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Effect of sodium silicate on early growth stages of wheat under drought stress

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Abstract
Wheat yield is decreasing due to climate change, and a method to prevent decreasing yield during drought stress is desirable. In this study, wheat cultivars (Koso and Jokyung) were treated with 15% polyethylene glycol-6000 (PEG) and PEG + Si solution (6.5, 8.7, 13.1 and 26.1 mM). The effect of Si treatment on the alleviation of drought stress was measured using the germination test, shoot relative water content (RWC), seedling stage observation, and quantitative real time polymerase chain reaction (qRT-PCR). The results of root/shoot length ratio and shoot length ratio showed that Si treatment induced the alleviation of drought stress in Jokyung cultivar. The result of qRT-PCR showed the alleviation of drought stress in Koso cultivar. In addition, the results with shoot RWC and seedling stage observation showed that the alleviation effects of Si treatment was observed with both Koso and Jokyung cultivar at the high concentration of Si (26.1 mM). All these results suggest that Si treatment at a high concentration could be employed to alleviate drought stress in wheat.

Keywords Relative water content, Seedling stage, Silicate, Water shortage, Wheat cultivar

Introduction

The extent of damage caused by climate change increases every year, and drought is one of the most serious causes of damage. In the current century, the average temperature of the earth's surface is predicted to increase by 4.0 °C. As the average temperature rises by 1 °C, crop yields were reported to be reduced by 3–4% globally [1, 2]. Thus, climate change is expected to induce many detrimental effects globally, especially on agricultural industries [3]. Wheat is one of the most important staple foods, with approximately half of the world's population depending on it [4]. Among cultivated wheat varieties, consumption of bread wheat (*Triticum aestivum*) has increased because of its dominance as a staple food [5]. While wheat consumption has increased every year, wheat production is now decreasing. One factor causing the decrease in wheat yield is drought.

Silicon (Si) is the second most abundant element in the soil [6]. It has been widely reported that Si can stimulate plant growth and alleviate various biotic and abiotic stresses [7]. For example, Si can benefit plants exposed to abiotic stresses such as heavy metals, droughts, and heat. In addition, it is known that Si increases the robustness of cell walls in plants and accumulates in the gaps between cells and outer layers while increasing leaf size, thickness, and dry weight [8]. Thus, Si is an important nutrient for plants. Especially, many previous studies have reported the beneficial effects of Si in sorghum, rice, and potato regarding growth under drought stress [9–11]. However, only 0.03% of Si is present in the biosphere [12]. The solubility of Si is very low because Si is almost always present in the soil in combination with other elements [13]. The

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concentration of Si that plants can absorb is very limited; only 0.1–0.6 mM Si is absorbed from water. Therefore, a fertilizer containing soluble silicate is helpful for plant growth [14].

Plant growth in abiotic stress (i.e., salt and drought stress) has known to induce many changes of the expression of numerous genes or proteins. One example of such change is observed with MYB protein which forms the largest transcription factor families [15]. The MYB transcription factors were involved in abiotic stress tolerance and hormone signal transduction [16]. Although these transcription factor families have numerous genes, a few studies were performed regarding plant responses [17]. For example, one MYB gene (*TaMYBsdul*) was identified under drought and salt stress such that it suggested as a potential marker about drought and salt stress [18].

Previous studies have found that Si helps to alleviate drought stress in wheat during early growth [19]. However, few studies have examined what kind of effect Si has on the expression of genes related to drought stress. In the present study, we examined the effect of Si on drought stress during the initial growth of wheat at the molecular level and the physiological level. The two wheat cultivars used in this study were selected by difference in their protein content. Koso and Jokyung wheat cultivars contain approximately 7.2% (i.e., soft flour) and 13.3% (i.e., hard flour), respectively [20]. Changes in the expression of related genes and physiological characteristics such as germination rate, root and shoot length ratio, and relative water content (RWC) of two wheat cultivars were studied.

Materials and methods

Germination test

A germination test was performed according to methods described in a previous study [21]. The two wheat cultivars (Koso and Jokyung) used in this experiment were provided by the National Institute of Crop Science (Wanju-gun, Korea). Briefly, seeds of Koso and Jokyung were sterilized with 5% NaClO in a 50 mL conical tube and washed with sterilized distilled water three times. After being stored at 23 °C for 1 day, germination tests were performed using a paper towel (GeneAll, Seoul, Korea). A solution of sodium silicate (28%, Odus, Eumseong-gun, Korea) was used for Si treatments with the appropriate dilution. For the drought stress test, seeds of Koso and Jokyung wheat were treated with sterilized distilled water, 15% polyethylene glycol-6000 (PEG), and PEG + Si solution (6.5, 8.7, 13.1, 26.1 mM). For each treatment, five seeds were randomly selected. After 7 days, the germination test was concluded, and the number of germinated wheat seeds was counted, along with measurements of the length of roots and shoots.

Drought stress in the seedling stage

The degree of drought stress was measured according to a published procedure [22] with modifications. Each treatment consisted of 30 sterilized wheat seeds contained in a plastic pot (Φ75 mm × 66 mm) with 25 g of soil (Sunshine® Mix #4, Sun Gro Horticulture, Agawam, MA, USA). Phenotypes were compared between pots. Thirty milliliters of water were sprayed on the surface of each pot every 2 days for 8 days. From day 10, treatment solutions of 50 mL of water, PEG, and PEG + Si solution were administered every 2 days until day 20. Pictures of seedling plants were taken 1, 4, 7, and 10 days after the treatment. After the treatment of drought stress to the seedlings, some leaves were immediately sampled and frozen in a deep freezer (VT-78, My-Bio, Seoul, Korea) at −75 °C to measure the degree of gene expression. Other leaves were used to measure the shoot RWC as described below.

Relative water content

Shoot relative water content (RWC) was measured for each treatment that received water, PEG, or PEG + Si solution (26.1 mM). Shoot fresh weight (FW) was recorded using excised shoots from test pots taken 10 days after the first drought stress. The excised shoot was put into a 50 mL conical tube containing 5 mL of tap water. The conical tubes were stored in a refrigerator for 24 h at 4 °C to measure turgid weight (TW). After drying for 72 h at 60 °C (BI-600 M, Jeio Tech, Seoul, Korea), dry weight (DW) was recorded. RWC was calculated according to the following formula [23]:

$$\mathrm{RWC}\ (\%) = [(\mathrm{FW} - \mathrm{DW}) / (\mathrm{TW} - \mathrm{DW})] \times 100$$

Quantitative RT-PCR (qRT-PCR)

Total RNAs were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) from the shoots of seedlings that received water, PEG, or PEG + Si solution (26.1 mM) for 10 days. cDNA was synthesized with the first stand synthesis kit (GeneAll, Seoul, Korea). The cDNA value was quantitatively measured using a nanodrop spectrophotometer (Colibri LB 915, JC Bio, Seoul, Korea) and used in qRT-PCR. Amplifications were performed in 50 cycles using the CFX connect Real-Time PCR (Bio-Rad, Hercules, CA, USA) with BrightGreen 2 × qPCR MasterMix (ABM, Vancouver, Canada). The genes examined were the wheat (*Triticum aestivum* L.) genes TC225859(TC1), TC277220(TC2), TC326615(TC3), TC282418(TC4), and BT008981(BT6) whose expression has previously been shown to be altered under drought stress [18]. The primer sequences used for qRT-PCR are

Table 1 Primers used in PCR and RT-PCR analyses

Gene	Primer	Sequence of primer 5′-3′
TC1	TC335859 F	TCGGACTTCGTTGACAATACTC
	TC335859 R	CGAGTTCGTGCTTGGTTTAAG
TC2	TC277220 F	GGTGTTTCTAAAGTCCCCAGTTAGC
	TC277220 R	GGTATTGCGTGTAAGCGTGCTC
TC3	TC326615 F	TGCAGTTTGAAGAGTCATGGAATG
	TC326615 R	GCGCAGGAGCTTACGCAAC
TC4	TC282418 F	TGGGCATGTCGAAGCTAAG
	TC282418 R	CCTGGAAAGCCGATTGTC
BT6	BT008981 F	CAGTTCGATCTCCCGTTCTC
	BT008981 R	ATCATGCATTTCTTCCGAGTTC
ACT	AB181991 F	GTGCCCATTTACGAAGGATA
	AB181991 R	GAAGACTCCATGCCGATCAT

listed in Table 1. Actin was used as a housekeeping gene [24]. Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [25].

Data analysis

Data were statistically treated by an analysis of variance (ANOVA) and the least significant difference test at a probability level of 0.05. An ANOVA F-test was used to separate means in the case of a significant effect.

Results

Root length, shoot length, and germination rate of the Koso and Jokyung cultivars were measured for comparing physical difference under drought stress [21] (Table 2). For the Koso cultivar, root lengths of the control and PEG treatment groups were very similar, while shoot length decreased in the PEG treatment group, meaning that shoot length was affected by drought stress due to the PEG treatment. When treated with increasing concentrations of Si, root length only decreased gradually. In contrast, with PEG treatment, the shoot length of the Koso cultivar decreased suddenly from about 7.3 cm to approximately 3.2 cm, while shoot length was maintained up to the treatment of 13.1 mM Si and decreased to about 2.0 cm with the treatment of 26.1 mM Si. For the Koso cultivar, the germination rate was about 82% for the control and PEG treatment. The germination rates increased to about 89% for the 6.5 mM Si treatment and then decreased with further increases in Si concentration. For the Jokyung cultivar, the effects on root length, shoot length, and germination rate were quite different from those of Koso cultivar for the same treatment. That is, root length in the PEG treatment group was greater than that in the control group. Then, root length decreased gradually with further increases in Si concentration.

Table 2 Root length, shoot length, and germination rate of wheat seedlings after Si treatment

Cultivar	Treatment	Root length (cm)	Shoot length (cm)	Germination rate (%)
Koso				
	Control*	7.37^{a**}	6.48^{a}	82.5^{a}
	PEG	7.37^{a}	3.26^{b}	81.3^{a}
	6.5 mM	6.75^{ab}	3.27^{b}	89.4^{b}
	8.7 mM	5.83^{b}	3.15^{b}	87.5^{b}
	13.1 mM	6.00^{b}	3.18^{b}	81.3^{a}
	26.1 mM	4.30^{c}	2.03^{c}	68.1^{c}
Jokyung				
	Control	9.60^{ab}	9.17^{a}	93.8^{a}
	PEG	12.29^{c}	6.98^{cd}	88.8^{ab}
	6.5 mM	11.69^{c}	7.42^{c}	86.9^{b}
	8.7 mM	10.44^{b}	6.52^{de}	91.3^{ab}
	13.1 mM	8.68^{a}	5.99^{e}	89.4^{ab}
	26.1 mM	10.17^{b}	8.30^{b}	91.3^{ab}

* Each cultivar was treated with sterilized distilled water (control), 15% polyethylene glycol-6000 (PEG), and PEG + Si of 6.5, 8.7, 13.1 and 26.1 mM

** Within each wheat cultivar and measured parameter, values followed by the same small letter are not significantly different ($p = 0.05$)

Shoot length decreased from about 9.2 cm for the control group to 6.0–7.0 cm with the PEG treatment and Si treatment (6.5, 8.7, 13.1 mM) groups. However, the shoot length for the 26.1 mM Si treatment group increased to 8.3 cm, suggesting that a higher concentration of Si might be helpful for alleviating drought stress. The germination rate of the Jokyung cultivar was not affected by PEG treatment and Si treatment, suggesting that the Jokyung cultivar is resistant to drought stress or that Si treatment helps alleviate drought stress.

The root/shoot length ratio (RSLR) of the Koso and Jokyung cultivars was calculated from the values obtained in Table 2 (Fig. 1). The RSLR is an important information which indirectly shows the extent of drought stress alleviation [26]. The RSLRs of the Koso cultivar for the control group were close to 1.0 but increased to about 2.0 with PEG treatment and the four increasing concentrations of Si. However, the RSLR values for the treatments were not statistically different (Fig. 1A). The RSLRs of the Jokyung cultivar increased to about 1.7 for the PEG treatment group and decreased gradually with increasing concentration of Si. The RSLR of the Jokyung decreased to about 1.2 with the treatment of 26.1 mM Si (Fig. 1B). These results suggest that the Si treatment is helpful for alleviating drought stress for the Jokyung cultivar but not for the Koso cultivar. For the Koso cultivar, the shoot length ratio of the PEG treatment was about 48.8% of the control. The shoot length ratios of the 6.5, 8.7,

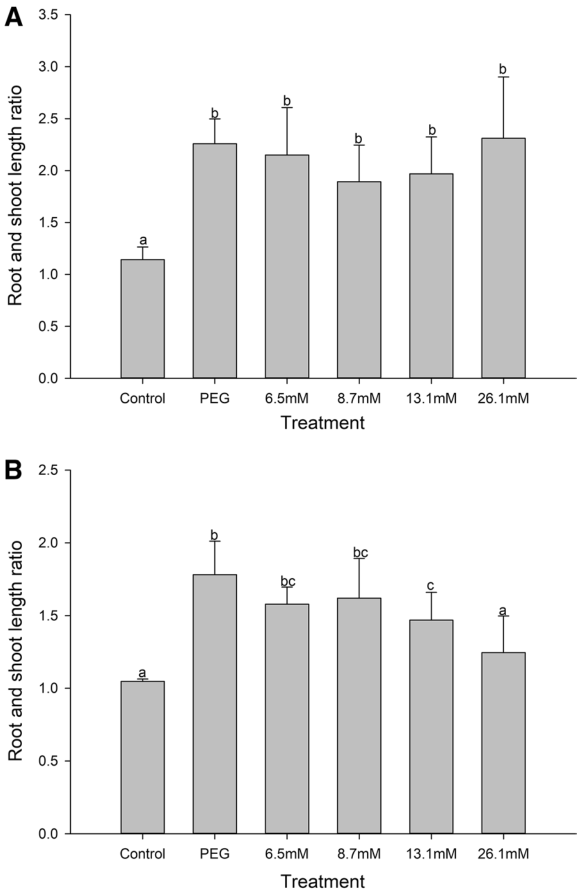

Fig. 1 The effect of Si treatment on root/shoot length ratio under drought stress. The wheat cultivars Koso (**A**) and Jokyung (**B**) were compared. Each group was treated with sterilized distilled water (control), 15% polyethylene glycol-6000 (PEG), and PEG + Si of 6.5, 8.7, 13.1 and 26.1 mM. Values followed by the same small letter are not significantly different ($p = 0.05$)

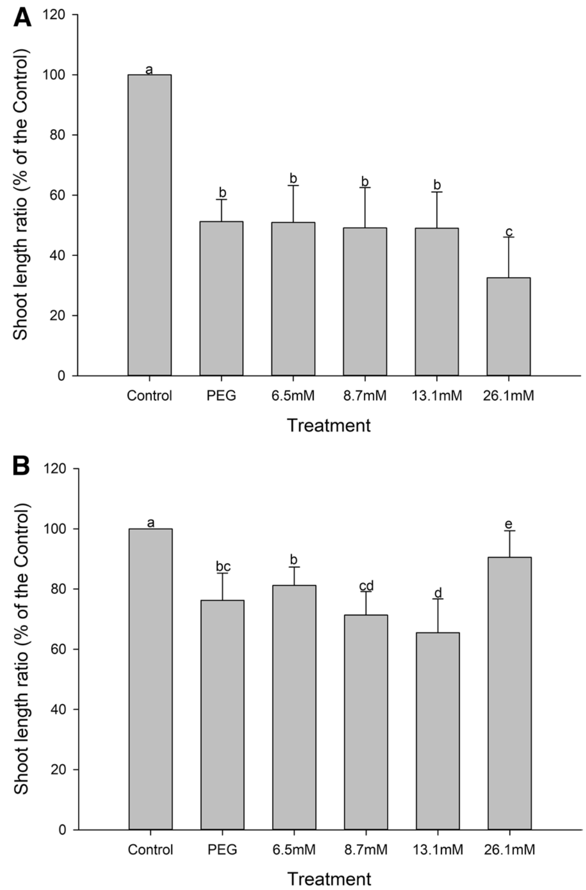

Fig. 2 The effect of Si treatment on shoot length ratio (%) under drought stress. The wheat cultivars Koso (**A**) and Jokyung (**B**) were compared. Each group was treated with sterilized distilled water (control), 15% polyethylene glycol-6000 (PEG), and PEG + Si of 6.5, 8.7, 13.1 and 26.1 mM. Values followed by the same small letter are not significantly different ($p = 0.05$)

13.1 mM Si treatment groups were not statistically different from the shoot length ratio of the PEG treatment group. However, the shoot length ratio of the 26.1 mL Si treatment was 30.0% of the control (Fig. 2A). These results suggest that Si treatment could not help alleviate the degree of drought stress in the Koso cultivar. For the Jokyung cultivar, the shoot length ratio of the PEG treatment was 76.3% of the control. As the concentrations of Si increased from 6.5 to 13.1 mM, the shoot length ratio slightly decreased to about 67.0%. However, the shoot length ratio of the 26.1 mM Si increased to 90.54% of the control. These results suggest that a high concentration of Si treatment could help alleviate the degree of drought stress in the Jokyung cultivar.

Based on the observation displayed in Figs. 1 and 2, the Si concentration of 26.1 mM was selected for investigating the effect of Si on the shoot RWC of the two wheat cultivars (Fig. 3). If drought stress of wheat is alleviated, wheat would contain more water in their leaves. Thus, comparison of shoot RWC indicates the comparative water content of wheat leaves under drought stress [27]. The RWC values of the Koso cultivar were 96.0%, 84.4%, and 91.4% for control, PEG treatment, and 26.1 mM Si treatment, respectively. The RWC values of the Jokyung cultivar were 93.3%, 75.1%, and 87.0% for the control, negative control (PEG treatment), and 26.1 mM Si treatment, respectively. Thus, the RWC values decreased with PEG treatment and increased with Si treatment. These results suggest that the extent of drought stress was lowered by the Si treatment in the two wheat cultivars.

One of methods to confirm the alleviation of drought stress is the direct observation of their phenotypic changes [22]. The time-dependent phenotypic changes of two wheat seedlings, which were under 15% PEG-stimulated drought stress, were observed with a pot test over 10 days. As shown in Fig. 4, the appearance of

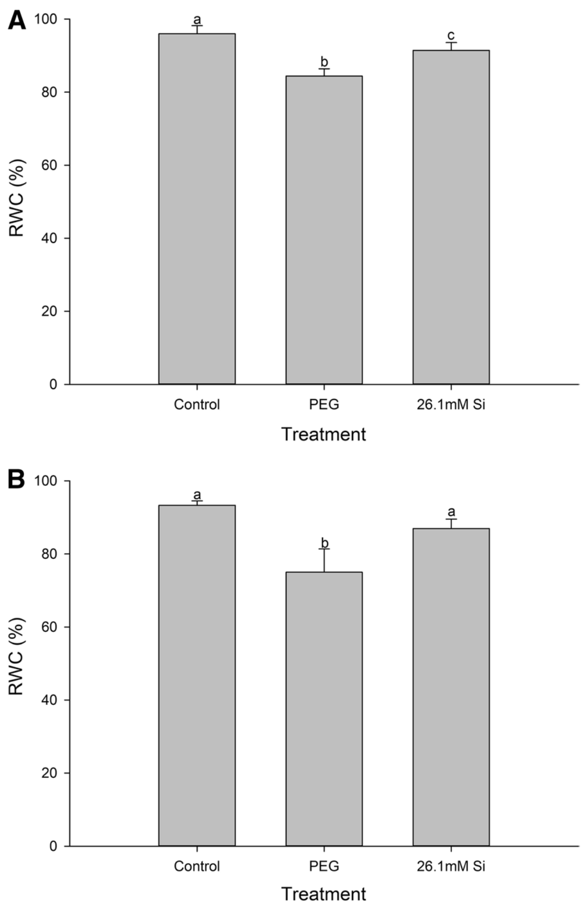

Fig. 3 The effect of Si treatment on relative water content (RWC) under drought stress. The wheat cultivars Koso (**A**) and Jokyung (**B**) were compared. Each group was treated with sterilized distilled water (control), 15% polyethylene glycol-6000 (PEG), and PEG + Si of 26.1 mM. Values followed by the same small letter are not significantly different ($p = 0.05$)

wheat shoots did not differ on day 1 among the control, PEG treatment, and Si treatment (26.1 mM). However, a noticeable change was observed on days 4, 7, and 10. Overall, the wheat shoots of the PEG treatment seemed to have more significant phenotypes, such as withered leaves, curled leaves, and shorter plant height. Wheat seedling status on day 10 seemed to be better in the order of control > Si treatment > the PEG treatment for the two cultivars. The extent of recovery from the PEG treatment was similar for both cultivars. These results suggest again that the Si treatment helped to protect the wheat seedlings from drought stress due to the PEG treatment. As observed in the germination test results (Table 2, Figs. 1 and 2), the Si treatment was effective at alleviating drought stress due to the PEG treatment for the Jokyung cultivar but not the Koso cultivar. However, the results of the RWC (Fig. 3) and phenotypic change (Fig. 4) show that Si treatment can effectively alleviate drought stress in both cultivars.

qRT-PCR analysis was conducted to examine the variation in the expression for five selected genes after treatment (Fig. 5). The five genes (TC1, TC2, TC3, TC4, and BT6) are known to be related to drought stress. The five genes used in the present study were confirmed as a marker for drought stress response [18]. For the Koso cultivar, the relative RNA expression levels of the five genes were much higher with the PEG treatment than in the control. However, with the Si treatment of 26.1 mM, the relative RNA levels decreased to about the same level as the control for genes TC2, TC3, TC4, and BT6, or were much lower than the control for TC1. In contrast, Si treatment for the Jokyung cultivar had no specific effect on the relative RNA expression levels of the five genes (Fig. 6).

Discussion

A paper towel test was performed to determine the effect of Si treatment on the RSLR and germination rate of two wheat cultivars. Drought stress was induced by the application of 15% PEG. It was evident that Si treatment had a more positive effect on the Jokyung cultivar than the Koso cultivar (Table 2 and Figs. 1 and 2). The RSLR of wheats which were growth in normal conditions has approximately close to 1 [26]. Unlike in the Koso cultivar, the RSLR for the Jokyung cultivar increased by the PEG treatment and gradually decreased with an increasing concentration of Si. The RSLR was not statistically different between control and the 26.1 mM Si treatment, suggesting that drought stress was relieved with Si treatment for the Jokyung cultivar. The RSLR of the Koso cultivar was not statistically different between the PEG treatment and the Si treatment (Fig. 1).

The shoot lengths in drought condition are decreased compared to those in normal condition due to the lack of nutrients for growth [27, 28]. For the Koso cultivar, the shoot length ratio decreased to about 50% of control with the PEG treatment and was not affected by the Si treatment (Fig. 2A). For the Jokyung cultivar, the shoot length ratio of the Si treatments (6.5, 8.7, 13.1 mM) was 70–80% of the control (Fig. 2B). However, the shoot length ratio noticeably recovered with the Si treatment of 26.1 mM. This result indicates that for the Jokyung cultivar, the relatively higher concentration of Si (26.1 mM) might be needed to have a positive effect on the shoot length ratio.

The shoot RWC might be more important than the other indicators for mitigating drought stress [29]. This is because most plants try to conserve turgor pressure to maintain their metabolic activity by the continuous absorption of moisture [30]. For the two wheat cultivars tested, RWC decreased with PEG treatment and

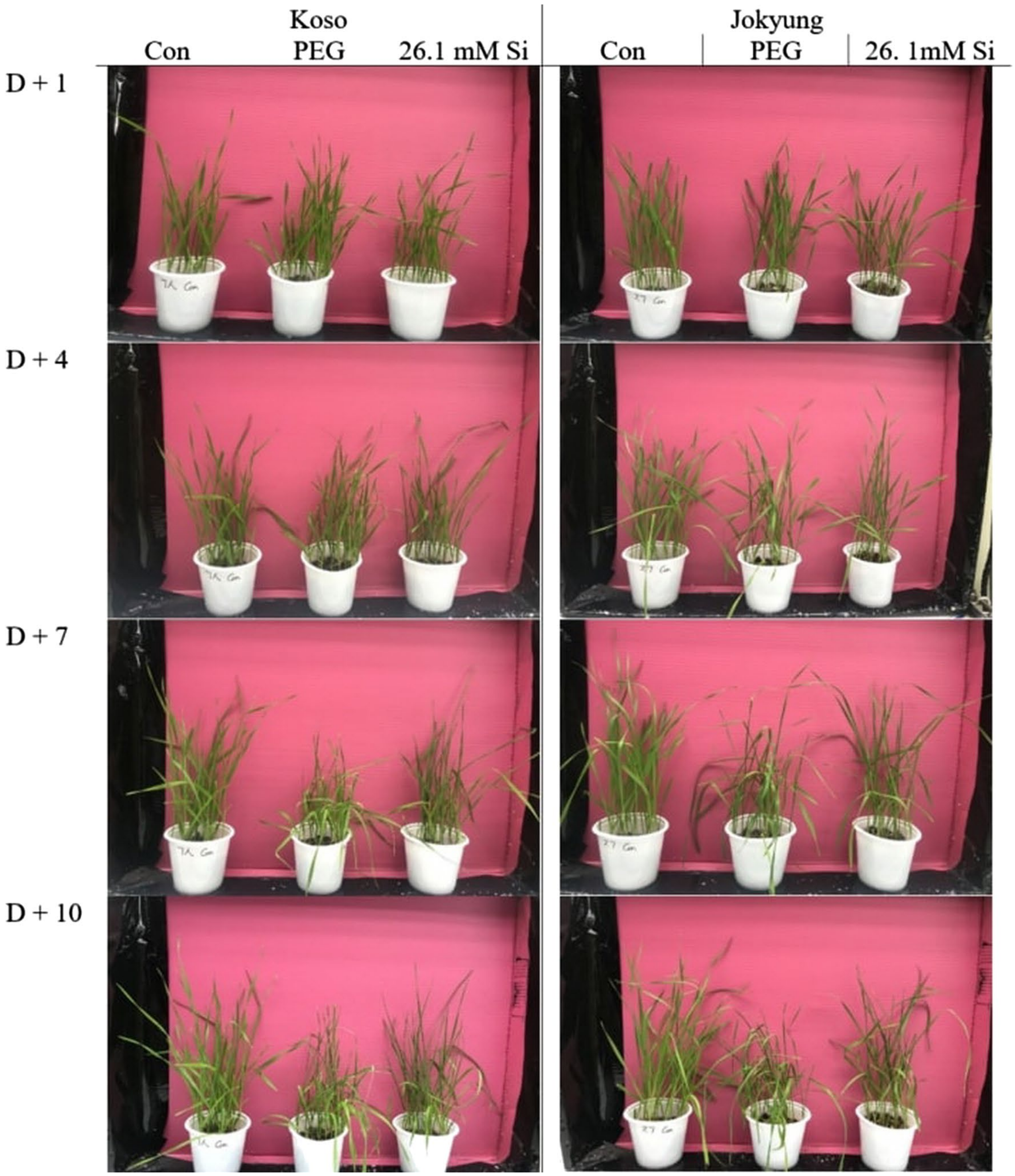

Fig. 4 The effect of Si treatment on phenotypic changes of wheat under drought stress. The wheat cultivars of Koso (**A**) and Jokyung (**B**) were compared. Each group was treated with sterilized distilled water (control or CON), 15% polyethylene glycol-6000 (PEG), and PEG + Si of 26.1 mM

increased with the Si treatment of 26.1 mM (Fig. 3). That is, the Si treatment had a positive effect on the RWC for both cultivars. This result contrasts with the results in Figs. 1 and 2 because the Si treatment did not increase the RSLR and shoot length ratio, the experiments of which were performed on a paper towel. The test of RWC was performed with a pot test containing soil. Although various paper towel tests are convenient, they might not be enough to mimic the actual growth of a plant in soil. Thus, caution needs to be used when interpreting the results from paper towel tests. The shoot RWC is known to be correlated with the water holding capacity of a plant [31]. Because the Si treatment of 26.1 mM increased the RWC, it is expected that by enhancing the water holding

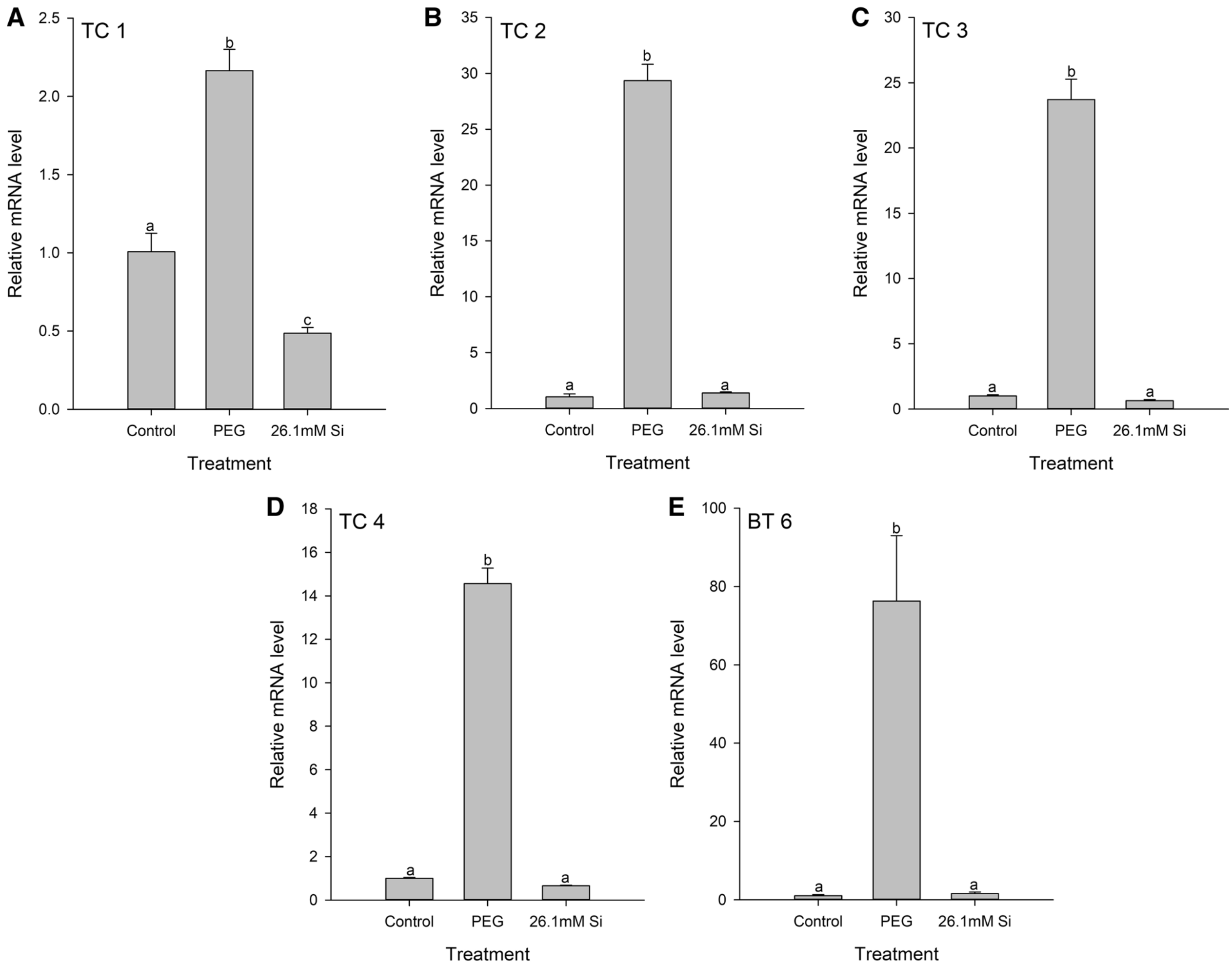

Fig. 5 The effect of Si treatment on the expression of genes for the Koso cultivar under drought stress. The treatments were sterilized distilled water (control), 15% polyethylene glycol-6000 (PEG), and PEG + Si of 26.1 mM. Values followed by the same small letter are not significantly different ($p = 0.05$)

capacity of wheat, the Si treatment could contribute to an increase in wheat yield under drought stress.

The results in Fig. 3 were supported by the effect of Si treatment on the phenotypic changes of wheat seedlings, as shown in Fig. 4. It is difficult to quantify the outer appearance in the photograph, but it is obvious that Si treatment helped seedlings recover from wheat shoot damage by drought stress due to the PEG treatment, which can be observed more clearly on day 7 and day 10. This phenotypic change might be correlated with the expression of genes related to drought stress [18]. In previous studies with *Triticum aestivum* L., it was shown that the gene expression of TC1, TC3, and BT6 increased but that of TC2 and TC4 decreased during drought. However, the results of this study have shown that the relative mRNA expression of TC2 and TC4, as well as TC1, TC3, and BT6, increased under drought stress for the Koso cultivar. Upon treatment with Si, the relative mRNA expression decreased back to almost the same level as in the control (Fig. 5). It is speculated that the mRNA expression of the two additional genes (TC2 and TC4) might be different from that in the previous study due to differences in the wheat cultivar tested. The expression of various genes related to drought for the Jokyung cultivar was also examined but no specific trend was observed, suggesting that there could be differences in the expression of the five genes among different wheat cultivars (Fig. 6).

In the present study, the relief of drought stress by Si treatment was tested for the two wheat cultivars. The paper towel test and the pot test were used to measure the RSLR, the shoot length ratio, and the shoot RWC. The results obtained for the pot test showed that Si treatment caused the phenotypic changes of wheats that contained low proteins or high proteins. This result is important because any changes due to

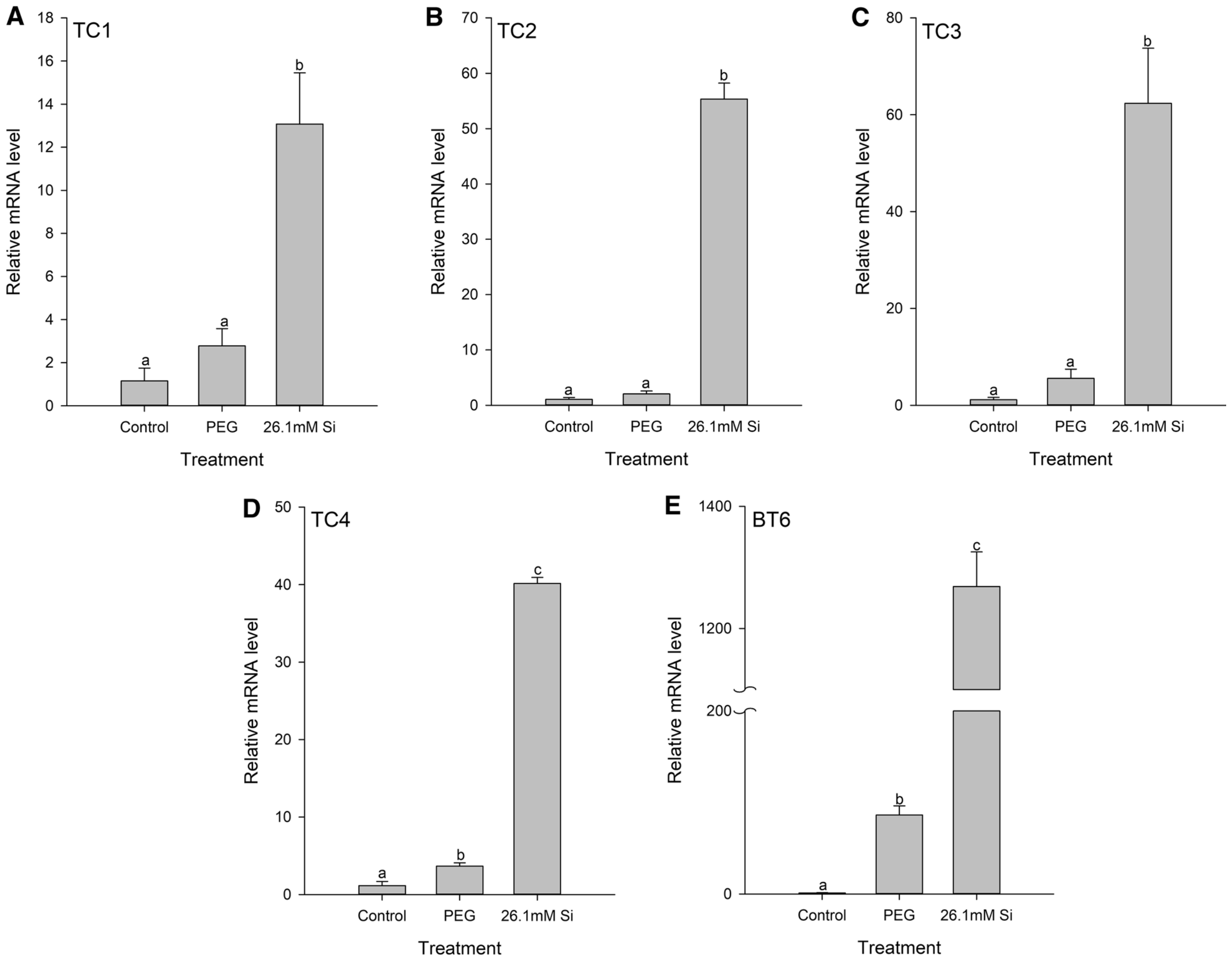

Fig. 6 The effect of Si treatment on the expression of genes for the Jokyung cultivar under drought stress. The treatments were sterilized distilled water (control), 15% polyethylene glycol-6000 (PEG), and PEG + Si of 26.1 mM. Values followed by the same small letter are not significantly different ($p = 0.05$)

drought stress have not much meaning if there are no visible changes. Thus, the phenotypic changes become important information about the benefits of Si treatment under drought stress. Additionally, the increase in the shoot RWC also indicated the alleviation of drought stress. These results are matched with those of phenotypic changes. However, the gene expression related to drought stress only matched the phenotypic changes observed in the Koso cultivar whose flour is used to make snack. This means that other combination of genes might be necessary to be matched with the phenotypic changes for Jokyung wheat cultivar. The measurement of RSLR and the shoot length ratio in control by the paper towel test was convenient and less time-consuming but, in some cases, this might not reflect the results of the pot test and gene expression study. However, for the purpose of comparison, both the paper towel test and the pot test might be necessary to observe the usefulness of Si treatment for other wheat cultivars. That is, we have found that every measurement was differentially affected by wheat cultivars. Thus, more factors (for example, spring/fall wheat and protein content) need to be examined to explain the reason of Si treatment effect on various other wheat cultivars in the future study. All the results obtained in the present study indicated that Si treatment might be effective in mitigating drought stress during wheat growth.

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Authors' contributions
SG performed experiments and wrote the paper. Hyeri and JC helped the preparation of experiments. BC and HJ revised the manuscript. NC and CH

edited, revised the manuscript and supervised the work. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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