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Effects of Micronutrients on Seed Yield and Yield Components of Alfalfa

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ABSTRACT

Effects of micronutrients [molybdenum (Mo), boron (B), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu)] on seed yield and yield components (seed yield per unit area, fertile shoots per unit area, number of racemes per shoot, number of pods per raceme, number of seeds per pod and 1000-seed weight) of alfalfa were investigated in the northwest region of China, over two seasons (2003–2004) on a torrifluvent soil and a site of 1720 m altitude. After two years study, it demonstrated that molybdenum and boron were more critical for alfalfa seed production. Molybdenum increased seed yield by 27–47%, the number of racemes per shoot by 38–55%, the number of pods per raceme by 100–133%, the number of seeds per pod by 48–61% and 1000-seed weight by 24%, and boron increased seed yield by 22–35%, fertile shoots per unit area by 17–35%, the number of racemes per shoot by 38–64%, the number of pods per raceme by 100%, the number of seeds per pod by 41–52% and 1000-seed weight by 16%. Iron, manganese, and copper had any effect on yield components, but they had no effect on seed yield. Zinc had no positive effect on seed yield and yield components. Among seed yield components, the number of pods per raceme was the most important factor to determine alfalfa seed yield. Results from this study will be used to recommend further evaluation of molybdenum and boron across a number of environments around the world and as a basis for developing recommendations to the alfalfa seed production industry.

Keywords: alfalfa, boron, copper, influence, iron, manganese, micronutrients, molybdenum, seed yield, yield components, zinc

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INTRODUCTION

Micronutrients are essential for normal physiological activity of plants (Bai, 1992) and have the same importance as macronutrients (Razmjoo and Henderlong, 1997). They affect root growth (Mohamed et al., 2005), plant growth (Baligar and Fageria, 2005), seed germination and yield of plants (Ge et al., 1999). In groundnut (*Glycine Willd.*), micronutrients are useful for promoting growth, increasing the number of pods and elevating productivity (Zhen et al., 2005). They increased the number of fertile shoots and seed germination of rice (*Oryza sativa* L.) (Xiao et al., 2004). Boron (B) increased the number of bolls per plant, number of bolls per square meter, mean boll weight, lint, and seed yield of cotton (Dordas, 2006a).

In seed production of legume crops, the widely studied and micronutrients utilized are mainly molybdenum (Mo), B, manganese (Mn), zinc (Zn), iron (Fe), and copper (Cu). Wang et al. (1997, 2003), Zhang et al. (2001), and Jia et al. (2005) reported that B, Mo, Zn, Mn, and Fe increased plant height, the number of fertile shoots and seed yield of soybean (*Glycine max* Merr.). Fan et al. (2003) reported that Zn and B increased seed yield of mung bean (*Phaseolus aureus* Roxb.) by 30%. And Bonilla et al. (2004) reported that B increased seed germination of pea (*Pisum sativum*). But Nautiyal and Chatterjee (2004) reported that Mo decreased seed yield and deteriorated the quality of Chickpea (*Cicer arietinum* L.).

Studies on micronutrients and alfalfa also showed that micronutrients affected plant height, seed germination and yield of alfalfa. Ge et al. (1999), Wang et al. (2003) and Liu et al. (2005) reported that B, Zn, and Mo elevated plant height of alfalfa. Zhao and Hong (1998) reported that 0.05% ammonium molybdate elevated seed germination rate of alfalfa and Zhang et al. (2005) reported that low concentration of Zn (80 mg/L) promoted seed germination of alfalfa and high concentration (600 mg/L) prohibited it. Ge et al. (1999) and Dordas (2006b) reported that B promoted alfalfa growth, improved seed set and increased seed yield of alfalfa. But studies also showed that Mo, Zn, Fe, and B had no influence on alfalfa plant height and seed yield (Liu and Yang, 1993; Guo, 2004). Therefore, the aim of this study was to see if Mo, B, Mn, Zn, Fe, and Cu have any influence on seed yield of *Medicago Sativa* cv. 'Gannong No.3' under field conditions and to describe in detail how micronutrients affected seed yield and yield components. Furthermore, the canopy structure was studied in order to identify the most important yield component which determined seed yield of alfalfa.

MATERIALS AND METHODS

Soil and Climate

The field experiment was established in Wuwei city of Gansu province, located in the northwest region of China. The climate of this region is very suitable for

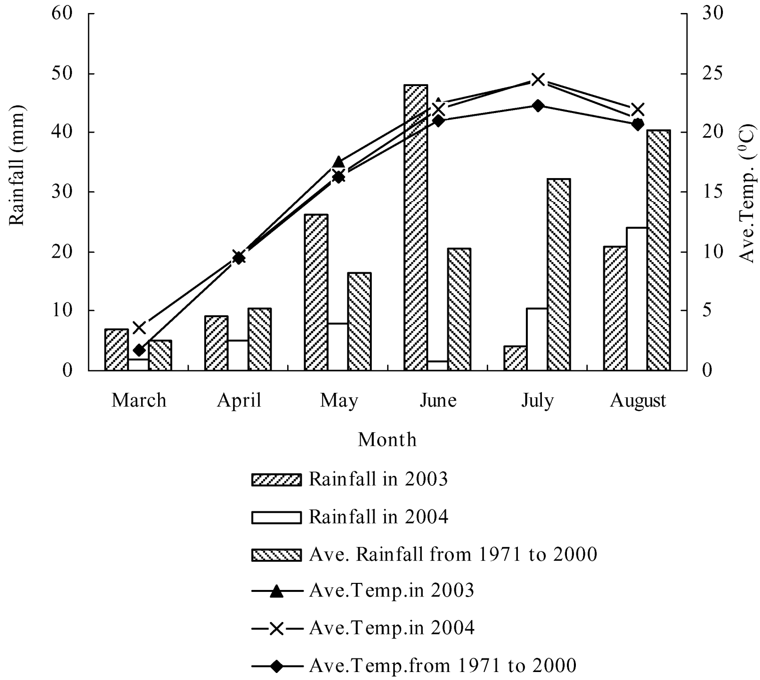

Figure 1. Comparison of rainfall and average temperature in 2003 and 2004.

alfalfa seed production, characterized by a warm summer growing season and very cold winter. Altitude is 1720 m, the annual rainfall of 160 mm is summer dominant and the annual average temperature is 7.7°C (average temperature of the coldest month is −15.4°C and warmest month is 29°C). There is a frost free period of 156 days, solar radiation is 6000 MJ/m^2 and sunshine time is 3051 h. The average rainfall and temperature of growing seasons in 2003 and 2004 was shown in Figure 1.

The soil type is torrifluvent (Shi et al. 2004), a shallow, uniform soil comprising 66.1% sandy loam, 18.5% clay, and 15.4% silt loam. The soil has a pH range of 7–8, bulk density of 1.15g/cm^3, organic matter content of 2.121 g/kg, total nitrogen (N) of 0.28 g/kg, soluble nitrogen of 0.035 g/kg, and total phosphorus (P) of 0.612 g/kg. Existing micronutrient levels were 0.31 ppm Mo, 0.4 ppm B, 0.42 ppm Zn, 1.0 ppm Cu, 1.2 ppm Mn, and 0.53 ppm Fe. Of these analyses only soil B appeared to be a limiting factor according to the published critical soil B levels (0.5 ppm).

Experimental Design

The experiment was a randomized complete block design with three replicates. Plot size was 4 × 5m, and row spacing was 80 cm. *Medicago Sativa* cv. 'Gannong No. 3' is a new commercial cultivar and widely grown in the northwest region of China. In our experiment, it was sown at 2.5 kg ha^{-1} and at a depth of 2–3 cm on April 15, 2003.

Application of Micronutrients Treatments

In each plot, 240 kg phosphorus pentoxide (P_2O_5) ha^{-1} and 94 kg N ha^{-1} were applied as superphosphate and urea, respectively, and fertilized before sowing in 2003, besides 240 kg P_2O_5 ha^{-1} was top dressed at the branching stage in 2004. Irrigation rate was 900 m^3 ha^{-1} and flooded at the branching stage of alfalfa in 2003 and 2004. Foliar application of 0.05% ammonium molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, Mo], 0.38% boric acid (H_3BO_3, B), 0.76% iron sulfate ($FeSO_4$, Fe), 0.5% zinc sulfate ($ZnSO_4$, Zn), 0.76% manganese sulfate ($MnSO_4$, Mn), and 0.5% cupric sulfate ($CuSO_4$, Cu) for three times at the flowering stage in both years. Spraying volume was 750 Liters/ha. Volumes of each micronutrient were 0.38, 2.85, 5.70, 3.75, 5.70, and 3.75 Liters ha^{-1}, respectively. A control plot (CK) was included which did not receive any micronutrient.

Measurements of Seed Yield And Yield Components

In 2003 and 2004, at full-flowering stage, 50 cm long row was randomly chosen as sample in each plot. The number of shoots with racemes were counted and converted to the number of fertile shoots per square meter. Then in each sample, the total number of racemes was counted and divided by the number of shoots to obtain the number of racemes per shoot. At pod setting stage, the total number of pods was counted and divided by the number of racemes to obtain the number of pods per raceme. At seed maturity, aboveground biomass was harvested, sun-dried, threshed and weighed to obtain seed yield. The total number of seeds was counted and divided by the number of pods to obtain the number of seeds per pod. To each examined index, the average of three replicates was the mean value of this index.

Determination of Phenophase, Plant Height, And Seed Germination

In 2003 and 2004, during growing seasons of alfalfa, phenophase was observed. At seed maturity stage, plant height of each plot was determined. Seed germination of each treatment was determined according to the method of (Schuab et al., 2007).

Statistical Analyses

Analysis of Variance was carried out using the GLM Procedure of SAS (SAS, 1989). Correlation and regression analysis was carried out using SPSS (SPSS, 1984).

RESULTS

Seed Yield

For 2003 and 2004 growing period, Mo increased seed yield by 27–47%, B increased seed yield by 22–35% ($P < 0.05$). Seed yields of 2004 were significantly higher than that of 2003 and treatments that had higher seed yields in 2003 also had higher yield seed yields in 2004 (Table 1). Correlation analysis showed that seed yields of two years were positively correlated ($P < 0.01$, $R = 0.938$), regression equation was $y = -1708.8+11.1281 \times -0.0116 \times^2$.

Yield Components

Number of Fertile Shoots Per Square Meter

In two years, B increased the number of fertile shoots per square meter by 17–35%, Cu increased them by 10–29% and Fe increased them by 4–22% ($P < 0.05$) (Figure 2).

Table 1
Plant height, seed germination and yield of alfalfa in 2003 and 2004

	Plant height (cm)		Seed germination (%)		Seed yield (kg/ha)	
Micronutrients	2003	2004	2003	2004	2003	2004
Control	121	124	65.5	60.3	344[c]	753[C]
Mo	119	122	65.2	60.3	506[a]	956[A]
B	122	124	63.4	56.0	464[b]	921[B]
Fe	118	121	68.4	64.0	327[c]	749[C]
Zn	120	125	67.3	62.7	350[c]	800[C]
Mn	123	126	64.8	58.0	346[c]	753[C]
Cu	121	122	64.9	60.1	363[c]	806[C]

Within a column means followed by different capital letter are significantly different at the 0.01 level ($P < 0.01$), different lowercase letters are significantly different at the 0.05 level ($P < 0.05$), and same letters are not significantly different ($P \geq 0.05$).

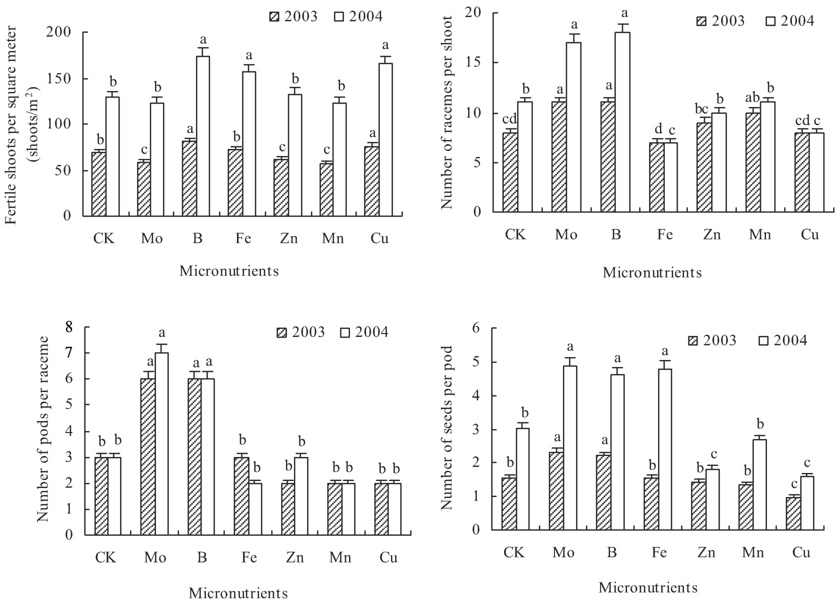

Figure 2. Influence of micronutrients on seed yield components of alfalfa. In each figure above, values are the average of three replicates, vertical bars represent standard error and different letters on the same bar means significantly different at the 0.05 level ($P < 0.05$).

Number of Racemes Per Shoot

In two years, B increased the number of racemes per shoot by 38–64%, Mo increased them by 38–55%, and Mn increased them by 25% ($P < 0.05$) (Figure 2).

Number of Pods Per Raceme

In two years, Mo increased the number of pods per raceme by 100–133% and B increased them by 100% ($P < 0.05$) (Figure 2).

Number of Seeds Per Pod

Mo increased the number of seeds per pod by 48–61%, B increased them by 41–52% and Fe increased them by 58% ($P < 0.05$) (Figure 2).

1000-Seed Weight

In two years, Mo increased 1000-seed weight by an average of 24%, B increased it by 16%, and Fe increased it by 13%.

Linear Regressions of Seed Yield And Yield Components

Yield components of alfalfa are mainly seed yield per unit area (y), fertile shoots per unit area (x_1), the number of racemes per shoot (x_2), the number of pods per raceme (x_3), the number of seeds per pod (x_4), and 1000-seed weight (x_5). In our experiment, the number of pods per raceme was positively correlated with seed yield at the 0.01 level (Table 2).

While doing regression analyses on yield components, all the other variances were rejected, except for the number of pods per raceme. Coefficients of this variance and seed yield were showed in Table 3. According to the model 1 in Table 3, the regression equation of seed yield was $y = 645.903 + 93.667x_3$.

Phenophase, Plant Height, and Seed Germination

After two years of experiments, micronutrients had no effects on alfalfa phenophase. Differential analysis showed that, there were no significant differences among micronutrients treatments and years (Table 1). Results also showed that seed germination of all micronutrients was similar to the control (Table 1).

DISCUSSION

Influence of Micronutrients on Seed Yield

de Oliveira et al. (1998) reported that fertilizing with Mo increased bean seed yield by 37%. Dordas (2006b) reported that B application increased seed yield of alfalfa by an average of 37%. Similar results received from our experiment and demonstrated that Mo and B increased seed yield of alfalfa by 22–47%. Besides, our experiment showed that the other micronutrients (Cu, Zn, Mn, and Fe) had no effect on alfalfa seed yield. But Heitholt et al. (2002; 2003) reported that seed yield of soybean increased while Cu, Mn, Zn, and Fe applied individually. Differences may come from different plant cultivars, soil conditions, and application doses of Cu, Zn, Mn, and Fe.

Influence of Micronutrients on Seed Yield Components

Our experiment showed that Mo, B, Fe, Zn, Mn, and Cu had different effects on yield components of alfalfa. Mo increased the number of racemes per shoot by 38–55%, the number of pods per raceme by 100–133%, the number of seeds per pod by 48–61%, and 1000-seed weight by 24%. B increased fertile shoots per unit area by 17–35%, the number of racemes per shoot by 38–64%, the number of pods per raceme by 100%, the number of seeds per pod by

Table 2
Correlations of seed yield and yield components of alfalfa

	Fertile shoots per unit area	Number of racemes per shoot	Number of pods per raceme	Number of seeds per pod	1000-seed weight	Seed yield
Fertile shoots per unit area	1.000	−0.038	−0.063	0.117	0.168	0.059
Number of racemes per shoot		1.000	0.938**	0.531	0.654	0.891*
Number of pods per raceme			1.000	0.648	0.818*	0.919**
Number of seeds per pod				1.000	0.879**	0.448
1000-seed weight					1.000	0.346
Seed yield						1.000

*mean correlation is significant at the 0.05 level; **mean correlation is significant at the 0.01 level.

Table 3
Coefficients of number of pods per raceme and seed yield of alfalfa

		Unstandardized Coefficients		Standardized coefficients		
	Model	B	Std. Error	Beta	t	Sig.
1	Constant number of	645.903	58.346		11.070	0.000
	pods per raceme	93.667	14.395	0.946	6.507	0.001

41–52% and 1000-seed weight by 16%. Fe increased fertile shoots per unit area by 4–22%, the number of seeds per pod by 58%, and 1000-seed weight by 13%. Mn increased the number of racemes per shoot by 25%. Cu increased fertile shoots per unit area by 10–29%. And Zn had no positive effect on any yield components. Similar results had been reported by Ziaeian and Malakouti (2001), Xiao et al. (2004), and Dordas (2006b), which reported that Fe, Mn, Zn, and Cu fertilizer significantly increased the number of seeds per spikelet and 1000-grain weight of wheat, the number of fertile shoots of rice, and the percent of pods per raceme of alfalfa.

Correlations of Seed Yield and Yield Components

In alfalfa seed production, seed yield were highly correlated with the number of seeds per pod (Kowithayakorn and Hill, 1982), seed weight per raceme and the number of seeds per raceme were highly correlated with seed yield per plant (Bolanos-Aguilar et al., 2001), seed yield per unit area was positively correlated with the number of seeds per raceme ($R = 0.593^*$), number of pods per raceme ($R = 0.602^*$), and seed weight per raceme ($R = 0.685^{**}$) (Sengul, 2006). Similar results derived from our experiment and showed that alfalfa seed yield was significantly correlated with the number of pods per raceme ($R = 0.919^{**}$) and the number of racemes per shoot ($R = 0.891^*$). But Rosellini et al. (1994) reported that only the number of seeds per pod was significantly correlated with seed yield of alfalfa.

Influence of Micronutrients on Phenophase, Plant Height, and Seed Germination

Bonilla et al. (2004) reported that B increased seed germination of pea, Jia et al. (2005) reported that Zn, Mn, and Fe increased plant height of soybean, and Dordas (2006b) reported that B improved seed germination and increased seed vigor of alfalfa. However, our experiment showed that micronutrients had no effect on phenophase, plant height, and seed germination of alfalfa. Differences may come from the less sensitive of 'Gannong No. 3' alfalfa to micronutrients. This inference needs to be proved furthermore.

Better Micronutrients to Increase Alfalfa Seed Yield

In the most parts of the world, alfalfa grown suffered from B deficiency and role of B could not be replaced by other elements (Victor and Shorrocks, 1997). And Moore (2005) reported that more critical micronutrient for alfalfa was B and it was more likely to be deficient under drought conditions. Ge et al. (1999) also reported that Mo was the most important micronutrient in alfalfa seed production. Similar results received from our experiment and showed that B and Mo were more critical micronutrients for alfalfa seed production, which increased seed yield by increasing fertile shoots per unit area, the number of racemes per shoot, the number of pods per raceme and the number of seeds per pod. Further research is recommended to investigate the effect of different concentrations of B and Mo across a number of environments around the world as a basis for developing recommendations to the alfalfa seed production industry.

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REFERENCES

Bai, B. Z. 1992. *Plant Physiology*. Beijing: China Agricultural Press.

Baligar, V. C., and N. K. Fageria. 2005. Soil aluminum effects on growth and nutrition of Cacao. *Soil Science & Plant Nutrition* 51: 709–713.

Bolanos-Aguilar, E. D., C. Huyghe, D. Djukic, B. Julier, and C. Ecalle. 2001. Genetic control of alfalfa seed yield and its components. *Plant Breeding* 120(1): 67–72.

Bonilla, I., A. El-Hamdaoui, and L. Bolaños. 2004. Boron and calcium increase pea (*Pisum sativum*) seed germination and seedling development under salt stress. *Plant and Soil* 267: 97–107.

de Oliveira, W. S., L. W. Meinhardt, A. Sessitsch, and S. M. Tsai. 1998. Analysis of Phaseolus–Rhizobium interactions in a subsistence farming system. *Plant and Soil* 204: 107–115.

Dordas, C. 2006a. Foliar boron application affects lint and seed yield and improves seed quality of cotton grown on calcareous soils. *Nutrient Cycling in Agro Ecosystems* 76: 19–28.

Dordas, C. 2006b. Foliar boron application improves seed set, seed yield, and seed quality of alfalfa. *Agronomy Journal* 98: 907–913.

Fan, F., Q. G. Zhang, and N. Zhang. 2003. Influence of Al, Zn and B micronutrients on seed yield of mung bean (*Phaseolus aureus* Roxb.). *Sinica of Inner Mongolia University for the Nationalities* 18: 248–252.

Ge, G. P., J. H. Gao, and Y. C. Zhao. 1999. Initial report on B and Mo increasing alfalfa seed yield. *Hei Long-Jiang Journal of Animal Science and Veterinary and Medicine* 1: 17.

Guo, X. 2004. Approaches on application of trace element fertilizers (Fe, Zn, B, Mo) to artificial grassland. *China Herbivores* 24(4): 42–44.

Heitholt, J. J., J. J. Sloan, and C. T. Mackown. 2002. Copper, manganese and zinc fertilization effects on growth of soybean on a calcareous soil. *Journal of Plant Nutrition* 25: 1727–1740.

Heitholt, J. J., J. J. Sloan, C. T. Mackown, and R. I. Cabrera. 2003. Soybean growth on calcareous soil as affected by three iron sources. *Journal of Plant Nutrition* 26: 935–948.

Jia, C. X., Y. X. Jia, and S. M. Li. 2005. Studies on effect of Zn, Mn and Fe fertilization on soybean in Huanghuai Area of China. *Soybean Journal* 2: 13–15.

Kowithayakorn, L., and M. J. Hill. 1982. A study of seed production of lucerne under different plant spacing and cutting treatments in the sowing year. *Seed Science and Technology* 10(1): 3–12.

Liu, G. H., Z. H. Qu, K. Wang, and J. G. Han. 2005. Effects of boron, molybdenum and zinc fertilizer application on the yield of alfalfa. *Journal of Hebei North University (Natural Science Edition)* 21(5): 48–51.

Liu, F. T., and Z. Z. Yang. 1993. Effects of boric acid on alfalfa seed yield. *Xinjiang Animal Husbandry* 3: 19.

Mohamed, M., El-Fouly, and A. F. A. Fawzi. 1995. Higher and better yields with less environmental pollution in Egypt through balanced fertilizer use. *Nutrient Cycling in Agroecosystems* 43(1): 1–4.

Moore, C. L. 2005. Irrigated alfalfa management under drought conditions. British Columbia Ministry of Agriculture and Lands: Drought management factsheet 5: 25–34.

Nautiyal, N., and C. Chatterjee. 2004. Molybdenum stress-induced changes in growth and yield of chickpea. *Journal of Plant Nutrition* 27: 173–181.

Razmjoo, K., and P. R. Henderlong. 1997. Effect of potassium,sulfur, boron, and molybdenum fertilization on alfalfa production and herbage macronutrient contents. *Journal of Plant Nutrition* 20: 1681–1696.

Rosellini, D., F. Veronesi, and M. Falcinelli. 1994. Seed yield components in lucerne materials selected for seed yield. *Rivista di Agronomia* 28(1): 43–49.

SAS. 1989. *SAS User's Guide: Statistics, Version 6*. Cary, NC, SAS Institute.

Schuab, S. R. P., A. L. Braccini, C. A. Scapim, J. B. França-Neto, D. K. Meschede, and M. R. Ávila. 2007. Germination test under water stress to evaluate soybean seed vigor. *Seed Science and Technology* 35(1): 187–199.

Sengul, S. 2006. Using path analysis to determine lucerne (*Medicago sativa* L.) seed yield and its components. *New Zealand Journal of Agricultural Research* 49(1): 107–115.

Shi, X. Z., D. S. Ding, and W. X. Sun. 2004. Comparison studies on soil classification system of Chinese and the United States. *Chinese Science Bulletin* 13: 25–35.

SPSS. 1984. SPSS, Version 10.0. Chicago, IL: SPSS Institute.

Victor, M., and H. Shorrocks. 1997. The occurrence and correction of boron deficiency. *Plant and Soil* 193: 121–148.

Wang, J. A., J. Xu, and H. L. Ning. 2003. Influence of micronutrients on quality and other properties of soybean (*Glycine max* Merr.). *Soybean Science* 22: 273–277.

Wang, Y., J. Sun, and J. L. Wu. 1997. Influence of Zn, Mn and Mo on yield and quality of soybean (*Glycine max* Merr.). *Journal of Shanxi Agricultural University* 17: 116–119.

Xiao, S. L., Q. Wang, and X. J. Zhang. 2004. Studies on application effect of making-up micronutrients on crops. *Agricultural Science of Hunan* 4: 23–25.

Zhang, C. R., H. Li, and L. J. Xia. 2005. Effects of zinc and cadmium on seed germination and seedling growth of alfalfa. *Acta Agricultural Boreali-Sinica* 20(1): 96–99.

Zhang, X. M., Y. L. Zhen, and F. Q. Wang. 2001. Yield-increasing effect of micronutrients on soybean (*Glycine max* Merr.). *Journal of Hei long-jiang Agricultural University* 13(3): 10–12.

Zhao, H. Q., and F. S. Hong. 1998. Effects of Mo on seed germination and hay yield of alfalfa. *Grassland of China* 1: 74–79.

Zhen, Z. G., Y. Duan, and F. Wu. 2005. Influence of Zn, B, Mo and Ca fertilization on yield and quality of groundnut (*Glycine* Willd.). *Soil and Fertilizer* 3: 49–50.

Ziaeian, A. H., and M. J. Malakouti. 2001. Effects of Fe, Mn, Zn and Cu fertilization on the yield and grain quality of wheat in the calcareous soils of Iran. *Plant Nutrition* 92: 840–841.