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SILICON ALLEVIATES THE ADVERSE EFFECTS OF SALINITY AND DROUGHT STRESS ON GROWTH AND ENDOGENOUS PLANT GROWTH HORMONES OF SOYBEAN (*GLYCINE MAX* L.)

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Abstract

Agricultural industry is subjected to enormous environmental constraints, particularly due to salinity and drought. We evaluated the role of silicon (Si) in alleviating salinity and drought induced physio-hormonal changes in soybean grown in perlite. The plant growth attributes i.e., shoot length, plant fresh weight and dry weight parameters of soybean improved with elevated Si nutrition, while they decreased with NaCl and polyethylene glycol (PEG) application. The adverse effects of NaCl and PEG on plant growth were alleviated by adding 100 mg L^{-1} and 200 mg L^{-1} Si to salt and drought stressed treatments. It was observed that Si effectively mitigated the adverse effects of NaCl on soybean than that of PEG. The chlorophyll contents were found to be least affected as an insignificant increase was observed with Si application. Bioactive GA_1 and GA_4 contents of soybean leaves increased, when Si was added to control or stressed plants. Jasmonic acid (JA) contents sharply increased under salinity and drought stress but declined when the plants were supplemented with Si. Similarly, free salicylic acid (SA) level also increased with NaCl and PEG application. However, free SA level further increased with the addition of Si to salt treated plants, but decreased when Si was given to PEG treated plants. It was concluded that Si improves physio-hormonal attributes of soybean and mitigate adverse effects of salt and drought stress.

Introduction

Silicon is mostly present in soil solution as silicic acid (H_4SiO_4) at the concentrations of 0.1-0.6 mM and is readily absorbed so that terrestrial plants contain it in appreciable concentrations, ranging from 1% to 10% or even higher of the dry matter. This difference of Si levels in different plant species have been attributed to the Si uptake ability of the roots (Takahashi *et al*., 1990; Parveen & Hussain. 2008.). Silicon deposition in the tissues help to alleviate water stress by reducing transpiration rate, improve light interception characteristics by keeping the leaf erect, increase resistances to diseases pests and lodging, remediate nutrient imbalances, and there are other documented beneficial effects (Epstein, 1994; Savant *et al*., 1997; Ma *et al*., 2001). Silicon presence in the cell wall fibre makes the cell wall tough and resistant to pest and pathogens attacks. Despite of the prominence of Si as a mineral constituent of plants, Si is not considered as "essential" nutrient, for any terrestrial higher plants except members of the Equisitaceae and is thus not included in the formulation of any of the commonly used nutrient solutions (Epstein, 1994).

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However, the importance of Si cannot be overlooked due to its beneficial effects on plant growth and development, as Si promotes growth by altering the levels of endogenous growth hormones. The plant growth hormones, such as gibberellins, jasmonic acid (JA) and salicylic acid (SA) play a favourable role in the growth and development of plant. Gibberellins (GAs) influence stem elongation, flower and fruit development, and seed germination (Ross *et al*., 1997). Jasmonic acid (JA) is a naturally occurring plant growth regulator (PGR) found in higher plants (Creelman *et al.,* 1992). JA has been reported to induce a wide range of physiological and developmental responses in plants (Xiang & Oliver, 1998; Engelberth *et al*., 2001) and thus JA has been implicated as important signal molecules, mediating induced defences of soybean against herbivores and pathogens. Salicylic acid (SA) is also known as an important signal substance that induces systemic acquired resistance (SAR) against pathogens in plant (Cameron *et al*., 1999; Siegrist *et al*., 2000). SAR is an inducible defense mechanism and plays an important role in defending the plants from pathogenic attacks (Durner *et al*., 1997). Salicylic acid is involved in local and systemic resistance to pathogens, including induction of pathogenesis-related (PR) proteins in plants, such as tobacco, cucumber, tomato and *Arabidopsis* (Delaney *et al*., 1995).

The effect of Si on growth and physiological processes is not well understood yet sparsely investigated (Nwugo & Huerta, 2008). Current study was aimed to evaluate the effect of Si, NaCl induced salt stress and PEG induced drought stress on growth and endogenous GA, JA and SA levels of soybean cultivar Daewonkong. Furthermore, the role of silicon in salinity and drought stress alleviation was also investigated as little information is available on the subject.

Materials and Methods

This experiment was arranged as complete randomized block design (CRBD), and consisted of 9 treatments, 3 replications per treatment and each replication comprised 9 plants (27 plants per treatment). Seeds of famous Korean soybean cultivar Daewonkong were procured from Plant Genetics Lab., Department of Agronomy, Kyungpook National University, Korea.

Growth conditions: Seeds were surface sterilized with 5% NaClO for 15 minutes and then rinsed with double distilled water. Seeds were sown in plastic pots (5.5 L) filled with perlite as a growth medium. The experiment was conducted under green house conditions with a temperature of 30±2ºC and 45±5% humidity. A 100 ml of Hoagland solution (Hoagland & Arnon 1950) was given to plants 4 times i.e., at emergence and 1st, 2nd and 3rd week after emergence. The first two Hoagland solution doses were of half strength while the later two of full strength.

Silicic acid (H_4SiO_4) was applied @100 mg L^{-1} and 200 mg L^{-1}, while each pot received 300 ml of silicon solution. For salt stress induction, 100 mM of NaCl solution, while for drought stress induction, 12% of PEG (10000 MW) solution was given to plants 20 days after sowing (DAS). Two doses of salt stress or drought stress (300 ml each) were given to each pot and the stress condition prevailed for two weeks till harvesting of soybean plants (34 DAS). NaCl and PEG were applied in the beginning and middle of the two weeks stress period.

Hormonal analysis: Nine plants per treatment were harvested 24 hr after NaCl and PEG application and immediately frozen in liquid nitrogen and stored at minus 70ºC. The plant samples were lyophilized in freeze drier (Virtis, SP Industries Inc.). The leaves of the lyophilized plant samples were crushed to powder for the analysis of gibberellins, jasmonic acid and salicylic acid.

Analysis of bioactive gibberellins: The endogenous GAs levels were quantified according to the protocol of Lee *et al.*, (1998). Extracted GA_1 and GA_4 were subjected to reverse-phase C18-HPLC. The GA_1 and GA_4 were chromatographed on a 3.9 x 300 m Bondapak, C_{18} column (Waters Corp., Milford, MA, USA) and eluted at 1.5 ml min-1 with the following gradient: 0 to 5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5 to 35 min, linear gradient from 28 to 86% MeOH; 35 to 36 min, 86 to 100% MeOH; 36 to 40 min, isocratic 100% MeOH. The fractions were then prepared for gas chromatograph/mass spectrometer (GC/MS) with selected ion monitoring (SIM) (6890N network GC system and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA). For GA_4 quantification, 1 ul of sample was injected in a 30 m × 0.25 mm (i.d.), 0.25 μm film thickness DB-1 capillary column (J & W Scientific Co., Folsom, CA, USA). The GC oven temperature was programmed for a 1 min hold at 60°C, then to rise at 15°C min^{-1} to 200°C followed by 5°C min^{-1} to 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a Mass Selective Detector with an interface and source temperature of 280°C, an ionizing voltage of 70 eV and a dwell time of 100 ms. GA_1 and GA_4 was quantified with GC-MS SIM using $[17, 17\text{-}^2H_2]$ –GA_4 (20 ng) as internal standard (obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia). The endogenous GA_1 and GA_4 content were calculated from the peak area ratios of 508/506 and 286/284, respectively.

Analysis of jasmonic acid: The endogenous JA level was extracted according to the protocol of McCloud & Baldwin (1997). The extracts were then analyzed by GC-MS (6890N network GC system, and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA). To enhance the sensitivity of the method, spectra were recorded in the selected ion mode i.e. in case of JA determination, monitored the fragment ion at m/z= 83 amu corresponding to the base peaks of JA and $[9, 10\text{-}^2H_2]$-9, 10-dihydro-JA (Koch *et al.*, 1999). The amounts of endogenous JA were calculated from the peak areas of JA in comparison with the corresponding standards. Three replicates per treatment were used for determination of JA.

Analysis of free salicylic acid: Free SA was extracted and quantified as described by Enyedi *et al.*, (1992) and Seskar *et al.*, (1998). Powder of leaf tissues (0.1 g) was sequentially extracted with 90 and 100% methanol by centrifuging at 10,000 rpm. The combined methanol extracts were vacuum dried. Dry pellets were resuspended in 2.5 ml of 5% Trichloroacetic acid. The supernatant was partitioned with Ethyl acetate: cyclopentane: isopropanol (100:99:1, v/v). The top organic layer containing free SA was transferred to a 4 ml vial and dried with nitrogen gas. The dry free SA was again suspended in 1 ml of 70% Methanol. HPLC condition was maintained at fluorescence detector (Shimdzu RF-10AXL, with excitation 305 nm, and emission 365 nm). The separation was done on a C18 reverse-phase HPLC column (Waters Corp., Milford, MA, USA).

Measurement of plant growth: The plant length, shoot and root fresh and dry weights were measured for harvested soybean plants while chlorophyll content of fully expanded leaves was analyzed with the help of chlorophyll meter (Minolta Co., Ltd, Japan). Three replicates of 6 plants each per treatment were randomly selected for measuring growth parameters. The dry weights were measured after drying the samples at 70°C for 48 hr in an oven (Bohm, 1979).

Statistical analysis: The data was statistically analyzed for standard deviation, using MS-EXCEL software. The mean values were compared, using the Duncan's multiple range test (DMRT) at $p<0.05$ (ANOVA SAS release 9.1; SAS, Cary, NC, USA).

Results

Silicon and growth of soybean under salinity and drought: Current study showed that silicic acid enhanced plant growth when applied under control conditions, while its application under plant stress condition alleviated the adverse effects of salinity and drought. The shoot length significantly increased with Si as maximum shoot length of 52.6 cm was observed with double Si application as compared to control. Shoot length significantly decreased with sole NaCl and PEG treatment (Table 1).

The shoot fresh and dry weight attributes significantly improved when Si applied singly or with salt stress. However, it significantly declined with sole NaCl or PEG application. Almost similar results were recorded for root fresh and dry weight parameters. The chlorophyll contents decreased under salinity and drought stress, although decline observed in the chlorophyll contents under stress condition was not significant. We observed that sole NaCl effect was more detrimental than PEG in soybean.

Silicon enhanced bioactive GA_1 and GA_4 contents of soybean: The endogenous bioactive GA_1 and GA_4 contents of Soybean leaves increased with elevated Si, while decreased with NaCl and PEG application as compared to control. Under stress condition, addition of basic and double Si amounts to plants enhanced GA_1 and GA_4 contents, though basic Si was more effective than double Si application. Addition of Si to PEG stressed soybean plants enhanced GA_4 levels but a decline in GA_1 content was observed with elevated Si nutrition (Fig. 1). The amount of GA_4 was found to be much higher than GA_1 in all treatments.

Silicon decreased endogenous JA contents under stress: The endogenous JA contents of soybean leaves increased with basic and double Si as compared to control. It was noted that JA content increased in plants treated with PEG and NaCl, as maximum JA contents were found in plants treated with PEG (102.48 ng/g), followed by NaCl (62.49 ng/g) (Fig. 2). An addition of Si to both NaCl and PEG stressed plants decreased the level of JA content of soybean leaves.

Silicon differentially affected salicylic acid under salinity and drought: Sole application of Si enhanced endogenous SA contents of soybean, while NaCl decreased it as compared to control. Under salinity stress, Si increased free SA contents, although the amount of SA was higher in plants treated with basic Si (100 mg L^{-1}) than double Si (200 mg L^{-1}). Under drought, a significant increase in SA contents was recorded. However, in contrast to salinity, an application of Si to drought stressed plants decreased the free SA contents of soybean leaves (Fig. 3).

Table 1. Silicon promoted growth of cv. Daewonkong under salinity and drought stress.

Treatment	Conc./unit	Shoot length (cm)	Shoot weight (g plant^{-1})		Root weight (g plant^{-1})		Chl. Content
			FW	DW	FW	DW	
Control	0	48.85^{ab}	6.02^{b}	1.77^{b}	14.37^{ab}	2.06^{a}	25.9^{a}
NaCl	100 (mM)	38.9^{c}	5.29^{b}	1.48^{b}	10.69^{b}	1.65^{b}	23.9^{a}
PEG	12 (%)	36.3^{c}	5.12^{b}	1.39^{b}	11.64^{b}	1.75^{b}	25.4^{a}
Silicon	100 (mg L^{-1})	49.03^{ab}	6.69^{b}	2.02^{a}	16.36^{a}	2.16^{a}	28.4^{a}
Silicon	200 (mg L^{-1})	52.6^{a}	8.2^{a}	2.24^{a}	16.75^{a}	2.11^{a}	27.6^{a}
NaCl+ Silicon	100 + 100	45.9^{ab}	6.73^{b}	1.89^{ab}	15.45^{a}	2.0^{a}	27.4^{a}
NaCl+ Silicon	100 + 200	40.4^{bc}	6.85^{b}	1.87^{ab}	15.85^{a}	1.98^{ab}	26.9^{a}
PEG+ Silicon	12 + 100	42.3^{bc}	5.99^{b}	1.5^{b}	11.45^{b}	1.64^{b}	27.5^{a}
PEG+ Silicon	12 + 200	41.8^{bc}	5.49^{b}	1.64^{b}	10.85^{b}	1.46^{b}	25.03^{a}

*In a column, treatment means having a common letter(s) are not significantly different at the 5% level by DMRT. FW stands for fresh weight; DW stands for dry weight.

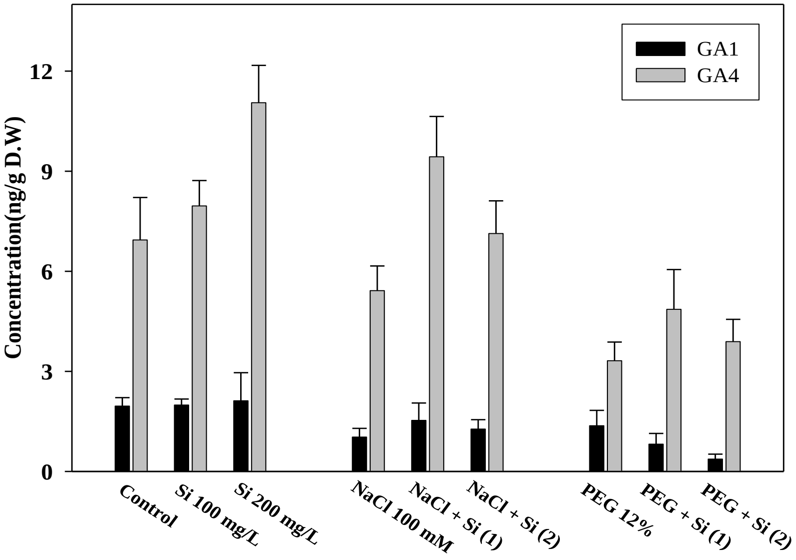

Fig. 1. Endogenous bioactive GA$_1$ and GA$_4$ contents of soybean leaves in response to NaCl and NaCl+Si or PEG+Si. Error bars show standard deviations. Si (1) and Si (2) stand for Si 100 mg/L and Si 200 mg/L respectively.

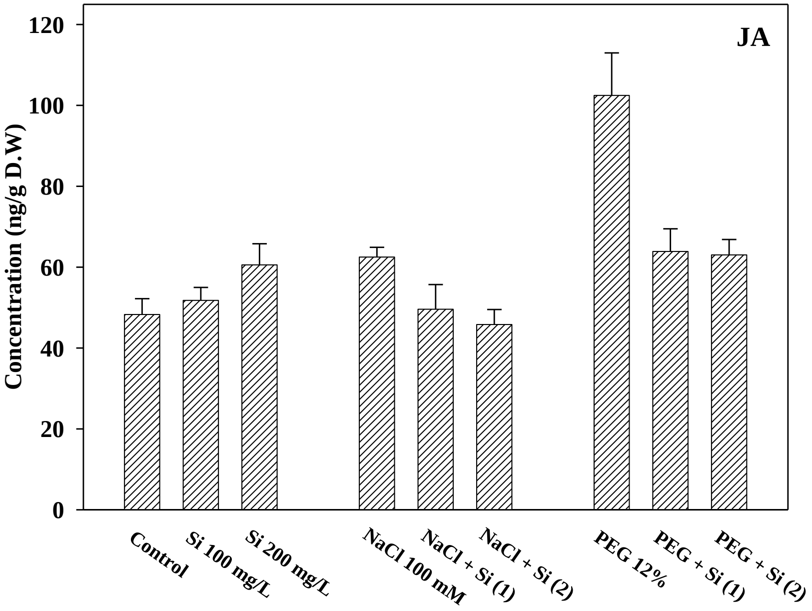

Fig. 2. Endogenous JA contents of soybean leaves in response to NaCl and NaCl+Si or PEG+Si. Error bars show standard deviations. Si (1) and Si (2) stand for Si 100 mg/L and Si 200 mg/L respectively.

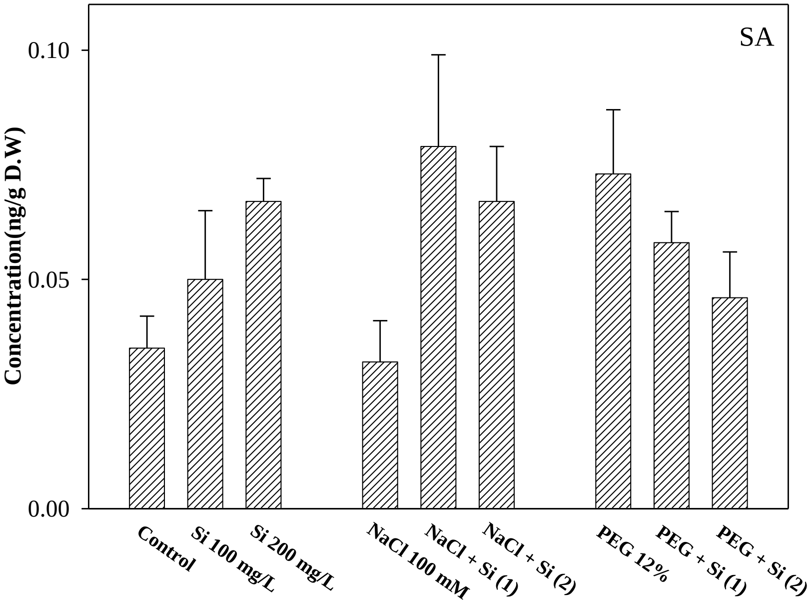

Fig. 3. Endogenous SA contents of soybean leaves in response to NaCl and NaCl+Si or PEG+Si. Error bars show standard deviations. Si (1) and Si (2) stand for Si 100 mg/L and Si 200 mg/L respectively.

Discussion

Plants grown in agricultural systems are exposed to many environmental stresses limiting their growth potential. Plant growth promotion is usually due to the improvement of plant resistance to abiotic stresses such as salinity or water stress, and biotic stresses such as pathogens and herbivores (Epstein, 1999) and there are ample evidences that Si, plays a favorable role in plant growth, mineral nutrition, mechanical strength and resistance to fungal diseases. In the current study, silicic acid enhanced plant growth when applied under control conditions, while its application under plant stress condition alleviated the adverse effects of salinity and drought. However, it was found that the role of Si in the alleviation osmotic stress was not as significant except its favorable increase of plant length. We also found that the Si is more efficient in alleviating salt stress as compared to drought stress in soybean. An increase in growth of salt and drought stressed soybean plants with the addition of Si in the growth medium, may be due to the fact that Si improves photosynthesis rate, which was related with leaf ultra-structure, chlorophyll content, and ribulose biphosphate carboxilase activity. Similar results were also documented in barley and cucumber (Adatia & Besford, 1986; Liang, 1998). Salt and drought stress adversely effect plant growth attributes and results of current study confirm that all growth variables decreased with NaCl and PEG application.

Under salt stress condition, the osmotic pressure in the soil solution exceeds the osmotic pressure in plant cells due to the presence of higher concentrations of salts, and thus, reduces the ability of plants to take up water and minerals like K^+ and Ca^{2+} (Munns *et al.*, 2006). On the other hand, Na^+ and Cl^- ions can enter into the cells and have their direct toxic effects on cell membranes, as well as on metabolic activities in the cytosol (Hasegawa *et al.*, 2000). The primary effects causes a reduction in cell, assimilate production and membrane function, as well as decreased cytosolic metabolism and production of reactive oxygen intermediates (ROS). As a result, in extreme cases, the plants may die under salt stress. Current study confirms previous reports, which suggested that salt stress reduced the biomass of tomato (Kaya *et al.*, 2001), pea (Ahmad & Jhon, 2005) and rice (Yeo *et al.*, 1999), although shoot dry weight was more sensitive to salinity than root dry weight (Essa, 2002). The chlorophyll contents are also sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in several plants, such as pea (Ahmad & Jhon, 2005), wheat (Ashraf *et al.*, 2002), rice (Anuradha & Rao, 2003) and tomato (Al-Aghabary *et al.*, 2004). In the current study, the chlorophyll contents decreased under salinity and drought stress, while its concentration slightly increased in the presence of Si, although the variations in the chlorophyll contents were not significant under different treatments. These findings are in agreement with reports suggesting that silicate partially offsets the negative impact of NaCl stress, which increased tolerance of tomato plants to NaCl salinity by raising SOD and CAT activities, chlorophyll content and photochemical efficiency of PSII (Al-Aghabary *et al.*, 2004).

Plant hormones influence physiological processes at low concentrations either in distant tissues to which they are transported or in the tissue where synthesis occurred (Davies, 1995a). It has been suggested that, hormones only provide a "turn on" or "turn off' signal and that the actual information is provided by the cell. This scenario is similar to that of calcium, which is now thought to be an intermediate in some hormonal responses (Davies, 1995b). Gibberellins regulate all aspects of the life history of plants, from seed germination to vegetative growth and flowering (Ritchie & Gilroy, 1998). In the current study, the endogenous bioactive GA_1 and GA_4 contents increased with elevated Si, while decreased with NaCl and PEG application as compared to control. This clearly suggests that GAs have no ample role in salt and drought stress alleviation and their metabolism is greatly reduced under stress condition. However, an increase in bioactive GAs in plants treated with Si under stress condition, narrates that Si alleviated the adverse effect of NaCl and PEG on gibberellin metabolism in soybean.

On the other hand, endogenous JA contents of soybean significantly increased with NaCl and PEG application, while decreased when Si was added to NaCl and PEG treated plants. In current investigations, an increase in JA levels with Si nutrition further strengthen the role of Si as an efficient element for improvement of plant resistance to abiotic stresses such as salinity or water stress. Our current findings confirm the previous reports of Wang *et al.*, (2001), who demonstrated that JA generally increase in plants in response to elevated salinity stress. Similarly, Kramell *et al.*, (2000) found a rapid increase in endogenous JA content in barley leaf segments subjected to osmotic stress with sorbitol or mannitol. However, our present results do not coincide with Kramell *et al.*, (1995), who observed that endogenous jasmonates did not increase when treated with a high NaCl concentration.

The application of SA has resulted in tolerance of plants to many biotic and abiotic stresses including fungi, bacteria, viruses (Delany *et al.*, 1994), chilling, drought and heat (Senaratna *et al.*, 2003). As SA was effective in inducing stress tolerance when applied as a soil drench (Senaratna *et al.*, 2000), foliar or seed treatment (Aldesuquy *et al.*, 1998) it appears that SA has a regulatory role in activating biochemical pathways associated with tolerance mechanisms (Sticher *et al.*, 1997). Current investigation confirmed previous report of Wang *et al.*, (2001), which demonstrated that JA generally increased and indole-3-acetic acid (IAA) and salicylic acid (SA) declined in response to salinity. The important role of SA in protecting plant is probably played by its ability to induce expression of genes coding not only for pathogenesis related proteins (PR-proteins**)** but also the extension gene, as found in *Arabidopsis* (Merkouropoulos *et al*., 1999; Nakashima *et al.*, 2000; Kidokoro *et al.*, 2009).

It was concluded that the inclusion of Si in soybean nutrition under stressed environmental conditions are beneficial, as our study showed that Si significantly improved growth attributes and effectively mitigated the adverse effects of NaCl induced salt stress and PEG induced drought stress. We also found that Si was more effective in alleviating salinity than drought stress. However, further studies are needed for a better understanding of the physiological or biochemical roles of silicic acid in higher plants at molecular level.

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