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Full Length Research Paper

Boron affects pollen viability and seed set in sunflowers

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Boron (B) is an essential micronutrient for plant growth and development of sunflower (*Helianthus annuus* L.). Most sunflowers growing areas in Thailand are in coarse texture soils with low B availability. The objectives of this experiment were (1) to evaluate the response of sunflower to B applications and (2) to determine the optimum B levels for sunflower under field conditions in sandy loam soil. This experiment was conducted at Suranaree University of Technology, Nakhon Ratchasima, Thailand, in January 2011. Two varieties of sunflower, "S473" and "Pacific 77", were grown in sandy loam soil with available B of 0.14 ppm. Five levels of B (0, 3.13, 6.25, 9.38 and 12.50 kg B ha^{-1}) were applied as borax ($Na_2B_4O_7.10H_2O$). The data was recorded for leaf B concentration, B uptake, pollen viability and seed set. They were highly correlated between pollen viability and seed set. Pollen viability and seed set of both varieties responded to B application, but pollen viability was more responsive. In addition, variety "S473" responded to B application more than "Pacific 77". Regression analysis showed that the levels of B application produced maximum pollen viability ranged between 5.6 and 11.3 kg B ha^{-1}, but B application at higher rates tended to decrease pollen viability and seed set.

Key words: *Helianthus annuus* L., borax, pollen viability, seed set, 2,3,5-triphenyltetrazolium chloride.

INTRODUCTION

Boron (B) is one of the micronutrients required for normal growth and plant development of many crops. However, the optimum quantity range of proper B application is rather narrow, because high concentrations become toxic to plants (Goldberg and Glaubig, 1985). The roles of B in plant has been proposed including functions in cell wall structure, cell wall synthesis, sugar translocation, cell division, enzymatic reactions and plant growth regulation (Blevins and Lukaszewski, 1998). Boron has also been reported to be required for flowering, pollen germination, pollen tube growth and seed development (Cakmak and Römheld, 1997). However, many functions of B in plant growth are not fully understood.

Sunflower is one of the most sensitive crops to B deficiency (Rerkasem, 1986). Boron deficiency symptoms in sunflower become evident on leaves, stems, reproductive parts, dry matter, yield components and seed yield (Rerkasem, 1986; Blamey et al., 1997). Asad et al. (2002, 2003) reported that B requirement of sunflower during reproductive growth is higher than during vegetative growth.

At flowering, B deficiency can affect pollen viability and abortion of stamens and pistils which contribute to poor seed set due to malformed capitulums and consequently low seed yield (Dell and Longbin, 1997; Chatterjee and Nautiyal, 2000). In Thailand, sunflower is usually grown in well drained sandy soils which are most likely B deficient, because of leaching. Low yield with high percentage of unfilled grain are usually found in sunflower grown in this area which might be partly contributed by low B availability. Under B deficient conditions, foliar application of B increased vegetative and reproductive dry mass of sunflower (Asad et al., 2003). Rerkasem (1986) reported the responses of sunflower to low levels of B in sandy soil in the Northern part of Thailand. To overcome this problem, B was applied at planting date at the rate of 10 kg B ha^{-1}, which increased seed yield by 10.5%. However, little information exists on the B requirements of sunflower grown in sandy loam soil in other parts of

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Table 1. Physical and chemical properties of soil samples (0 to 30 cm) from experimental field at NakhonRatchasima, Thailand.

Soil property	Unit	Mean	Optimal level
pH	-	7.25	6.0 - 7.0
EC	dS/cm	0.12	2.0
Organic matter	%	1.54	2.0 - 3.0
Available P	mg kg^{-1}	50	35 - 60
Exchangeable K	mg kg^{-1}	176	100 - 120
Exchangeable Ca	mg kg^{-1}	1306	800 - 1500
Exchangeable Na	mg kg^{-1}	42	-
Available S	mg kg^{-1}	134	-
Available B	mg kg^{-1}	0.14	0.26
Textural class	Sandy loam soil	-	-

Thailand. The objectives of this study were: (1) to evaluate the effect of different levels of B on pollen viability and seed set of two sunflower varieties and (2) to determine optimum B levels for sunflower under field condition in a sandy loam soil.

MATERIALS AND METHODS

The experiment was conducted during the cropping season of 2011 at the experimental field of Suranaree University of Technology, Nakhon Ratchasima, in the Northeastern part of Thailand. Soil was collected from 0 to 30 cm layer for physical and chemical analysis. The soil type is classified as Typic Haplustulf (Soil Survey Staff, 2010). Physical and chemical properties of the soil were as follows: pH, 7.25; organic matter, 1.54%; exchangeable potassium (K), calcium (Ca), and sodium (Na) were 176, 1,306, and 134 ppm, respectively; available phosphorus (P), sulphur (S) and B concentrations were 50, 42 and 0.14 ppm, respectively (Table 1).

The soil was slightly basic, low in organic matter, total nitrogen (N) and B. These values revealed the low to medium fertility classes suggested for soils in the Northeastern Thailand. Factorial treatments (2 sunflower varieties and 5 levels of B application) were arranged in a randomized complete block design experiment with three replications. Sunflower varieties, "S473" (synthetic variety) and "Pacific 77" (hybrid variety), were used in this study. Five levels of B (0, 3.13, 6.25, 9.38 and 12.50 kg B ha^{-1}) were applied at planting as borax ($Na_2B_4O_7.10H_2O$).

Basal fertilizers were also applied at planting at the rate of 120 kg ha^{-1} of N, 45 kg ha^{-1} of P_2O_5 and 45 kg ha^{-1} of K_2O.The plot size was 4 × 5 m and three seeds were sown in one hill with the plant spacing of 70 × 30 cm. Seedlings were thinned to a plant per hill at 15 days after planting.

Data was recorded for leaf B concentration, B uptake, pollen viability and seed set. The viability of pollen grains was examined by 2,3,5-triphenyltetrazolium chloride (TTC). At R5.1 stage (Schneiter and Miller, 1981), anthers of both varieties in each replication were randomly collected. They were placed in a solution of 0.5% TTC for 60 min. To determine viability, pollen grains were counted under a microscope. Pollen grains were considered viable if they turn red, whereas those that remained translucent were dead (Shivanna and Rangaswamy, 1992). At R5.1 stage, leaf B concentration was analyzed. In each treatment, the youngest fully expanded leaves from the top (3rd and 4th leaves) were collected and dried at 70°C for 48 h. The dried leaves were finely milled and digested in 0.01 M $CaCl_2$. Boron analysis was based on the Azomethine-H method (Bingham, 1982). Percent seed set was determined at maturity by randomly sampling, ten plants per plot. Average quantities of total B uptake were estimated based on the total of plant dry matter and B concentration of plant tissue (Yermiyahu et al., 2001).Data collected were subjected to analysis of variance (ANOVA) using R statistical software (Zuur et al., 2009). The Duncan's multiple range test was performed to determine significant differences among B application levels. Correlation and regression analysis were carried out to exhibit the relationship between the observed traits determined. Then, the optimal B levels for sunflower varieties were determined from the regression equations.

RESULTS

No visual B deficiency symptoms were found on leaves of sunflower grown at various levels of B application. However, there were significant differences (Table 2) for pollen viability and seed set of sunflower varieties grown at various B levels.

Pollen viability

Data on pollen viability are shown in Table 2 and Figure 1. There were significances in pollen viability in response to applied B compared with control. Sunflower varieties grown in the low B (without added B) soil exhibited low pollen viability, while B application caused an increase in pollen viability. When B supply was increased from 0 to 9.38 kg B ha^{-1}, pollen viability increased from 71.93 to 98.33%. These gave an increase of 34.76 and 19.83% relative to the control for S473 and Pacific 77, respectively. Pollen viability of S473 increased to 96.93% at 9.38 kg B ha^{-1} after which there was a decline in the pollen viability with further increase in B levels. However, pollen viability of Pacific 77 increased up to 98.0% at 6.25 B ha^{-1} and did not decrease with further increase in B levels. Addition of B was sufficient to correct the low level of B in the soil. However, negative effect of pollen viability was observed when the level of B in the soil was increase up to 12.50 kg ha^{-1} in S473 variety.

Table 2. The effects of B applications on pollen viability, seed set, leaf B concentration and B uptake of sunflower varieties“S473” and ”Pacific 77”.

Variety	Applied B level (kg B ha^{-1})	Pollen viability (%)	Seed set (%)	B concentration (mg kg^{-1})	B uptake (mg plant^{-1})
S473	0	71.93 ± 2.4^{d}	63.00 ± 1.9^{bc}	32.40 ± 4.1^{e}	4.67 ± 0.8^{c}
	3.13	82.60 ± 1.9^{bc}	63.47 ± 3.1^{bc}	44.20 ± 3.6^{de}	6.57 ± 0.7^{bc}
	6.25	85.26 ± 1.6^{bc}	67.53 ± 3.2^{ab}	47.40 ± 2.9^{cde}	6.64 ± 1.4^{bc}
	9.38	96.93 ± 1.1^{a}	72.13 ± 4.6^{a}	50.04 ± 3.0^{bcd}	9.17 ± 0.5^{a}
	12.50	81.73 ± 1.2^{c}	71.00 ± 3.6^{a}	44.77 ± 3.5^{de}	7.94 ± 1.6^{ab}
Pacific 77	0	82.06 ± 1.9^{bc}	60.26 ± 2.4^{c}	33.87 ± 3.4^{de}	4.88 ± 0.7^{c}
	3.13	89.40 ± 1.7^{abc}	62.73 ± 1.8^{bc}	57.23 ± 1.9^{ab}	5.85 ± 1.2^{bc}
	6.25	98.00 ± 1.0^{a}	65.00 ± 2.8^{bc}	57.98 ± 2.1^{ab}	6.18 ± 0.6^{bc}
	9.38	98.33 ± 0.7^{a}	63.89 ± 2.8^{bc}	58.46 ± 2.2^{ab}	6.65 ± 1.3^{bc}
	12.50	98.13 ± 0.7^{a}	62.67 ± 2.9^{bc}	61.06 ± 2.9^{a}	6.84 ± 0.3^{bc}
F-test		**	*	**	*

*, **Significant differences at 0.05 and 0.01 levels, respectively. Means within a column followed by the different letters are significantly different at P < 0.05.

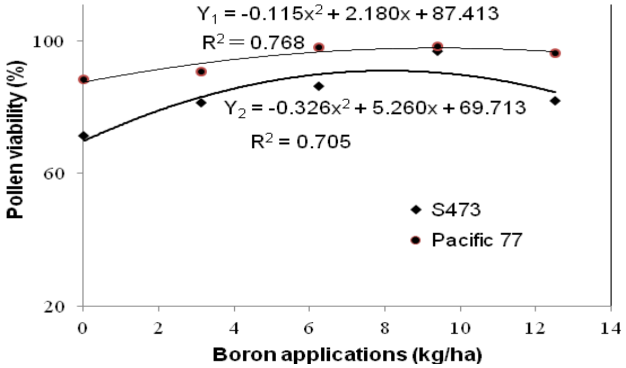

Figure 1. The relationship between pollen viability and B application levels.

Seed set

Effects of B levels on the seed set of both varieties were significantly different. Seed set of both varieties increased with the increase in the application of B up to 9.38 kg B ha^{-1}. Pacific 77 applied with 6.25 kg B ha^{-1} gave the highest seed set of 65.00%, whereas S473 variety recorded the highest seed set of 72.13% at 9.38 kg B ha^{-1}. Application of B up to 12.50 kg B ha^{-1} resulted in no further benefit, but tended to decrease percentage of seed set in comparison with 9.38 kg B ha^{-1}.

Leaf B concentration

There was low B concentration in leaves for both varieties grown under no B application. Increase in B application levels resulted to significant increase in leaf B concentration. Maximum B concentration of S473 (50.04 mg kg^{-1}) was obtained at B application level of 9.38 kg B ha^{-1}, while Pacific 77 (61.06 mg kg^{-1}) was obtained at 12.50 kg B ha^{-1}. It was observed that the B concentration in B added plants of Pacific 77 was greater than in S473, but the B concentrations in both varieties were similar in the control plants (no B application).

B uptake

In this study, B uptake of both varieties significantly increased with increase in B application levels. Among B application levels, minimum B uptake in sunflower leaves was recorded in the control for both varieties (4.67 and 4.88 mg plant^{-1}). Maximum B uptake of 9.17 mg plant^{-1} was obtained at 9.38 kg B ha^{-1} for S473. Further increase in B applications tended to decrease B uptake in S473. For Pacific 77, B uptake gradually increased with increase in B applications and reached the maximum level (6.84 mg plant^{-1}) at the highest B application level.

Correlation analysis

The correlation coefficients between traits are shown in Table 3. There was a strong positive association (r=0.876**) between the concentration of B in leaves at flowering stage and B uptake. There was a positive correlation between pollen viability and seed set (r=0.636*) and highly positive correlation between pollen viability and B concentration (r=0.844**) and B uptake (r=0.798**). Seed set had positive correlation with B concentration (r=0.534*) and had significant positive correlation with B uptake (r=0.863**). The results revealed

Table 3. Correlation coefficients among pollen viability, seed set, B concentration and B uptake of two sunflower varieties at different B levels.

Trait	Pollen viability	Seed set	B concentration	B uptake
Pollen viability	1.000	0.636*	0.844**	0.798**
Seed set		1.000	0.534*	0.863**
B concentration			1.000	0.876**
B uptake				1.000

*, ** Significant differences at 0.05 and 0.01 levels, respectively.

that seed set and pollen viability are also highly correlated with B uptake.

Regression analysis

The regression analysis result (Figure 1) showed that pollen viability could be used for prediction of optimum B levels for sunflower grown in sandy loam soil (R^2>70%). The optimum B application levels determined from the range of B application that gave 98% of maximum pollen viability ranged between 5.6 to 11.3 kg B ha^{-1} for Pacific 77 and 5.7 to 10.4 kg B ha^{-1} for S473.

DISCUSSION

There was a clear increase in B concentration, B uptake, pollen viability and seed set with the increasing levels of B application in both varieties.The results revealed high levels (32 to 60 mg kg^{-1}) of B concentrations in sunflower leaves, whereas critical concentration of 29 to 34 kg B ha^{-1} in the top mature leaf blades of sunflower have been reported (Blamey et al., 1997). In wheat, B requirement in anthers for successful seed set is 10 mg B kg^{-1} dry matter (Rerkasem et al., 1997).

In this study, increase in B applications caused an increase in B concentration and B uptake in both sunflower varieties. Boron concentration and B uptake in sunflower were closely related to pollen viability and seed set (Table 3). It can be concluded that increase in uptake of B enhanced the pollen viability and seed set. The efficacy of B on the pollen viability and grain set of wheat were studied by several researchers (Cheng and Rerkasem, 1993; Rerkasem et al., 1997). It is revealed that B tended to increase the pollen viability and grain set of wheat due to B role in plant growth and development. It has been reported that B plays essential roles in the structure and function of cell walls, cellular membranes, translocation of sugars, fruit and seed development (Cakmak and Römheld, 1997). Boron deficiency caused poor anther, pollen development, floret fertility and low grain set (Cheng and Rerkasem, 1993; Rerkasem et al., 1993; Huang et al., 2000). Rerkasem et al. (1997) also found that B influenced the male reproductive organs. Boron deficiency caused floret sterility which was mainly due to sterile pollen. They also reported that pollen of wheat required 8 to 10 mg B kg^{-1} in order to avoid grain yield losses from sterility. In this study, there was high correlation between pollen viability and seed set, but pollen viability was more responsive to B application than seed set in both varieties. Many factors adversely affect the formation of seed in sunflowers, such as B deficient, water deficit and high temperature. In many cases, B deficiency is associated with the induction of pollen sterility and low seed set and its impact may be exacerbated by environmental factors. Therefore, pollen viability would be a better parameter for determination of optimum B application levels.

At the highest level of B application (12.50 kg B ha^{-1}), the pollen viability was decreased in sunflower variety S473 (Figure 1). This could be due to toxic effect as a result of excess B (Chatterjee and Nautiyal, 2000). However, it was not the direct toxic effect of B on pollen viability, because B concentration and uptake in S473 decreased at the highest rate of B application. The reduction of B uptake at high rate of B application might be due to the toxic effects of B in the roots which could prohibit the nutrient uptake and plant growth (Lukaszewski and Blevins, 1996). This corresponds with the results of Oyinlola (2007), who reported that B application at high levels (12 kg B ha^{-1}) resulted in toxicity symptoms, low plant growth and yield reduction of sunflower grown in sandy loam soil. In addition, soil application of B has been reported to improve the harvest index, seed set, seed yield and oil content in sunflower (Rerkasem, 1986; Oyinlola, 2007; Al-Amery et al., 2011).

The results also exhibited the genotypic differences in B sensitivity which S473 is more sensitive than Pacific 77 to B applications. With added B, S473 responded to higher levels of B than Pacific 77. In addition, the results indicated that S473 variety had better pollen viability and seed set than Pacific 77 on soils with optimal B, butworse on soils with low and high levels of B.

Conclusion

The results of this study indicated that B application at planting date could increase pollen viability and percent seed set of sunflower. However, the application at high rate (more than 11.3 kg B ha^{-1}) resulted in no further benefit, but tended to decrease B uptake, pollen viability and seed set. From the result of the regression analysis, it could be concluded that, in this area, the optimum levels of B application for sunflower variety “Pacific 77” is 5.6 to 11.3 kg B ha^{-1}, while for variety “S473” is 5.7 to 10.4 kg B ha^{-1}.

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