

Effect of Silicon Application on Wheat Under Boron Stress

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Membran damage,
Relative water content,
Chlorophyll content

Abstract: In this study, effects of different silicon (Si) concentrations (0.75, 5, 10 mM) on wheat under boron(B) stress, investigated by means of some stress indicators such as tissue length, malondialdehyde (MDA), ion leakage, relative water content (RWC) and total chlorophyll content. Stress mitigating effects of silicon were observed mainly as reductions on membrane damage with reduced MDA concentrations and decreased ion leakage levels. Although necrosis was present on shoot tissues due to the boron stress, no significant change observed on shoot and root length with the application of Si. On the contrary, relative water content (RWC) has changed positively with the implementation of Si under boron stress. Furthermore Si has enhanced RWC level in Si+B co-applied sets to the level of control plants. Total chlorophyll content increased with the existence of 0.75 and 5 mM Si, while 10 mM Si had no effect on the chlorophyll content under boron stress. Overall, silicon was found to have a potential to alleviate boron stress in wheat production.

Silisyum Uygulamasının Bor Stresi Altındaki Buğday Üzerindeki Etkisi

Anahtar Kelimeler

Bor toksisitesi,
Silisyum uygulaması,
Buğday bor stresi,
Membran hasarı,
Bağlı su içeriği,
Klorofil içeriği

Özet: Bu araştırmada, bor (B) stresi altındaki buğday bitkisinde farklı silisyum (Si) konsantrasyonlarının (0.75, 5, 10 mM) etkisi, doku uzunluğu, malondialdehit (MDA), iyon sızıntısı, bağlı su içeriği (RWC) ve toplam klorofil içeriği gibi bazı stres göstergeleri aracılığıyla incelenmiştir. Silisyumun stres azaltıcı etkisi membran hasarının iyileştirilmesi ile MDA ve iyon sızıntısı seviyelerinin pozitif düzenlenmesi üzerinde gözlenmiştir. Bor (B) stresi nedeniyle gövde dokularında nekroz meydana gelmesine rağmen silisyum uygulamasının sürgün ve kök uzunlukları üzerinde önemli bir değişiklik gerçekleştirmediği gözlemlenmiştir. Ancak tam aksine bağlı su içeriği (RWC), bor stresi altında silisyum uygulanması ile pozitif olarak değişmiştir. Ayrıca silisyum ve bor stresinin (Si+B) birlikte uygulandığı setlerde silisyum, bağlı su içeriğini kontrol bitkilerindeki seviyelere kadar artırmaktadır. Toplam klorofil içeriği 0.75 ve 5 mM silisyum varlığı ile artarken 10 mM silisyumun bor stresi altında klorofil içeriğine herhangi bir etkisi olmamıştır. Genel olarak, silisyumun buğday üretiminde bor stresini azaltıcı bir potansiyele sahip olduğu bulunmuştur.

1. Introduction

Boron (B) is a necessary microelement for plant growth. B has numerous functions over plant metabolism and physiology [1]. Important example for B in plants emerged as structural and functional substance of cell walls. Boron (B) provides structural component of cell walls with rhamnogalacturonan II complex [2,3]. Boron toxicity is changeable among plants but difference between toxicity and deficiency becomes more sensitive when considering internal

boron concentration. Furthermore as reported information by Inal et al, B concentration decreased due to probable creation of B-Si (boron-silicate) complexes both in soil and within plant when presence of Si [4]. Including Turkey there is a lot of country has boron toxicity problem such as America, China, South Africa especially in arid and waterless region [5,6,7]. wheat is classified as low boron requirements plant. In soil just a few mg/kg boron may cause considerable yield losses [8]. Therefore the result of present investigation is substantial.

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Earth's crust contains silicon (Si) as a second abundant element. Plants can absorb silicon from soil when pH is under 9 as a monomeric molecule $[\text{Si}(\text{OH})_4]$ silicic acid [9,10]. Before 2 decades ago it was believed that silicon has no effect for direct growth but now a days when plants deal with abiotic and biotic stress factors silicon helps to exceed unfavorable conditions [11,12,13,14]. Si plays a role over apoplastic bypass flow to decrease and partial blockage. Thus provides reduction of heavy metal transport from root to leaves [15,16]. Most of plants undergo various stresses in their life span, therefore, Si has the potential to be used in agriculture. Si still remains a lot of unknown features in plant growth [17,18].

The point of this study is to understand the role of silicon under boron stress. Effects of co-application of different doses of silicon and boron in wheat were investigated. Our recent study [19] showed that Si has a potential to change boron transporter and some aquaporin gene expressions. The present study showed that under different silicon and boron applications, RWC, ion leakage, MDA and chlorophyll contents of wheat plants varied significantly. This study will be a guide for further studies to comprehend the roles of Si over B stress.

2. Material and Method

2.1. Plant materials, growth conditions and stress treatments

Plant materials as seeds of wheat (*Triticum aestivum*) cv. Ayyıldız were indulgently supplied by East Anatolian Agricultural Research Institute of Turkey. Firstly for prevention of contamination the seeds were superficially sterilized with 10 % sodium hypochlorite solution afterwards three to four times washed out with autoclaved distilled water. Seedlings were grown in plastic pots (Figure 1) loaded with perlite in a stable growth chamber at $23 \pm 1^\circ\text{C}$ with 8 hours dark and 16 hours light ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$) photo-cycle. Experiment was initiated mainly as seed germination with $\frac{1}{2}$ Hoagland's solution [20]. Germinated seeds became larger on 12th day of growth, subsequently boron stress was started by implementations of $\frac{1}{2}$ Hoagland's solution including determined boron (7.5 mM) concentration for 3 days. Control sets also were performed with seedlings which were irrigated with $\frac{1}{2}$ Hoagland's solution regularly. Silicon (0.75, 5, 10 mM) was added as sodium silicate ($\text{Na}_2\text{Si}_2\text{O}_7$) solution from the initiation of seedling germination. Furthermore in overall, five individual treatments were given as Control, 0 Si+7.5 mM B, 0.75 mM Si+7.5 mM B, 5 mM Si+7.5 mM B, 10 mM Si+7.5 mM B. Si and B represent silicon and boron respectively. Plants were reaped 15th day of growth which means that three days exposure of boron stress. Experiment was carried out for per sets of analysis at least 3 times.

2.2. Growth parameters

Shoot and root pieces of wheat were taken out from perlite after 15 days of developed seedlings and tissue lengths were measured (Figure 1).



Figure 1. Wheat plants under boron stress
*Pots from left to right belong to 7.5 mM B, 0.75 mM Si+7.5 mM B, 5 mM Si+7.5 mM B, 10 mM Si + 7.5 mM B respectively (Figure 1).

2.3. Relative water content

Leaf tissues were used to obtain relative water content (RWC) values. Firstly wet mass weight was measured before immersion in water for 24 hours at 23°C . After turgid mass was determined by weighing hydrated tissues. Shoot tissues were afterwards dried in an incubator at 60°C for 48 hours later weighed to assess the dry masses. Smart and Bingham [21] method was used to calculate relative water content via $\text{RWC} (\%) = \frac{\text{WM}-\text{DM}}{\text{TM}-\text{DM}} \times 100$ formula.

2.4. Determination of membrane damage

Membrane damage was evaluated by quantification of electrolyte leaked from both shoot and root tissues pursuant to the method of Nanjo et al [22]. Thermo Scientific Orion 013016MD conductivity meter was used to evaluate electrical conductance to tissues in 0.4 M mannitol.

2.5. Determination of malondialdehyde (MDA) content

Lipid peroxidation was evaluated by prediction of the malondialdehyde (MDA) content through the method of Ohkawa et al. [23]. Tissues were blended in 5 % trichloroacetic acid (TCA). The blended tissues were centrifuged at 12000 rpm for 15 minutes. Thiobarbituric acid added in trichloroacetic acid (1/4 w/w) then supernatant in same amount was incubated 25 minutes at 96°C on a dry heat block. The purpose of obtaining supernatant in samples, 10000 rpm for 5 minutes centrifuged then absorbance of supernatant was assessed at 532 nm afterwards to determine and subtract non-specific turbidity absorbance was read at 600 nm. Malondialdehyde contents were calculated by using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

2.6. Total chlorophyll content

Total chlorophyll content was determined by using methanol based method [24]. Prepared tissues homogenized with methanol were centrifuged at 4000 rpm for 5 minutes at 23 °C. Acquired supernatant were mixed with methanol then absorbance readed at 653 and 666 nm. After calculate of chlorophyll a and chlorophyll b. Total chlorophyll content has been determined.

2.7. Data analysis

Experiments were conducted with 3 replicates per analysis. The importance of the implementation effect was determined at 5 % prospect level by utilize Tukey test of one-way ANAVO by the assistance of SPSS 15.

3. Results

The goal of the research was to comprehend the role of Si under boron stress. Therefore, tissue length, malonedialdehyde (MDA), ion leakage, relative water content and total chlorophyll content analysis were (Table 1) performed in wheat (*Triticum aestivum*) cv. Ayyıldız tissues.

Present study showed that effect of Si, on both MDA and ion leakage in shoot tissues mitigated stress impact under boron toxicity. Although in root tissues, change in MDA level was not statistically important, ion leakage levels clearly showed positive effects of Si under boron stress.

Although shoot and root tissue length data were not found statistically important, leaf tip necrosis was observed in boron and co-applied Si+B plants. Even though there were necrosis over all boron treated plant leaves even at the presence of Si, total chlorophyll content has changed significantly.

All doses of Si application improved relative water content (RWC) under boron stress when compared to control plants, while under 7.5 mM B treatment without silicon existance, change was found statistically significant. Presence of Si increased water retaining capacity as much as control plants, under boron stress.

Shoot tissue MDA contents indicated a substantial reduction under the Si + B co-presence when compared to only B treated plant. However, in root tissues there were no substantial change which is statistically significant.

All analysis were performed under three different (0.75 mM, 5 mM, 10 mM) Si concentrations and under 7.5 mM boron stress. According to our findings Si helps to alleviate B toxicity. In general increasing Si concentration has some effect over MDA content of shoot tissues. 0.75 mM Si and 5 mM Si applications had more positive impact when compared to 10 mM Si application. Moreover, there were significant changes in relative water contents. All concentration of Si helped the plants to deal with boron stress and provided better water retaining capacity as much as to the level of control plants.

Si also had a positive impact on ion leakage levels in shoot tissues. Although the levels were not reduced to the level of control plants, all Si concentrations significantly decreased leakage under B toxicity.

Furthermore, interestingly in root tissues both 0,75 mM and 10 mM Si applications had more positive impacts when compared to 5 mM Si application under boron stress. Also all Si treatments decreased ion leakage levels when compared to only B application.

Although total chlorophyll content negatively affected from boron stress, presence of 0.75 mM and 5 mM Si increased chlorophyll contents under boron toxicity, while 10 mM Si didn't have any impact on chlorophyll contents.

Table 1. Conducted analysis on shoot and root tissues

Shoot tissues	Tissue length(cm)	MDA(nmol/g)	Ion leakage %	RWC %	Total Chlorophyll
C	28,81±0,21	8,02±0,41 _b	12,06±2,69 _b	88,81±0,94 _a	13,69±0,45 _a
0 Si+7.5 B	27,50±0,24	9,86±0,13 _a	26,28±1,16 _a	78,86±0,86 _b	10,78±0,40 _b
0.75 Si+7.5 B	27,91±0,29	8,03±0,34 _b	18,17±1,22 _{ab}	85,63±1,12 _a	11,69±0,34 _{ab}
5 Si+7.5 B	26,91±0,64	8,24±0,14 _b	20,04±1,30 _{ab}	86,65±0,85 _a	12,71±0,72 _{ab}
10 Si+7.5 B	28,40±0,69	8,94±0,42 _{ab}	19,90±2,43 _{ab}	84,14±1,27 _a	10,72±0,47 _b
Root tissues	Root length (cm)	MDA(nmol/g)	Ion leakage %		
C	14,10±0,95	4,97±0,31	24,17±0,98 _c	-	-
0 Si+7.5 B	12,95±0,55	6,04±0,69	30,87±0,31 _a	-	-
0.75 Si+7.5 B	13,80±1,09	5,43±0,48	27,97±0,36 _b	-	-
5 Si+7.5 B	13,76±0,98	5,35±0,26	28,98±0,56 _{ab}	-	-
10 Si+7.5 B	13,44±0,73	5,32±0,21	27,52±0,28 _b	-	-

* Treatments C, B, Si+B indicates; normal growth (control), 7.5 mM B application, simultaneous application of 0.75 mM Si and 7.5 mM B, 5 mM Si and 7.5 mM B, 10 mM Si and 7.5 mM B respectively.

*MDA and RWC stands for malonedialdehyde and relative water content respectively.

* The values appointed by distinct characters are meaningfully different on 5 % significance level.

4. Discussion and Conclusion

Boron toxicity is notably limiting wheat production. The toxicity symptoms of B were seen as general firstly chlorosis and later necrosis at the leaf tips in wheat [3,25,8]. Although necrosis was observed due to the application of boron stress, no significant difference on shoot and root lengths was detected in another study performed on barley, which were quite similar to our findings [19]. Therefore probably three days of stress treatment didn't show any difference in tissue length. Furthermore other studies also supporting that B toxicity importantly diminish shoot growth [4,26,27]. Stress mitigating effect of Si was monitored on MDA level in shoot tissues which was parallel to other studies [28,29,30]. However, no substantial change found statistically important in root tissues MDA level.

Based on the present work both MDA and ion leakage analysis were consistent meaning of understand membrane damage with boron stress. Which affect ameliorated by Si treatment of both two analysis. It was supported by previous studies [4,26,30]. Plants water retain capacity has decreased due to boron stress. Si has positive effect over RWC even increased water retain capacity as much as control plant under boron stress with the three different application dose. Stress alleviating effects of silicon can be associated with the competitive role of silicon for the transport via boron transporters under toxic boron levels [19].

Boron stress were dwindle total chlorophyll content when compared with control and 7.5 mM B treated plants, chlorophyll content affects negatively. Which were also expressed by reid [31]. Presence of 0,75 mM and 5 mM Si increased chlorophyll content under boron stress while 10 mM Si didn't make any difference. Similar results were supported by Eraslan [32].

Previous investigations emphasis that silicon were beneficial against environmental stress by amelioration of oxidative damage with the inducing efficient plant antioxidative defense system [28,32,33,34]. The consistency of present study analysis were approved that Si has a positive role of boron toxicity. Another study on barley which was quite similar to present study, determined that Si were change gene expressions profile under boron stress. Moreover more specifically silicon were changed aquaporin and boron transporter gene expression under boron toxicity [19]. This kind of change provides lower B concentration within plant tissues. Therefore silicon probably provide stress alleviation under B stress in present study. Overall, silicon was found to have a potential to alleviate boron stress in wheat production.

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