The response of antioxidant enzymes of three soybean varieties to molybdenum and boron in soil with a connection to plant quality

P. Liu[1, 2], Y.S. Yang[2], G.D. Xu[1], Y.H. Fang[3], Y.A. Yang[3]

[1]*Zhejiang Normal University in Jinhua, China*
[2]*Queens University in Belfast, UK*
[3]*Zhejiang University in Hangzhou, China*

ABSTRACT

The response of antioxidant enzymes and quality of three soybean varieties to Mo and/or B in soil has been studied in this paper. Pot experiments were set up with four treatments (control, +Mo, +B, +[Mo + B]) at four growth stages. The study showed that Mo and/or B increased antioxidant enzyme activities and protein content in the seeds. The variation and interaction between Mo and B in soil was explored with regard to their impact on the soybean quality. The Mo and/or B decreased oil-content, linoleic and linolenic acid and thus improve plant quality for human health. There were some dissimilarity in antioxidant enzymes and quality of plant between supplement of Mo and B in the soil, and the interrelation between Mo and B in the plant was co-supplementary to each other. Therefore, the quality of the soybean with Mo and B treatments was much improved than those with Mo or B alone. The study shows that the quality of soybean seeds has some stability as compared to physiological and growth characteristics of vegetative organs.

Keywords: SOD (superoxide dismutase); CAT (catalase); APX (ascorbate peroxidase); POD (peroxidase); protein; fatty acids composition; amino acids composition; oil

Molybdenum or boron deficiency of soil is a widespread and agriculturally important micronutrient disorder affecting the productivity of cultivated plants in many parts of the world (Liu 1991, Shorrocks 1997, Liu et al. 2000, Liu 2001). Mo or B could be applied either at seeding, as a foliar or soil-applied fertilizer to crops in Mo or B deficient soil and the crop yield can be greatly increased (Liu 1991, Liu 2001). Various studies have reported that the application of Mo or B enhances the yield of soybean that grows in the Mo or B deficient soil (Chu et al. 1963, Gupta and Lipsett 1981, Liu 2001, Guertal 2004). Our study showed that the yield of soybean with Mo and B increased more than that with Mo or B alone when the soil lacks in Mo and B (Liu 2000). There are still many questions surrounding plant Mo uptake and metabolism and the exact functional of B in the plant is not fully understood (Marschner 1995, Goldbach 1997, Liu et al. 2000, Hale et al. 2001, Liu 2002a, b). The physiology and quality of soybean responding to Mo and/or B fertilization needs further systematic study to examine the interactive and conjunctive effect of Mo and B.

Soybean seeds are put to a myriad of uses, both nutritional and industrial, and form a very important part of our diet and that of other animals due to its high levels of protein (40%) and fat (20%). However, if and how Mo or B affects quality of soybean has been controversial (Chu et al. 1963, Wu 1988, Liu 1991, Liu 2000, 2001) and their mechanism has not been well studied. For example, the effect of Mo on seeds oil, Liu (1991) observed that Mo increases soybean seeds oil, but Chu et al. (1963) and Wu (1988) reported contrary results.

The objectives of this study were to (1) determine the effect of Mo and/or B in soil on antioxidant enzymes, interactive effect between Mo and B on plant and quality of soybean; (2) determine the differences in growth, physiology and quality among three soybean varieties. Therefore, we investigated antioxidant enzymes activities (POD, CAT, SOD and APX) of leaves, content of oil and fatty acid and amino acid composition of seeds of three soybean varieties under different treatments of Mo and/or B.

Supported by the Natural Science Foundation of Zhejiang Province, Project No. 300284.

MATERIAL AND METHODS

Plant materials

The soybean [*Glycine max* (L.) Merrill] seeds of three varieties, Zhechun III, Zhechun II and 3811, used for the study were obtained from the seeds store, Zhejiang Academy of Agricultural Science, Hangzhou, China.

Plant growth condition

The seeds of the soybean were disinfected with 10% (w/v) H_2O_2 for 20 minutes and washed thoroughly with distilled water and sown in seedbeds filled with a mixture of perlite and vermiculite (1/1, v/v) in a greenhouse under natural light and air conditions, 25°C, 80% relative humidity. The germinated soybean seedlings were selected for uniformity. When the first trifoliate leaf was completely outspread and transferred to plastic growth pots (35cm diameter and 35 cm tall, a hole 2 cm drilled at bottom for drainage purpose) in a netted-greenhouse, a special greenhouse chamber, with glass roof and wire-netted sides for natural air and light conditions. To facilitate plant removal, the pots were lined with perforated plastic bags; there were four plants in each pot. The pots were moved and rearranged daily to receive a random distribution of growth condition in the netted-greenhouse. The plants were watered with 200–400 ml deionised water per pot each morning to avoid water stress and was increased as the plant grew.

Soil, 5 kg per pot, was topsoil of 0–20 cm deep collected from a field in Jiangshan, Zhejiang Province, China (28°45′N; 118°38′E). The soil belongs to subtropical alluvial red soils. The freshly collected soil was screened with an 8-mm sieve to remove stones, coarse plant roots and residues and then air-dried prior to further pot experiment. The screened and air-dried soil was ground to pass a 2-mm sieve for chemical analysis. The physico-chemical characteristics of the soil were analysed as: pH = 5.96, organic C = 18.2 g/kg, cation exchange capacity (CEC) = 39.4 mmol/kg, total N = 0.53 g/kg, hydrolytic N = 22.8 mg/kg, total P = 2.3 g/kg, available P = 60.4 mg/kg, available K = 147.9 mg/kg, effective Mo = 0.134 mg/kg, hot water soluble (HWS) B = 0.20 mg/kg.

Mo and B treatments

The experiment was run for 110 days with four treatments (CK, +Mo, +B, +[Mo + B]). CK is the control without application of Mo/B fertilizers and it is in the condition of Mo and B deficiency; three treatments (+Mo, +B and +[Mo + B]) were applied with various amounts of Mo and/or B fertilizers. +Mo is the adequate Mo treatment containing 0.0185 g Ammonium Heptamolybdate, $(NH_4)_6Mo_7O_{24}.4\ H_2O$; +B the adequate B treatment containing 0.08 g borax ($Na_2B_4O_7 \cdot 10\ H_2O$), and +[Mo + B] is the adequate Mo and B treatment containing 0.0185 g Ammonium Heptamolybdate and 0.08 g borax.

Sampling and plant growth analysis

Each treatment consisted of four replicates in a randomised block design including twenty in total and was sampled at four growth stages. Four sampling pots were selected randomly from the same treatment at the first five growth stages except for eight pots in the harvest maturity stage for sufficient plant sampling. Four growth stages were adopted (c.f. Fehr et al. 1971): V5 (5-triofolia stage), R1 (initiation of flowering stage), R4 (peak of podsetting stage) and R8 (harvest maturity stage). The antioxidant enzyme of the soybean leaves was measured at V5, R1 and R4. Protein content, oil content, fatty acid composition and amino acid composition of the soybean seeds were measured after harvest.

Antioxidant enzyme analysis

The activity of SOD (superoxide dismutase) was assayed according to Wang et al. (1983) in terms of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of NBT auto-oxidation under assay condition. SOD activity was expressed as a unit per milligram protein of soybean leaf. CAT (catalase) activity was measured by the method proposed by Zheng et al. (1991). One unit of CAT activity corresponded to the amount of enzyme that decomposes 1 μmol of H_2O_2 per minute under assay conditions. CAT activity was expressed as a unit per milligram protein of soybean leaf. Determination of APX (ascorbate peroxidase) activity was performed as described by Nakano and Asada (1981). One unit of APX activity was defined as the amount of enzyme that oxidizes 1 μmol of ascorbate per minute under assay condition. APX activity was expressed as a unit per milligram protein of soybean leaf. POD (peroxidase) activity was determined by the Amako (1994) method. POD activity was defined as the increase in absorbance recorded at one OD value of A_{470} per minute under assay condition. The method to determine the protein content of soybean leaf was the same one as soybean seeds measurement (see quality analysis).

Table 1. The response of antioxidant enzyme activities (SOD, POD, CAT and APX) of the soybean leaves to Mo/B for four treatments at three growth stages (V5, R1 and R4)

Antioxidant enzyme	Treatment	Soybean varieties								
		Zhechun III			Zhechun II			3811		
		V5	R1	R4	V5	R1	R4	V5	R1	R4
SOD (U/mg protein)	CK	38.5 ± 3.2	68.3 ± 7.8	71.3 ± 10.4	28.9 ± 1.2	46.7 ± 3.2	51.2 ± 6.6	51.5 ± 6.7	65 ± 4.3a	69.2 ± 4.3
	+Mo	80.7 ± 11.2**	165.1 ± 14.5**	187.6 ± 14.7**	68.2 ± 4.7**	105 ± 9.4**	118 ± 8.9**	68.9 ± 8.7*	113.1 ± 12.1**	124.3 ± 9.4**
	+B	62.5 ± 4.7**	110.5 ± 9.3**	122.7 ± 12.1**	45.8 ± 5.9**	73.1 ± 6.8**	80.4 ± 7.7**	62.4 ± 3.2*	77.3 ± 8.4*	84.1 ± 10.3*
	+[Mo + B]	106.4 ± 9.4**	188.2 ± 13.5**	216.3 ± 13.5**	95.2 ± 10.6**	144.3 ± 12.1**	162.2 ± 11.5**	88.1 ± 8.1**	152.5 ± 14.2**	169.4 ± 5.9**
CAT (U/mg protein)	CK	11.7 ± 0.92	17 ± 1.5	7.9 ± 9.1	9.2 ± 8.6	10.3 ± 8.7	5.6 ± 4.6	7.9 ± 1.3	9.5 ± 2.5	4.3 ± 0.8
	+Mo	20.9 ± 3.1*	23.3 ± 3.4*	14.5 ± 11.2**	15.1 ± 2.3*	17.3 ± 4.3*	12.2 ± 2.3**	12 ± 2.2*	13.5 ± 1.6*	10.3 ± 3.5**
	+B	27.3 ± 2.3**	34.6 ± 7.5**	23.9 ± 8.3**	18.2 ± 4.4**	27.8 ± 1.6**	15.7 ± 1.4**	18.1 ± 1.9**	23 ± 3.4**	14 ± 2.5**
	+[Mo + B]	29.7 ± 4.3**	47.7 ± 6.8**	27.6 ± 21.2**	22.4 ± 5.7**	29.4 ± 5.3**	18.6 ± 3.4**	23 ± 2.1**	26.1 ± 3.8**	15.9 ± 3.1**
POD (ΔA_{470}/mg protein/min)	CK	3.6 ± 0.7	9.5 ± 2.4	10.4 ± 1.8	1.9 ± 2.4	5.6 ± 3.7	8.1 ± 3.3	3 ± 0.8	5.7 ± 1.1	7.6 ± 1.4
	+Mo	6.7 ± 1.5*	17.5 ± 1.6*	19.6 ± 3.6*	3.9 ± 3.7*	10.6 ± 1.6**	10.5 ± 2.9*	3.4 ± 1.4	10.6 ± 1.6*	12.2 ± 2.4*
	+B	5.3 ± 2.4*	14.3 ± 1.7*	18.9 ± 6.3*	3.2 ± 4.5*	8.3 ± 1.4*	10.1 ± 2.6*	3.2 ± 1.6	7.7 ± 2.3*	11.4 ± 3.1*
	+[Mo + B]	10.1 ± 2.3**	22.9 ± 2.6**	26.9 ± 5.3**	4.5 ± 6.4**	10.9 ± 4.5**	16.6 ± 3.4**	4.1 ± 0.7*	12.3 ± 4.7**	13.3 ± 2.3*
APX (U/mg protein)	CK	16.4 ± 3.5	13.7 ± 3.4	9.9 ± 1.6	17.6 ± 1.7	14.4 ± 1.3	10.1 ± 1.5	18.5 ± 4.5	15 ± 1.2	11.2 ± 2.6
	+Mo	22.3 ± 4.6*	18.8 ± 5.2*	14.3 ± 2.58*	20 ± 2.6	16.8 ± 2.4	12.8 ± 3.3	22 ± 4.1	18.2 ± 1.6	13.8 ± 3.1
	+B	19.5 ± 1.7*	16.4 ± 2.2*	12.3 ± 3.5*	18.2 ± 3.2	15.3 ± 2.3	11.5 ± 4.2	19.6 ± 3.2	16.2 ± 3.4	12.2 ± 1.6
	+[Mo + B]	27.2 ± 3.8**	23.3 ± 2.4**	17.5 ± 4.4**	22 ± 6.3*	18.6 ± 4.6*	14.3 ± 1.3*	25.2 ± 2.1*	20 ± 3.9*	15.5 ± 2.4*

The values are means ± *SE* (n = 4)
*indicates significantly different ($P < 0.05$) from control, **very significantly different ($P < 0.01$) from control treatment by *LSD*

Quality analysis

Protein, oil and fatty acid and amino acid were used as indicators of the seeds quality in the soybeans. Protein content of soybean seeds were quantified according to Bradford (1976), using bovine serum albumine (BSA) as a standard. The oil and fatty acid composition were measured by Analyzing and Testing Centre of Zhejiang University, China, with GC (HP5890 II gas chromatograph). The method of Sotelo et al. (1994) used in determining the amino acid content of the sample with a Beckman system (model 6300) high performance amino acid analyser.

Statistical analysis

The data obtained were analysed by a statistical analysis system program (SAS 1990) using an analysis of variance (ANOVA). All values shown in tables and graphs represent the means of four replicates. Error bars indicate SES. *LSD* was used to compare means among treatments.

RESULTS

Response of antioxidant enzymes activities to Mo and B

Activities of antioxidant enzymes of the soybean leaves, namely superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), were determined with the four treatments at three growth stages (Table 1). As a result of Mo and/or B effect with comparison to control on the antioxidant enzymes, the activities of SOD, POD, CAT and APX enzymes significantly ($P < 0.05$) or very significantly ($P < 0.01$) increased. Some difference was observed between Mo and B treatments. By contrast, the activities of SOD, POD and APX in +Mo increased greater than that in +B; but the activity of CAT in +B increased greater than that in +Mo. Significant differences ($P < 0.05$) were found in the activity of SOD, CAT and POD between +Mo, +B, +[Mo + B] and CK, while the difference between +[Mo + B] and CK was very significant ($P < 0.01$). Moreover, the variation in APX activity between +[Mo + B] and CK was very significant ($P < 0.01$) in Zhechun III or significant ($P < 0.05$) in Zhechun II and 3811; but significant ($P < 0.05$) variation in APX activity between +Mo, +B and CK was only in Zhechun III. There was some difference among the temporal changes of antioxidant enzyme activities in three growth stages. The activities of SOD and POD were increasing, but APX activity was decreasing from V5 to R4, with a peak of CAT activity at R1 and a valley at R4.

Response of protein of the soybean seeds to Mo and B

The effect of Mo/B on protein content of the soybean seeds is shown in Figure 1. There is a significant difference ($P < 0.05$) in the protein content between +[Mo + B] and CK, but not between +Mo, +B and CK. The protein content of the soybean seeds in +Mo is greater than that in +B. Among three soybean varieties, Zhechun III has the largest variations of the protein and 3811 the smallest among four treatments, but the differences are minor. The largest variations between the treatments are some 2.63%, 1.69% and 0.75% for Zhechun III, Zhechun II and 3811, respectively.

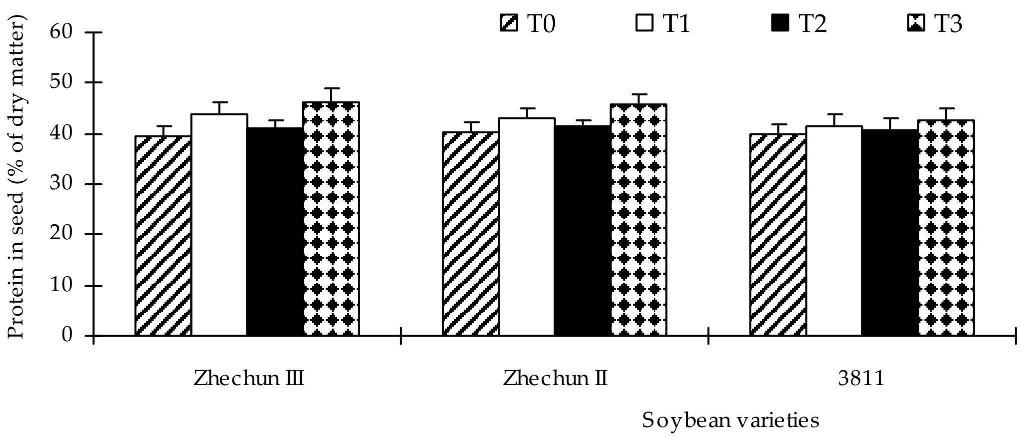

Figure 1. Protein content of the soybean seeds of three varieties (% of dry matter) with Mo/B effect for four treatments; the values are means ± *SE* ($n = 4$); bars showing the same letter code within each graph are not significantly different at $P > 0.05$ (*LSD*)

Table 2. Oil content (% of dry matter) and its fatty acid composition (% of total fatty acid) with Mo/B effect in the soybean seeds of three plant varieties; values represent the mean of four replicates; for a plant variety in four treatments

Soybean varieties	Treatment	Content of oil (% of dry matter)	Fatty acid composition (% of total fatty acid)				
			palmetic acid (16:0)	stearic acid (18:0)	oleic acid (10:1)	linoleic acid (18:2)	linolenic acid (18:3)
Zhechun III	CK	25.41a	13.25Bc	3.14Bc	21.89Bd	55.80Aa	5.89Aa
	+Mo	24.32b	26.95Aa	7.70Aa	36.55Aa	27.40Bc	1.37Bd
	+B	23.65bc	15.89Bb	4.07Bbc	26.40Bb	49.46Ab	4.16Ab
	+[Mo + B]	21.66c	14.29Bbc	5.35Bb	24.30Bc	53.20Aab	2.84Bc
Zhechun II	CK	27.38a	12.58Cc	2.44Bc	23.38Dd	52.13Aa	9.45Aa
	+Mo	25.62b	23.84Aa	6.58Aa	37.61Aa	28.30Cd	3.65Bc
	+B	24.05bc	16.40Bb	5.40Ab	30.28Bb	43.34Bc	4.57Bb
	+[Mo + B]	23.01c	17.63Bb	5.97Aab	28.65Cc	45.15Bb	2.59Bd
3811	CK	26.15a	11.86Cc	2.69Bc	28.75Cc	48.41Aa	8.27Aa
	+Mo	24.54b	24.24Aa	6.77Aa	39.45Aa	26.09Cc	3.43Bc
	+B	23.02bc	15.96Bb	4.00Bb	36.38Bb	38.74Bb	4.89Bb
	+[Mo + B]	22.04c	14.56Bb	4.21Bb	29.50Cc	47.69Aa	4.02Bbc

The value followed by the different letters indicates very significant ($P < 0.01$, capital letters) or significant ($P < 0.05$, small letters) by *LSD*

Effect of Mo and B on seed oil and its fatty acid composition

There were significant differences (P < 0.05) in seed oil (% of dry matter) or very significant differences (P < 0.01) in fatty acid composition (% of total fatty acid) among different treatments of the same plant variety (Table 2). The oil content of the soybean seeds decreased with Mo and/or B treatments; the oil decreasing amount in +B (B treatment) was greater than that in +Mo (Mo treatment) comparing to CK (control). The contents of oleic acid, palmetic acid and stearic acid increased with Mo and/or B treatments; the increases in +Mo treatment were greater than those in +B comparing to CK. However, the content of linoleic acid and linolenic acid of the seeds, which can impair the soybean quality, was reduced with the Mo/B treatments. The amount of seed oil in different treatments varies in the soybean varieties as an order of Zhechun II, Zhechun III and 3811, from large to small. The composition of oil among three soybean varieties does not follow a similar rule but varies with the various treatments.

There were different effects in +[Mo + B] treatment on different composition of oil when comparing to +Mo and +B treatments. The content of linoleic acid varies from +[Mo + B], +B to +Mo, the linolenic acid from +B, +[Mo + B] to +Mo, the palmetic acid and oleic acid from +Mo, +B to +[Mo + B] and the stearic acid from +Mo, +[Mo + B] to +B among the three treatments from large to small.

Mo and B on amino acid composition

Table 3 shows the effect of Mo and B on amino acid composition in the soybean seeds of three varieties. There are very significant ($P < 0.01$) variations in the total amino acid between three Mo/B treatments (+Mo, +B and +[Mo + B]) and control (CK), but not very significant for indispensable amino acid or amino acid. For example, glutamic acid of three varieties and indispensable amino acid of Zhechun III and Zhechun II have partially significant ($P < 0.05$) variations between +Mo, +B, +[Mo + B] and CK. The data observed from the study reveals that, except for proline, other amino acid compositions increase more or less in the +Mo, +B and +[Mo + B] treatments as compared to control. The increasing amounts in the treatments are +[Mo + B] > +Mo > +B. The increment in indispensable amino acid is greater than that of total amino acid in the three treatments. For example in Zhechun II, increases in the total amino acid are 13.41%, 10.46%, 21.96%; indispensable amino acid 19.14%, 14.97%, 30.26%

Table 3. The effect of Mo and B on amino acid composition of the soybean seeds of three varieties (g/100 g); values represent the mean of four replicates; for a plant variety in four treatments

Amino acid composition	Soybean varieties											
	Zhechun III				Zhechun II				3811			
	CK	+Mo	+B	+[Mo + B]	CK	+Mo	+B	+[Mo + B]	CK	+Mo	+B	+[Mo + B]
Asparagine	4.51c	5.17b	4.85b	5.56a	4.62b	4.95ab	4.87ab	5.42a	4.70a	4.88a	4.82a	5.10a
Threonine*	1.45c	1.75b	1.70b	1.88a	1.43b	1.68ab	1.76ab	1.86a	1.44a	1.66a	1.75a	1.79a
Serine	1.71b	2.34a	2.25a	2.38a	2.00a	2.24a	2.31a	2.36a	2.03a	2.21a	2.15a	2.34a
Glutamic acid	6.39b	7.86a	7.87a	8.14a	6.72b	7.46a	7.21a	7.97a	6.93b	7.38a	7.22a	7.87a
Glycin	1.62b	2.24a	2.15a	2.39a	1.74b	2.20a	2.13a	2.38a	1.81a	2.11a	2.07a	2.18a
Alanine	1.49b	2.00a	1.91a	2.11a	1.53b	1.91a	1.79a	2.02a	1.58a	1.80a	1.75a	1.92a
Cystine	0.39a	0.43a	0.40a	0.42a	0.42a	0.41a	0.37a	0.43a	0.41a	0.45a	0.41a	0.45a
Valine*	1.68b	2.04a	1.94a	2.16a	1.71b	2.15a	1.85a	2.26a	1.74a	1.89a	1.72a	1.95a
Methionine*	0.42a	0.60a	0.56a	0.69a	0.44a	0.56a	0.52a	0.68a	0.48a	0.56a	0.51a	0.60a
Isoleucine*	1.68b	2.32ab	2.28ab	2.51a	1.74b	2.24a	2.08a	2.45a	1.81a	2.09a	1.95a	2.15a
Leucine*	2.62a	3.25a	2.84ab	3.59a	2.85a	3.09a	2.99a	3.21a	2.89a	3.07a	2.97a	3.19a
Tyrosine	1.21b	1.64a	1.52a	1.78a	1.39a	1.62a	1.50a	1.72a	1.23a	1.56a	1.45a	1.68a
Phenylalanine*	1.85b	2.54a	2.42a	2.69a	1.92b	2.49a	2.33a	2.60a	1.99a	2.29a	2.22a	2.55a
Lysine*	2.01b	2.12b	2.13b	2.73a	1.98a	2.17a	2.36a	2.69a	2.00a	2.42a	2.31a	2.61a
Tryptophan*	0.38a	0.51a	0.45a	0.50a	0.42a	0.50a	0.47a	0.52a	0.45a	0.49a	0.46a	0.50a
Histidine	0.79b	1.13a	1.08a	1.21a	0.80b	1.10a	1.04a	1.17a	0.81a	1.10a	1.05a	1.13a
Arginine	2.75b	3.21a	3.12a	3.31a	2.89a	3.01a	2.94a	3.26a	2.96a	2.97a	2.76a	3.14a
Proline	1.47a	1.23a	1.43a	1.19a	1.65a	1.33a	1.52a	1.21a	1.72a	1.41a	1.51a	1.25a
Total amino acid	34.42Cd	42.38Bb	40.90Bc	45.24Aa	36.25Cc	41.11Bb	40.04Bb	44.21Aa	36.98Bc	40.34Ab	39.08Ab	42.40Aa
Indispensable amino acid	12.09c	15.13b	14.32b	16.75a	12.49c	14.88b	14.36b	16.27a	12.80b	14.47b	13.89b	15.34a

*indicates indispensable amino acid

The value followed by the different letters indicates very significant ($P < 0.01$, capital letters) or significant ($P < 0.05$, small letters) by *LSD*

for +Mo, +B and +[Mo + B], respectively. This demonstrates that Mo/B improves the composition of the soybean seeds for human health. For amino acid compositions of three soybean varieties, the highest is glutamic acid, then asparagines and the lowest cystine and methionine. The response of total amino aid and indispensable amino acid in three plant varieties to Mo/B is similar to that of root growth discussed above.

DISCUSSION

The effect of deficient, appropriate and excessive boron on activity of antioxidant enzymes was investigated in a number of studies (Cakmak and Römheld 1997, Garcia et al. 2001, Li et al. 2002, Karabal et al. 2003), but their results were not consistent in the changes of antioxidant enzyme. It is likely that changes of antioxidant enzymes in different plants in response to boron deficiency or excess are inconsistent. On the other hand, there is little literature on antioxidant response of plants to Mo except that Li et al. (2002) reported increased activity of CAT, SOD and POD in potato leaves by Mo. To the best of our knowledge, this study is the first report on antioxidant enzymes activities response in soybean with Mo and/or B. By comparison to the control, Mo and/or B cause significant ($P < 0.05$) or very significant ($P < 0.01$)

Table 4. Some major characteristics of vegetative organs and seed quality from four treatments of the varieties at harvest stage; they are the ratios of the largest to smallest values in four treatments for three soybean varieties

Soybean varieties	Main root length	Roots system volume	Dry weight of roots	Above-ground biomass	Leaf area	Protein content	Oil content	Indispens-able amino acid	Total amino acid
Zhechun III	1.62	2.00	1.73	1.91	1.87	1.17	1.17	1.31	1.39
Zhechun II	1.28	1.89	1.89	1.74	1.35	1.14	1.14	1.20	1.30
3811	1.18	1.35	1.35	1.58	1.43	1.07	1.19	1.15	1.20

changes in most activities of SOD, CAT, POD and APX (Table 1). Little was known about the differences between the effects of Mo and B in soil on antioxidant enzymes on a plant. But observation showed that the effect of Mo on SOD, POD and APX was larger than that of B and the effect of B on CAT was larger than that of Mo. Mo plays a key role in nitrogen metabolism through some important Mo-enzymes (Stallmeyer et al. 1999, Liu 2000, Liu and Yang 2001) and B indirectly involved in nitrogen metabolism (Goldbach 1997, Liu et al. 2000). Therefore, we found that an increase of content of protein, indispensable amino acid and total amino acid of seeds in the Mo treatment is greater than that in the B treatment compared to control (Figure 1, Tables 2 and 3). Furthermore, in this study, activities of antioxidant enzymes (POD, SOD, CAT and APX), seed oil, total amino acid and indispensable amino acid with Mo and B increased greater than that with Mo or B alone, and oil content with Mo and B decreased greater than that with Mo or B alone. This indicated that the interaction between Mo and B is positively correlated and co-supplementary.

Whether Mo or B affects the quality and their composition of the plant is controversial. For example, among the studies on the effect of B on rape quality, Liu and Yang (1999) summarised that some literatures shown that B altered the protein, oil and fatty acid composition in rape, but others demonstrated that the quality in rape could not be changed by B. Regarding the controversial issue on soybean quality response to Mo or B, some experiments showed that Mo decreased the content of oil, oleic acid and linoleic acid in soybean seeds (Chu et al. 1963, Wu and Xiao 1994, Liu 2001) and Liu (1991) reported that B decreased the content of nitrogen in soybean seeds. However, some other experiments found that Mo increased content of protein (Wu 1988) and oil (Liu 1991) in soybean seeds, and B increased the content of nitrogen in the soybean seeds (Wu 1988). Our study supported that quality is affected by Mo and B in soil. Experimental results (Figure 1, Tables 2 and 3) showed that Mo and/or B apparently improved quality and constitute by the increasing content of protein, oleic acid, palmetic acid, stearic acid, indispensable amino acid and total amino acid and decreasing the content of linoleic acid and linolenic acid.

Our work found that the difference of quality among different treatments was smaller than that of vegetative organs. This may indicate that the quality of soybean seeds has some stability as compared to the physiological and growth characteristics of vegetative organs. For example, the ratio of the largest/smallest of major physiological and growth characteristics of the vegetative organs from four treatments of same variety at R8 is larger than that of the quality characteristics of the seed (Table 4). Therefore, the seed quality is mainly controlled by gene of the varieties; however, the nutritional condition can affect the quality at certain extent. Appropriate Mo/B will be helpful to improve the seed quality and thus beneficial to human health.

REFERENCES

Amako K., Chen G.X., Asade K. (1994): Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. Plant and Cell Physiology, *35*: 497–504.

Bradford M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, *72*: 248–254.

Cakmak I., Römheld V. (1997): Boron deficiency-induced impairments of cellular functions in plants. Plant Soil, *193*: 71–83.

Chu C., Liang C.W., Chen E.F. (1963): Effect of minor element on the growth and yield of soybean in some important soil types of northeastern China. Acta Paediatrica Sinica, *11*: 417–425.

Fehr W.R., Canvins S., Burmood D.T., Pennington J.S. (1971): Stage of development descriptions for soybean *Glycine max* (L.) Merrill. Crop Science, *11*: 929–931.

Garcia P.O.C., Rivero R.M., Lopez-Lefebre L.R., Sanchez E., Ruiz J.M., Romero L. (2001): Response of oxidative metabolism to the application of carbendazim plus boron in tobacco. Australian Journal of Plant Physiology, *28*: 801–806.

Goldbach H.E. (1997): A critical review on current hypotheses concerning the role of boron in higher plants: suggestion for further research and methodological requirements. Journal of Trace Microprobe Technology, *15*: 51–91.

Guertal E.A. (2004): Boron fertilization of bentagrass. Crop Science, *44*: 204–208.

Gupta U.C., Lipsett J. (1981): Molybdenum in soils, plants, and animals. Advances in Agronomy, *34*: 73–115.

Hale K.L., McGrath S.P., Lombi E., Stack S.M., Terry N., Pickering I.J., George G.N., Pilon-Smits E.A.H. (2001): Molybdenum sequestration in Brassica species. A role for anthocyanins? Plant Physiology, *126*: 1391–1402.

Karabal E., Yücel M., Öktem A.H. (2003): Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Science, *164*: 925–933.

Li J., Li X.D., Zhang D.J. (2002): Effect of boron and molybdenum on tuber yield and active oxygen metabolism in potato (*Solanum tuberosum* L.). China Potato, *16*: 10–14.

Liu P. (2000): The effect of molybdenum and boron on nutritional and physiological mechanism of yield and quality in soybean. [Ph.D. Thesis.] Zhejiang University, Hangzhou.

Liu P. (2001): The research development of molybdenum and boron nutrition in soybean. China Agricultural Science Bulletin, *17*: 41–44.

Liu P. (2002a): Effects of the stress of boron on plants and the interaction between boron and other element. Agri-environmental Protection, *21*: 372–374.

Liu P. (2002b): Effects of the stress of molybdenum on plants and the interaction between molybdenum and other element. Agri-environmental Protection, *21*: 276–278.

Liu P., Wu J.Z., Yang Y.A. (2000): The research development of boron in soil and its effect in plant. Agri-environmental Protection, *19*: 119–122.

Liu P., Yang Y.A. (1999): The research development of physiological response of rape in the stress of low boron. China Journal of Oil Crop Science, *21*: 74–76.

Liu P., Yang Y.A. (2001): Research development of molybdenum in soil and its effect in vegetation. Agri-environmental Protection, *20*: 280–282.

Liu Z. (1991): Agricultural chemistry of microelement. Agriculture Press, Beijiang.

Liu Z. (1996): Soil microelement in China. Jiangsu Sci. Technical Press, Nanjiang: 25–47.

Marschner H. (1995): Mineral nutrition of higher plants. Academic Press, San Diego.

Nakano Y., Asada K. (1981): Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology, *22*: 867–880.

SAS (1990): SAS User's Guide: Statistics.Version 6. 4th ed. SAS Institute, Cary, NC.

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

Sotelo A., Hernandez M., Montalvo I., Sausa V. (1994): Amino acid content and protein biological evaluation of 12 Mexican varieties of rice. Cereal Chemistry, *71*: 605.

Stallmeyer B., Schwarz G., Schulze J., Nerlich A., Kirsh J., Mendel R.R. (1999): The neurotransmitter receptor-anchoring protein gephyrin reconstitutes molybdenum cofactor biosynthesis in bacteria, plants and mammalian cells. In: Proceedings of National Academy of Sciences, USA, *96*: 1333–1338.

Zheng S.X., Wang Y.R., Liu H.X. (1991): Some enzymatic reactions related to chlorophyll degradation in cucumber cotyledons under chilling in the light. Acta Phytotechnica Sinica, *17*: 177–182.

Wang A.G., Luo G.H., Shao C.B., Wu S.J., Guo J.Y. (1983): A study on the superoxide dismutase of soybean seeds. Acta Botanica Sinica, *9*: 77–84.

Wu M.C. (1988): Effect of trace elements on nitrogen metabolism of soybean. Soybean Science, *7*: 305–310.

Wu M.C., Xiao C.G. (1994): Study on molybdenum soybean. Soybean Science, *13*: 245–251.

Received on September 27, 2004

ABSTRAKT

Vliv molybdenu a bóru v půdě na hladinu antioxidačních enzymů v nadzemní biomase a kvalitu semen u tří odrůd sóje

Byl studován vliv molybdenu a bóru na hladinu antioxidačních enzymů v nadzemní biomase a na kvalitu semen u tří odrůd sóje. V nádobových pokusech byly použity čtyři varianty (kontrola, +Mo, +B, +[Mo + B]), přičemž odběry vzorků pro rozbory proběhly ve čtyřech růstových fázích. Aplikace samotného Mo nebo samotného B, jakož i kombinace Mo a B do půdy zvýšila aktivitu antioxidačních enzymů v biomase vegetativních orgánů i obsah bílkovin v semenech. Uvedené varianty aplikace Mo a B snížily obsah olejů i kyseliny linolové a linolenové, čímž se zvýšila kvalita semen z hlediska lidského zdraví. Mezi aplikacemi Mo a B byly však zaznamenány také významné rozdíly v hodnocených znacích, přičemž jako nejlepší se projevil účinek společné aplikace Mo a B. Kvalita semen sóje byla zvýšena mnohem více při aplikaci obou mikroprvků ve srovnání s variantami, kdy byl aplikován vždy jen Mo nebo B. Kvalita semen sóje se ukázala jako stabilnější faktor ve srovnání s fyziologickými a růstovými charakteristikami vegetativních orgánů.

Klíčová slova: SOD (superoxiddismutáza); CAT (kataláza); APX (askorbátperoxidáza); POD (peroxidáza); bílkoviny; mastné kyseliny; aminokyseliny; oleje

---

Corresponding author:

Y.S. Yang, Environmental Engineering Research Centre, Queen's University of Belfast, Belfast BT9 5AG, United Kingdom
phone: +44 (28) 9027 4298, fax: +44 (28) 9066 3745, e-mail: ys.yang@qub.ac.uk

---