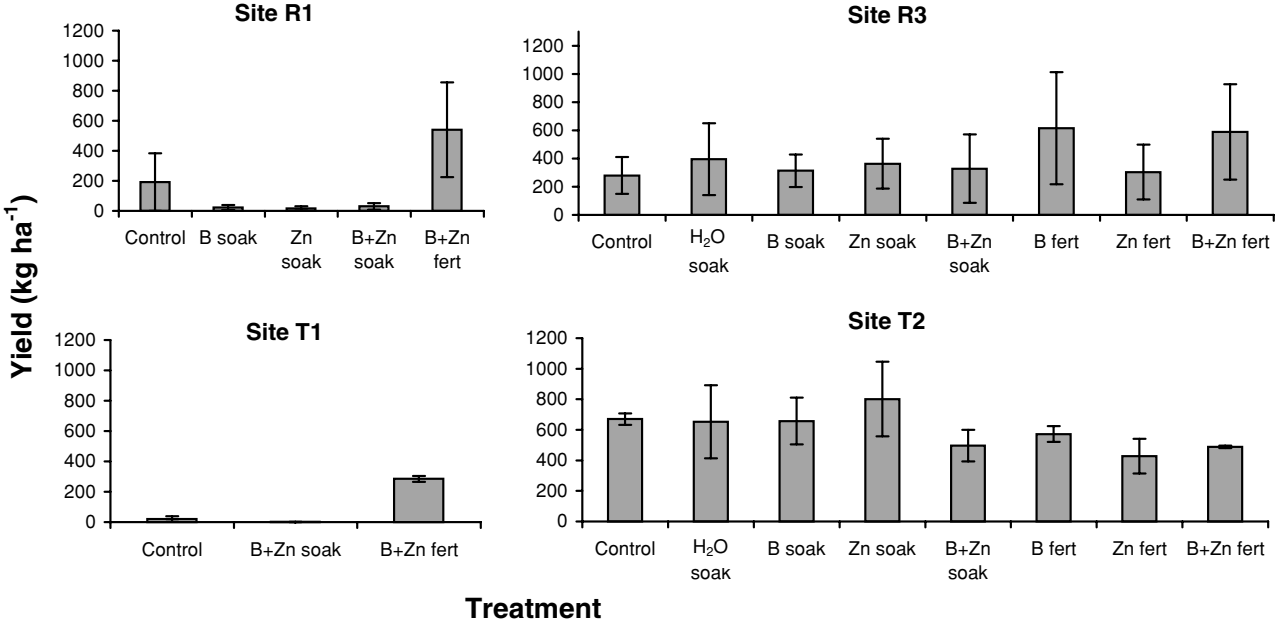

Figure 2. Chickpea grain yields by treatment at different experiment sites and years (see Table 2 for description). Bars represent mean of four (sites R1, R3) or three (sites T1, T2) replicates; error bars represent standard deviation. ANOVA revealed significant treatment differences at the 0.05 level at sites R1, R3 and T1, but no significant difference at site T2.

Table 5. Grain yields in the lentil, rice, and wheat experiments, showing averages across all treatments (since there was no statistical difference between treatments).

Site (year)	Crop	Grain yield (t ha^{-1})
R1 (1st)	lentil	0.45
R2 (2nd)	lentil	0.16
T1 (1st)	lentil	0.21
T3 (2nd)	lentil	0.49
M (2nd)	lentil	0.10
R4 (1st)	rice	1.3
R4 (2nd)	wheat	2.4

T1 ($P < 0.05$; Figure 2). At site R3, application of B fertilizer to the soil, alone or in combination with Zn fertilizer, significantly increased yield ($P < 0.05$), but application of Zn fertilizer did not. There were no significant treatment effects on yield at site T2.

Lentil, rice and wheat. There were no significant treatment effects on lentil yields in any of the five experiments in either year (Table 5). Likewise, there were no significant treatment effects on the yield of rice or wheat (Table 5). Most yields were well below achievable yields of approximately 1 t lentil and 5–6 t rice or wheat ha^{-1}.

Effects of micronutrient treatments on progeny seed nutrient content

Chickpea. B fertilization (alone or in combination with Zn) significantly increased ($P < 0.001$) the amount of B in chickpea seeds at all sites during both years (Figure 3). In contrast, B priming had no effect on the amount of B in the progeny seeds (Figure 3). There was no effect of any micronutrient treatment (fertilization or priming) on the content of Zn in chickpea seeds at any of the sites (Table 6).

Lentil. B fertilization (alone or in combination with Zn) significantly increased ($P < 0.001$) the amount of B in lentil seeds at all five sites during both years (Figure 4), although there was no effect of B fertilization on yield at any site (Table 5). In contrast to B fertilization, B priming had no effect on B content of progeny lentil seeds (Figure 4). There were small but significant ($P < 0.1$ at site R1, $P < 0.05$ at site R2) increases in Zn content of the seeds of Zn fertilized plants at two of the five sites (Table 6). As with chickpea, Zn priming had no effect on Zn content of progeny lentil seeds.

Rice. There was a significant ($P < 0.05$) increase in Mo concentration in seeds of rice plants fertilized with Mo (Figure 5), even though micronutrient fertilization did not increase yield (Table 5). Neither priming nor soil fertilization had an effect on B or Zn content of progeny rice seeds (Figure 5). Likewise, there was no effect of micronutrient fertilization on Mn or Cu content of rice seeds (Figure 5). Mo, Mn and Cu priming were not tested.

Wheat. There was a small but significant ($P < 0.05$) increase in B content in wheat seeds with B fertilization (alone or in combination with other micronutrients), in Zn content with Zn fertilization, and in Mo content with Mo fertilization (Figure 6), even though yield was not affected by any treatment (Table 5). Priming had no effect on the Zn or B content of progeny wheat seeds (Figure 6). As with rice, micronutrient

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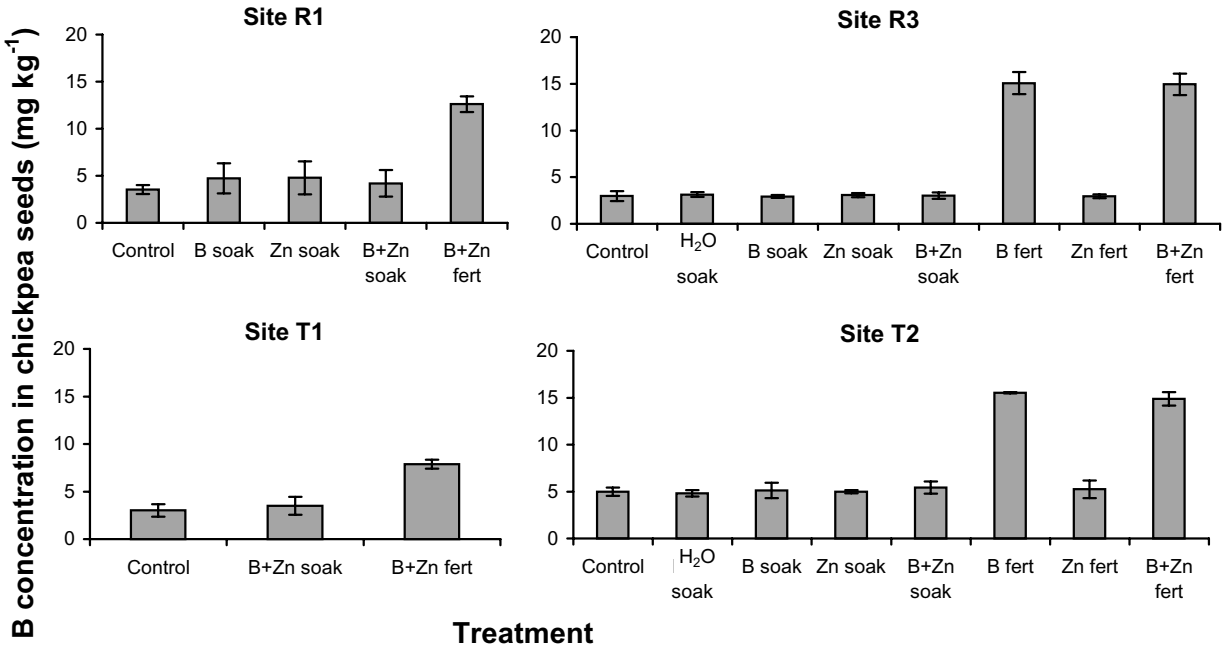

Figure 3. B concentration in chickpea seeds from different experiment sites. Each bar represents the mean of four (sites R1, R3) or three (sites T1, T2) field replicates; error bars indicate standard deviation. ANOVA revealed that at all sites the treatment effect was significant at the 0.001 level.

Table 6. Zn concentration (mg kg^{-1}) in chickpea and lentil seeds at the time of harvest.

Site (year)	Crop	Average of all not-significantly-different treatments†	Average of Zn-fertilized treatments where significantly different
R1 (1st)	chickpea	50	
R3 (2nd)	chickpea	50	
T1 (1st)	chickpea	50	
T2 (2nd)	chickpea	50	
R1 (1st)	lentil	59	69‡
R2 (2nd)	lentil	57	66§
T1 (1st)	lentil	70	
T3 (2nd)	lentil	60	
M (2nd)	lentil	45	

† For all sites except R1 (lentil) and R2, this value is an average of all treatments. At R1 (lentil) and R2, it is an average of all treatments except the Zn-fertilized ones.
‡ Difference significant by ANOVA at 0.1 level.
§ Difference significant by ANOVA at 0.05 level.

fertilization had no effect on the Mn or Cu content of wheat seeds (Figure 6). Mo, Mn and Cu priming were not tested.

Effect of micronutrient treatments on leaf tissue nutrient content

Lentil. Similar to treatment effects on B content of progeny seeds (Figure 4), B fertilization resulted in a large significant ($P < 0.05$) increase in B concentration in lentil leaf tissue at site M (measured at harvest time), but B priming had no effect (Figure 7). There were no significant treatment effects on Zn concentration in leaf tissue at site M (Figure 7).

Rice. Priming with B or Zn significantly ($P < 0.05$) decreased both B and Zn concentration in seedling leaf tissue at transplanting time compared to the unprimed control (Figure 7). The primed seeds produced larger plants, causing a dilution of the nutrients. This decrease in nutrient content was not related to the specific nutrient in the priming solution, since the B primed seeds showed a decrease in both B and Zn concentration in the leaves, and the Zn primed seed also showed a decrease in both B and Zn concentrations.

DISCUSSION

Development of a safe method of micronutrient seed priming

One objective of this study was to identify a micronutrient priming procedure that would allow for maximum micronutrient loading of seeds without causing toxicity. The results in Figure 1 show the conclusions of apparently successful laboratory experiments designed to identify such a procedure in terms of micronutrient priming solution concentrations. However, the failure of micronutrient primed chickpea seeds to emerge in the first season (Figure 2) revealed that there was some danger to micronutrient primed seeds in the field that had not been observed in the laboratory.

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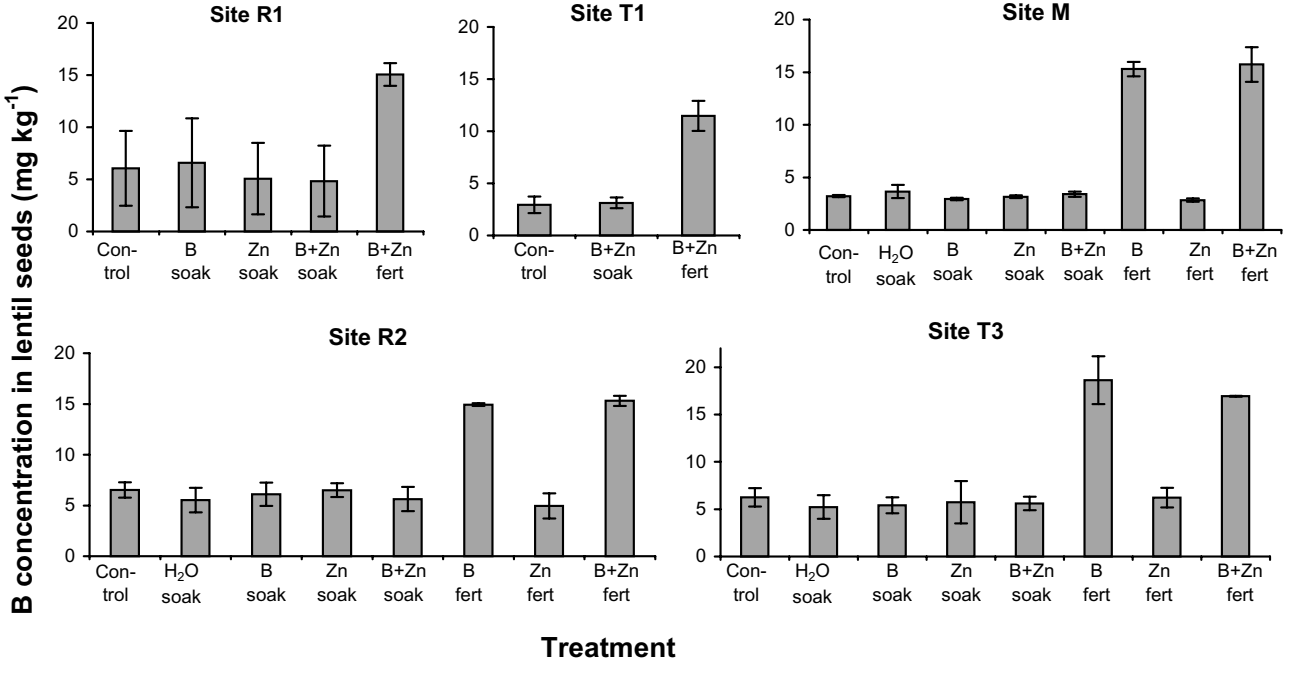

Figure 4. Concentration of B in lentil seeds by treatment at each experiment site and year (see Table 2 for descriptions). Bars represent mean of four (sites R1, R2), three (sites T1, M), or two (site T3) replicates. Error bars indicate standard deviation. ANOVA analysis showed a significant treatment difference at the 0.001 level at all sites.

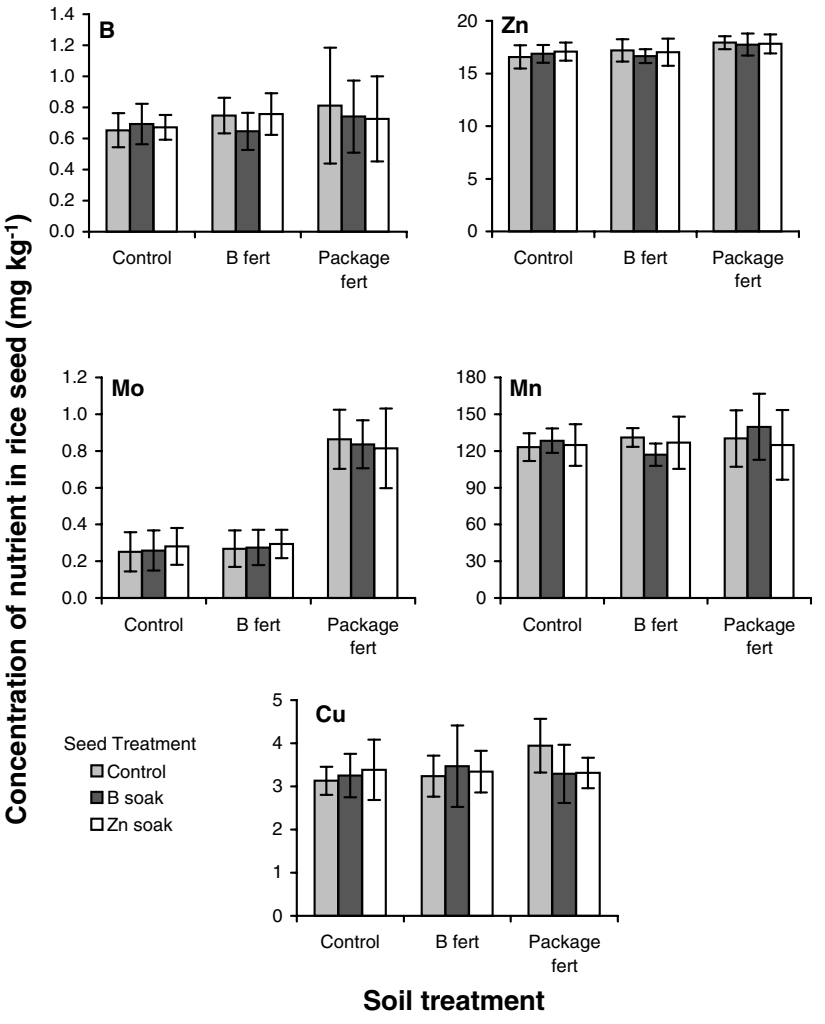

Figure 5. Concentration of the five package fertilizer micronutrients (B, Zn, Mo, Mn, Cu) in rice seed at harvest, by soil and seed treatment. Each bar represents the mean of five field replicates; error bars indicate standard deviation. Split-plot ANOVA revealed that of all the soil, seed, and interaction effects, only the soil treatment for Mo was significant (at the 0.05 level).

Perhaps there was a negative interaction between priming, low soil temperature and the seed fungicide treatment, and chickpea was more susceptible to this interaction than lentil. In spite of attempts to determine the precise conditions that resulted in seed death, this effect was not repeated in the second season, and its origin remains a mystery. Therefore, there might be some risk to farmers if they were to practice micronutrient priming of chickpea, and further experimentation is needed before promoting this technique. With any micronutrient seed priming treatment, it would be wise to test a few primed seeds in the soil for germination prior to priming the whole batch. It should be noted that in contrast to the results of the first season of

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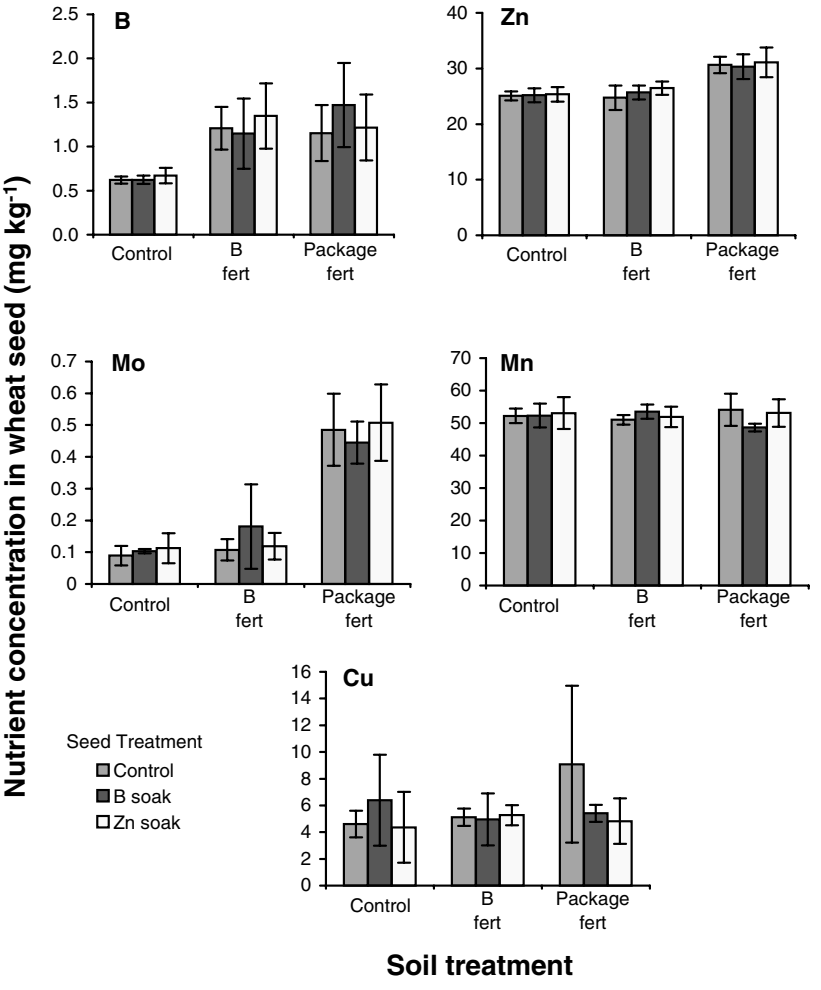

Figure 6. Concentration of the five package fertilizer micronutrients (B, Zn, Mo, Mn, Cu) in wheat seed at harvest, by soil and seed treatment. Each bar represents the mean of five field replicates; error bars indicate standard deviation. Split-plot ANOVA revealed that of all the soil, seed, and interaction effects, only the soil treatment effects for B, Zn and Mo were significant (at the 0.05 level).

our study, water priming of chickpea seeds has been quite successful in Bangladesh, leading to widespread adoption of the practice by farmers (Musa *et al*., 2001).

For the other crops tested – lentil, rice and wheat – the safe micronutrient seed priming method proved to be the 'medium' concentration of Zn, B and Mo (Figure 1), soaking the seeds for the time recommended for water priming according to species (Harris, 2001). It might be possible to use even higher concentrations of micronutrient in priming solutions for rice and wheat, since the seeds of these species absorbed much less of the nutrients during priming than did chickpea and lentil (Tables 3 and 4). Higher priming solution concentrations were not tested for rice and wheat in this study.

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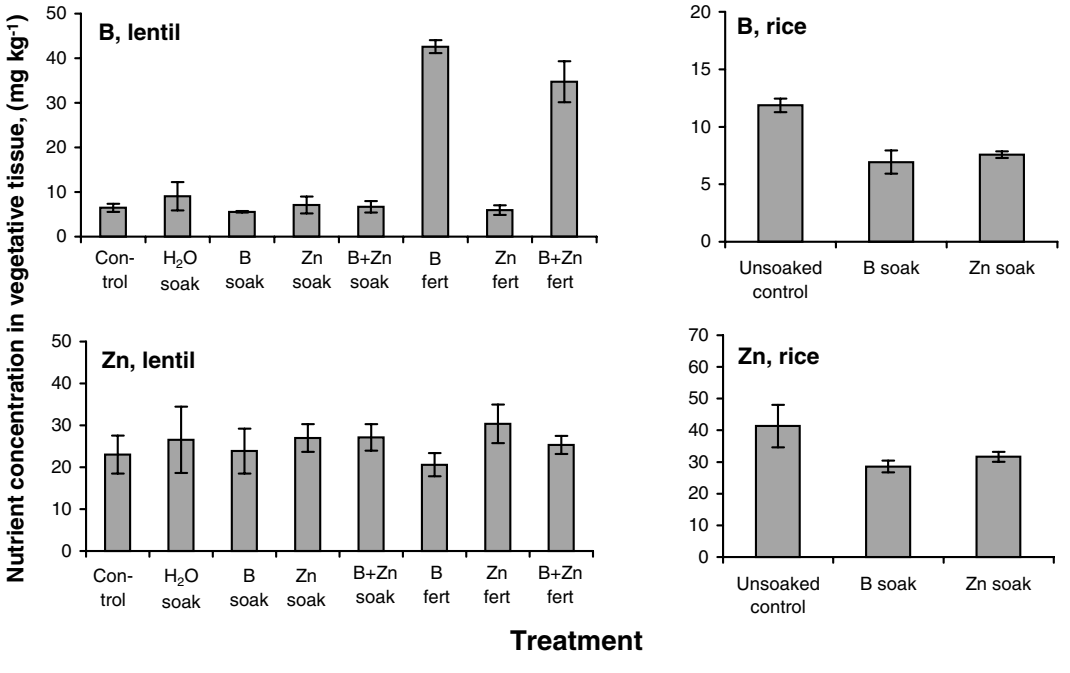

Figure 7. Concentration of B and Zn in vegetative tissues of lentil and rice. Lentil leaf tissue nutrient concentrations were measured at harvest time, site M, and rice leaf tissue at transplanting time. Treatment details are provided in Table 2. Each bar represents the mean of three replicates; error bars indicate standard deviation. ANOVA revealed a significant treatment effect at the 0.05 level for each figure except lentil Zn, for which there was no significant treatment effect.

Effect of micronutrient treatments on plant nutrition

A second objective of this study was to compare the effects of micronutrient seed priming with soil fertilization on improving plant micronutrient nutrition. Because of boron's phloem-immobility (Brown and Shelp, 1997), it is unsurprising that B priming did not increase grain yield in any species (Figure 2, Table 5), as B added to the seed during priming would not *a priori* be expected to be available to the plant during the critical time of flowering. In the case of chickpea, there was a yield response to B fertilization in three of the four experiments (Figure 2), which is consistent with the observation of very low available B in the unfertilized soil (Table 1), and resulted from a plentiful supply of B from the fertilized soil throughout the growing season. Since soil B content at site T2 was at a similarly low level to all of the other experimental sites, it is unclear why there was no yield response to B fertilization at that site. Presumably there was another factor limiting yield, or perhaps another factor mitigating the negative effect of low soil B (such as favourable weather for the translocation of B through the xylem). The B content of the control seeds at site T2 was slightly higher than at any of the other sites (Figure 3), even though soil B content was not higher (Table 1), which supports the idea that the plants were better able to obtain B from the soil at that site. There was a similar increase in B content of the progeny seeds with fertilization at site T2 as at the other sites (Figure 3), indicating a similar ability to take up additional B from soil. Since additional B uptake into seeds does not usually occur in B-sufficient plants (see discussion below), this observation implies that these plants were not B-sufficient, even though there was no yield response to B fertilization, which supports the idea that there was another factor limiting yield.

Despite the fact that soils at most sites were below critical deficiency levels of 0.2 mg B kg^{-1} and 0.5 mg Zn kg^{-1} (Table 1), there was no yield response to B fertilization in lentil, rice and wheat, or to Zn fertilization in any of the four species. The yields of all crops were low (Figure 2, Table 5), indicating that at most sites, there was some factor limiting agronomic productivity other than Zn and/or B nutrition. Perhaps responses to B and Zn fertilization would be observed if the crops were near their potential yields.

Because there was a yield response to B fertilization but not to B priming of chickpea at site R3 (Figure 2), it is concluded that B priming is not an effective way to provide B to plants. This conclusion is supported by the observation that there was no increase in B content of the progeny seeds or leaf tissue from B priming in any crop even though there were increases following B fertilization (Figures 3, 4, 6, and 7).

Similar to B, Zn priming had no effect on yield in any species (Figure 2, Table 5). However, since there was no yield response to Zn fertilization, either, no conclusion can be made about the effectiveness of Zn priming at improving Zn nutrition of plants. It is possible that Zn priming would affect yield of plants limited by Zn deficiency. But since there was no effect of Zn priming on the Zn content of progeny seeds at sites for which there were small increases following Zn fertilization (lentil: sites R1, R2, Table 6; wheat: Figure 6), it seems unlikely that Zn priming would be as effective as fertilization at mitigating the symptoms of Zn deficiency. Recently, however, yield gains associated

Table 7. The effectiveness of different methods of enriching seeds of several species with a variety of micronutrients.

		Method of enrichment	
		Sowing micronutrient-primed seed	Soil fertilization‡
Crop	Micronutrient	Enrichment factor compared to seed source†	
Rice	Zn	NE	NE
	B	NE	NE
	Mo	nd	3.2
	Mn	nd	NE
	Cu	nd	NE
Wheat	Zn	NE	1.2
	B	NE	2
	Mo	nd	4.6
	Mn	nd	NE
	Cu	nd	NE
Chickpea	Zn	NE	NE
	B	NE	3–5
	Mo	nd	4–10
Lentil	Zn	NE	0–1.3
	B	NE	3–5
	Mo	nd	6–10

† NE: No enrichment; nd: no data.
‡ Fertilization rates were: rice, wheat: 5.7 kg Zn, 1.1 kg B, 0.4 kg Mo, 8 kg Mn, 1.5 kg Cu ha^{-1}; chickpea, lentil: 11.4 kg Zn, 1.5 kg B, 2 kgMo ha^{-1}

with Zn priming in wheat and chickpea have been reported in Pakistan (Harris *et al*., 2004).

Effect of micronutrient treatments on enrichment of progeny seed

The third objective of this study was to compare the effectiveness of the different micronutrient treatments at enriching the progeny seed, for purposes both of agronomic productivity of the crop grown from those seeds and human nutrition of people eating these staples. Table 7 summarizes the results of all of the experiments in this study according to the level of enrichment of the progeny seed resulting from various micronutrient treatments. Sowing micronutrient-primed seed never resulted in enrichment of the progeny. Soil fertilization sometimes resulted in enrichment of progeny in Zn, always in Mo, and nearly always (in every crop except rice) in B.

The most interesting of these results is the enrichment of seeds with B by soil fertilization, because such enrichment is unexpected based on several experiments indicating that foliar- or soil-applied B is not translocated to the grain (Brown and Shelp, 1997; Ransome and Dowdy, 1987). There is no clear understanding of how the plant regulates uptake of B into the seed, but it does appear that there is some mechanism to do so, probably to prevent B toxicity. In most crop plants, B is most likely transported to the seed in small part through the phloem and in greater part through the xylem, or perhaps through xylem-to-phloem transport in the last step

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before unloading/loading from the phloem into the endosperm or cotyledons (Brown and Shelp, 1997). In the current study, B was probably transported into the seed through the xylem, and the higher supply of B in the soil resulted in higher B content in the xylem. Perhaps B can only be enriched in seeds if the non-enriched control treatment is severely B-deficient; i.e. if the soil is severely B-deficient.

Since the B content of grains is not known to have a significant impact on human nutrition, the ability to enrich seeds with B in some low-B environments is more likely to be of agronomic than human nutritional use. A logical next step would be to compare the growth of the seeds *in vivo* enriched with B with unenriched seeds grown on soil with and without B fertilizers.

In this study, further field experiments comparing Mo fertilization with sowing Mo primed or Mo *in vivo* enriched seed were not done due to lack of confirmed Mo-deficient soil, although soil pH was low enough at some sites to suggest the possibility of deficiency. However, Mo is likely to be more effectively added to legume seeds during priming than Zn or B due to the fact that it is needed primarily on the seed coat in order to enhance nodulation with nitrogen-fixing bacteria, which need Mo for the proper function of the nitrogen-fixing enzyme, nitrogenase (Marschner, 1995, p. 216). Even though Zn and B priming had minimal effect on Zn and B plant nutrition, Mo priming might have an effect on Mo nutrition, especially of grain legumes. Positive yield responses to Mo priming have recently been reported for chickpea in Bangladesh (Johansen *et al*., 2004).

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REFERENCES

Black, R. E. (1998). Therapeutic and preventive effects of zinc on serious childhood infectious diseases in developing countries. *American Journal of Clinical Nutrition* 68:476–479.

Brown, P. H. and Shelp, B. J. (1997). Boron mobility in plants. *Plant and Soil* 193:85–101.

Fuchs, G. J. (1998). Possibilities for zinc in the treatment of acute diarrhea. *American Journal of Clinical Nutrition* 68:480–483.

Harris, D. (1996). The effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi-arid Botswana. *Soil & Tillage Research* 40:73–88.

Harris, D. (1997). On-farm seed priming to accelerate germination in rainfed, dry-seeded rice. *International Rice Research Notes* 22:30.

Harris, D. (2001). On-farm seed priming: A key technology to improve the livelihoods of resource-poor farmers in marginal environments. *Bangor: UK Department for International Development and University of Wales*.

Harris, D., Joshi, A., Khan, P. A., Gothkar, P. and Sodhi, P. S. (1999). On-farm seed priming in semi-arid agriculture: Development and evaluation in maize, rice and chickpea in India using participatory methods. *Experimental Agriculture* 35:15–29.

Harris, D., Rashid, A., Arif, M. and Yunas, M. (2004). Alleviating micronutrient deficiencies in alkaline soils of North West Frontier Province of Pakistan: on-farm seed priming with zinc in wheat and chickpea. In *International Workshop on Agricultural Strategies to Reduce Micronutrient Problems in Mountains and Other Marginal Areas in South and South East Asia. Kathmandu, 8–10 September, 2004. Nepal Agricultural Research Council.*

Jeffrey, A. J. and McCallum, L. E. (1988). Investigation of a hot 0.01 M $CaCl_2$ soil boron extraction procedure followed by ICP-AES analysis. *Communications in Soil Science and Plant Analysis* 19:663–673.

Johansen, C., Musa, A. M., Kumar Rao, J. V. D. K., Harris, D., Ali, M. Y. and Lauren, J. G. (2004). Molybdenum response of chickpea in the High Barind Tract of Bangladesh and in Eastern India. In *International Workshop on Agricultural Strategies to Reduce Micronutrient Problems in Mountains and Other Marginal Areas in South and South East Asia. Kathmandu, 8–10 September, 2004. Nepal Agricultural Research Council.*

Khalid, B. Y. and Malik, N. S. A. (1982). Presowing soaking of wheat seeds in copper and manganese solutions. *Communications in Soil Science and Plant Analysis* 13:981–986.

Lindsay, W. L. and Norvell, W. A. (1978). Development of a DTPA test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* 42:421–428.

Lopes, A. S. (1980). Micronutrients in soils of the tropics as constraints to food production. In *Priorities for Alleviating Soil-related Constraints to Food Production in the Tropics*, 277–298. Los Baños: International Rice Research Institute.

Mahler, R. L., Naylor, D. V. and Fredrickson, M. K. (1984). Hot water extraction of boron from soils using sealed plastic pouches. *Communications in Soil Science and Plant Analysis* 15:479–492.

Mandal, B., Pal, S. and Mandal, L. N. (1998). Effect of molybdenum, phosphorus, and lime application to acid soils on dry matter yield and molybdenum nutrition on lentil. *Journal of Plant Nutrition* 21:139–147.

Marcar, N. E. and Graham, R. D. (1986). Effect of seed manganese content on the growth of wheat (*Triticum aestivum*) under manganese deficiency. *Plant and Soil* 96:165–173.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants*, 2nd ed. San Diego: Academic Press.

Musa, A. M., Harris, D., Johansen, C. and Kumar J. (2001). Short duration chickpea to replace fallow after aman rice: the role of on-farm seed priming in the High Barind Tract of Bangladesh. *Experimental Agriculture* 37:509–521.

Parera, C. A. and Cantliffe, D. J. (1994). Presowing seed priming. *Horticultural Review* 16:109–141.

Peeran, S. N. and Natanasabapathy, S. (1980). Potassium chloride pretreatment on rice seeds. *International Rice Research Newsletter* 5:19.

Ransome, L. S. and Dowdy, R. H. (1987). Soybean growth and boron distribution in a sandy soil amended with scrubber sludge. *Journal of Environmental Quality* 16:171–175.

Rawson, H. M. (1996). The developmental stage during which boron limitation causes sterility in wheat genotypes and the recovery of fertility. *Australian Journal of Plant Physiology* 23:709–717.

Rerkasem, B., Bell, R. W. and Loneragan, J. F. (1990). Effects of seed and soil boron on early seedling growth of black and green gram (*Vigna mungo* and *V. radiata*). In *Plant Nutrition–Physiology and Applications*, 281–285 (Ed. M.L. van Beustchem). Dordrecht: Kluwer Academic Publishers.

Roberts, W. O. (1948). Prevention of mineral deficiency by soaking seed in nutrient solution. *Journal of Agricultural Science* 38:458–468.

Savithri, P., Perumal, R. and Nagarajan, R. (1999). Soil and crop management technologies for enhancing rice production under micronutrient constraints. *Nutrient Cycling in Agroecosystems* 53:83–92.

Shankar, A. H. and Prasad, A. S. (1998). Zinc and immune function: the biological basis of altered resistance to infection. *American Journal of Clinical Nutrition* 68:447–463.

Sherrell, C. G. (1984). Effect of molybdenum concentration in the seed on the response of pasture legumes to molybdenum. *New Zealand Journal of Agricultural Research* 27:417–423.

Soltanpour, P. N. and Schwab, A. P. (1977). A new soil test for simultaneous extraction of macro- and micro-nutrient in alkaline soils. *Communications in Soil Science and Plant Analysis* 8:195–207.

Srivastava, S. P., Bhandari, T. M. S., Yadav, C. R., Joshi, M. and Erskine, W. (2000). Boron deficiency in lentil: Yield loss and geographic distribution in a germplasm collection. *Plant and Soil* 219:147–151.

Storer, D. A. (1984). A simple high sample volume ashing procedure for determination of soil organic matter. *Communications in Soil Science and Plant Analysis* 15:759–772.

Subedi, K. D., Gregory, P. J., Summerfield, R. J., and Gooding, M. J. (1998). Cold temperatures and boron deficiency caused grain set failure in spring wheat (*Triticum aestivum* L.). *Field Crops Research* 57:277–288.

van Campen, D. R. (1991). Trace elements in human nutrition. In *Micronutrients in Agriculture*, 2nd ed., 663–701 (Eds J. Mortvedt *et al.*). Madison: Soil Science Society of America, Inc.

Welch, R. M. (1986). Effects of nutrient deficiencies on seed production and quality. *Advances in Plant Nutrition* 2:205–247.

Welch, R. M., Combs Jr., G. F. and Duxbury, J. M. (1997). Toward a "greener" revolution. *Issues in Science and Technology* 14:50–58.

Wilhelm, N. S., Graham, R. D. and Rovira, A. D. (1988). Application of different sources of manganese sulphate decreases take-all (*Gaeumannomyces graminis* var. *tritici*) of wheat grown in a manganese deficienct soil. *Australian Journal of Agricultural Research* 39:1–10.