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EFFECT OF POTASSIUM ON SOYBEAN UNDER SHORT-TERM SALINITY

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ABSTRACT

A saline-tolerant soybean advanced line AGS313 from Bangladesh and a saline-sensitive variety Fukuyutaka from Japan were cultivated in pots under short-term saline condition to evaluate the effects of potassium (K^+) application on mitigation of salt stress effect. Three levels of K^+ (2, 4, and 8mM) were prepared in a half-strength 'Enshi' solution to apply on the pots from 13 days after seed sowing. The plants at the vegetative stage were subjected to 50 mM NaCl treatment for 15 days. The physiological responses such as photosynthetic rate, transpiration rate, relative water content, leaf water potential (LWP), and exudation rate were measured. The accumulation of Na^+ and K^+ in the leaves were also measured. The yield components were recorded at harvest. The results indicated that all the physiological parameters, except LWP were decreased by NaCl application, regardless of K^+ concentrations. Both the soybean varieties controlled Na^+ transport into their leaves. AGS313 accumulated the highest amount of K^+ in the leaves with 8 mM K^+ application regardless of NaCl treatment. Salinity affected number of pods in AGS313 only, while seeds per pod in Fukuyutaka. However, 100-grain weight was not affected by the salinity. Thus, the yield reduction for AGS313 and Fukuyutaka were attributed to number of pods and number of seeds per pod, respectively. Salinity damage on AGS313 yield was alleviated considerably by K^+ application. However, the yield improvement by K^+ was not significant. This inconclusive improvement was probably caused by the restricted Na^+ transportation under short-term salinity.

Keywords: *Glycine max,* salinity, ion accumulation, yield component

Introduction

Soybean (*Glycine max.* L) food products are very popular in most of the East Asian countries including Japan**.** Despite the high nutritional values with protein (40-45%), various minerals, and vitamins in soybean (Kaul and Das 1986), the popularity of this crop as human food is still low in the South Asian countries. Soybean has been cultivated since the early 1970s in Bangladesh when the Mennonite Central Committee (MCC), an American NGO, worked on improving the nutritional levels of the rural Bangladeshi people in the district of greater Noakhali after the birth of the nation in 1971. Until recently the cultivation of soybean was concentrated only in the greater Noakhali area. Later, the cultivation significantly expanded from only 5000 ha in 2005 (Satter *et al*. 2005) to 62500 ha in 2018-2019, to the districts of Barishal, Bhola, Chandpur, Laksmipur, Noakhali and even in the northern Bangladesh (BBS 2020). The expansion of soybean cultivation has been due to its high demand for animal feed. However, recently the awareness about the high nutritional status of soybean as human food is increasing in Bangladesh (Mannan *et al*. 2012).

Despite the increase in areas of soybean cultivation in different parts of Bangladesh, it is still concentrated mostly in the southern part of the country, which is very near to the coastal region. The salinity area has been increasing in Bangladesh due to intrusion of seawater into the fresh river water during the dry season, seepage of salty water due to dryness of the soil, rising of salt through capillary movement, and inundation of land due to cyclones (Mannan *et al*. 2012; Mondal *et al*. 2013). The yield of soybean was decreased even at 5 dS m^{-1} (≈50 mM NaCl) in saline condition (Maas and Hoffman 1977). Arable areas with over 5 dS m^{-1} salinity expanded to 40% of the total cultivated area in the southern part of Bangladesh (SRDI 2010), where the average soybean yield of Noakhali district was 0.76 t ha^{-1} (BBS 2020) in 2018-2019. This was much lower than the national average yield of 1.77 t ha^{-1} in 2018-2019 (BBS 2020). The average global yield was 2.77 t ha^{-1} in 2019-2020 (USDA 2021). Salt damage is the major factor for reducing soybean grain yield in Noakhali, Bangladesh.

Soybean is mostly sown in January after harvesting summer rice (aman rice) in Bangladesh. Saline concentration begins to increase from March in the dry season (Karim *et al.* 2012). Usually, this is the period just before or at the reproductive stage of soybean plants. However, in March-April heavy torrential rains may occur (Amin 2011). If rains, salt is leached and the saline effect will be lessened. Usually soybean is harvested in May.

Damage of plants due to salinity is dependent on both the salt concentration in the soil solution and the duration of exposure of the crop to salinity. Many works indicated that the salinity damage can be mitigated to some extent by employing different management techniques. For example, Ueda (2015) found a positive effect of calcium on lessening of salinity effect on soybean. Similarly, Hashi *et al*. (2015) reported the mitigation effect of salinity on soybean by potassium (K^+), while Khatun *et al*. (2016) reported the mitigation of that by the application of plant growth regulator. Also by selecting salinity tolerant soybean varieties, the yield can be improved to a great extent under saline condition (Ghassemi-Golezani *et al*. 2009, Mannan *et al*. 2012, Khan *et al*. 2015). Noticeably, all those studies were mainly focused under long-term salinity (Hashi *et al*. 2015, Ueda 2015, Khatun *et al*. 2016) and the study under short-term is rather limited. However, short term salinity may be happened due to different reasons, e.g. sudden strong high tide in the coastal area or cyclone that may carry salty seawater in the field. Therefore, this study was initiated to evaluate the effect of K^+ application on mitigation of short-term salinity (15 days) stress on two soybean varieties of very diverse origins, AGS313 from Bangladesh and Fukuyutaka from Japan.

Materials and Methods

Plant materials: Seeds of two soybean varieties of diverse origin, such as AGS313 collected from Bangladesh (Bangabandhu Sheikh Mujibur Rahman Agricultural University) and Fukuyutaka from Japan, were used as plant materials. AGS313 showed a relatively high salt tolerance compared to other varieties in Bangladesh (Karim *et al.* 2012, Mannan *et al.* 2012, Mannan *et al*. 2013a). In a study conducted in Japan, Fukuyutaka showed a relatively low salt tolerance compared to AGS313 (Ueda and Higuchi 2014). The seeds were sown on May 18, 2016 in 8-liter pots containing mountain soil. The plants were grown inside a vinylhouse under natural light at Kyoto University, Japan. Two plants per pot were allowed to grow. The day length for AGS313 was adjusted to 12.5 hours by interrupting natural light to promote flowering according to Ueda *et al*. (2014). From the 20th day till the end of the flowering stage, a dark curtain was used for floral initiation.

Saline water treatments: Three different concentrations of K^+ were prepared in half-strength 'Enshi' solution to apply on the pots from 13 days after seed sowing. That is a regular Enshi solution containing 4 mM K^+ ion (Takatsuji, 1997), 2 mM K^+ (i.e 1/2 of the regular amount of K^+), and 8 mM K^+ (i.e 2 times the regular amount of K^+). When the 1st trifoliate leaf appeared, i.e. 21 days after seed sowing, hardening of 50 mM NaCl treatment with the Enshi solutions of different K^+ concentrations was initiated. For the hardening of the plants, the salt solutions were applied with an increment of 10 mM NaCl every alternate day till the concentration attained to 50 mM. On 29 days after seed sowing, the plants at the vegetative stage were subjected to a 50 mM salinity treatment for 15 days. Salt solutions were prepared by dissolving a calculated amount of NaCl into the Enshi solution. One set of the pots, i.e. without NaCl treatment, was kept free from NaCl application and irrigated with only half the strength of Enshi solution. The treatment solutions were applied in excess so that the extra solutions dripped out through the holes at the bottom of the pots. The half strength of Enshi solutions with different levels of K^+ was irrigated for 81 days. Afterward, the solution was applied only 2 times per week, though the plants were irrigated regularly with tap water.

Measurement of physiological responses: Photosynthetic rate (Pn) and transpiration rate (TR) of the fully developed third uppermost leaves of four replicated plants were measured with a portable gas exchange system LI-6400 (Li-COR Inc, USA) at PPFD 500µmol $m^{-2}s^{-1}$ and 380ppm CO_2 during 09:00-12:00 h, two weeks after the initiation of the NaCl treatment.

Leaf water potential (LWP) of the 2nd or 3rd bottom leaves was measured at predawn according to Tyree and Hammel (1972) two weeks after the initiation of the NaCl treatment.

Relative water content was measured on the same day of LWP measurement using fully developed third uppermost leaves. Leaves were soaked in pure water for 24 hours in the dark, under laboratory condition for measuring turgid

weight. The RWC was measured using the following formula:

$$RWC(\%) = \frac{V_F - V_D}{V_T - V_D} X100$$

where, V_F=Fresh leaf weight, V_T=Turgid leaf weight, and V_D=Dry leaf weight.

Exudation rate (ER) was measured at 8:00 h – 9:00 h on the same day of measuring LWP and RWC following Mannan *et al*. (2013b).

Measurement of ion contents: To measure Na^+ and K^+, the extracts were first prepared in wet-ashing method with HNO_3. Leaf samples collected from 2nd or 3rd trifoliate leaves were over-dried at 70°C for one day. These samples were frozen in liquid nitrogen, ground to a fine powder using a mortar and pestle, and then oven-dried again at 70°C for 20 minutes. This sample powder of 100 mg was mixed with 2 mL nitric acid in a borosilicate heat-resistant test tube (Nichidenrika-Glass Co., Ltd., Kobe, Japan) and the mixture was kept overnight. The next day, the test tube was heated to 100°C to generate bubbles. When bubble generation ended, the tube was gradually heated to reach 150°C and was kept at this temperature until the solution volume decreased to *ca*. 0.5 mL. The solution was then cooled to room temperature, after which 0.1 N HNO_3 was added. Then the solution was filtered through No. 6 filter paper and made up to 50-mL volume with 0.1 N HNO_3. The contents of Na^+ and K^+ in the solution were determined by atomic absorption spectrophotometry (AA-6200, Shimadzu, Inc., Kyoto, Japan).

Measurement of yield components: After harvesting the plants, the number of pods $plant^{-1}$, seed pod^{-1}, 100-seed weight, and grain yield $plant^{-1}$ were recorded from 8 replicated plants.

Statistical analysis: Two levels on NaCl application with three levels of K^+ concentrations formed the six treatments. The 6 treatments were arranged in a randomized block design. The data were analyzed by two-way (NaCl application × K^+ concentrations) ANOVA of randomized block design. Then, the interaction was observed on AGS313 yield, and further analysis of multiple comparisons was conducted including the other measurements by using Tukey's test ($p < 0.05$).

Results and Discussion

The effects of K^+ levels under 50 mM NaCl treatment on photosynthesis, water relations, and leaf mineral ions content are presented in Fig. 1. The 15 days salinity affected significantly the photosynthetic rate and all the parameters of water relations except LWP in both the varieties. In AGS313 the maximum photosynthetic rate (21.2 μ mol $m^{-2}s^{-1}$) was recorded with 4 mM K^+ without NaCl treatment, while the minimum rate (12.8 μ mol $m^{-2}s^{-1}$) was recorded with 2 mM K^+ under 50 mM NaCl treatment, which was closely followed by 8 mM K^+ under the same level of salinity. In Fukuyutaka, the maximum photosynthetic rate (24.5 μmol $m^{-2}s^{-1}$) was recorded with 8 mM K^+ without NaCl treatment. The lowest photosynthetic rate (8.7 μmol $m^{-2}s^{-1}$) in Fukuyutaka was evident in treatment with 4 mM K^+ and 8 mM K^+ under 50 mM NaCl treatment. The maximum transpiration rate (5.9 mmol $m^{-2}s^{-1}$) in AGS313 was recorded without NaCl treatment with 2 mM K^+, while the minimum (3.6 mmol $m^{-2}s^{-1}$) was recorded with 2 mM K^+ under 50 mM NaCl treatment. In Fukuyutaka, the maximum transpiration rate (8.6 μmol $m^{-2}s^{-1}$) was recorded with 4 mM K^+ without NaCl treatment. The lowest transpiration rate (1.7 μmol $m^{-2}s^{-1}$) was recorded with 8 mM K^+ under 50 mM NaCl treatment.

The maximum RWC (94.3%) was recorded in AGS313 with 2 mM K^+ without NaCl treatment. Similarly, in Fukuyutaka, the maximum RWC (98.4%) was recorded with 2 mM K^+ without NaCl treatment. The minimum RWC (80.1%) was recorded in AGS313 with 2 mM K^+ followed by that with 8 mM K^+, and in Fukuyutaka (81.3%) with 4 mM K^+ followed by 8 mM K^+ under 50 mM NaCl treatment. The LWP was not affected by the 50 mM NaCl treatment in both the varieties. The ER was lowered significantly by 50 mM NaCl treatment, and the maximum ER was recorded with 4 mM K^+ without NaCl treatment in AGS313 (1.44 g h^{-1}) which was closely followed by 2 mM K^+ without NaCl treatment. In Fukuyutaka, the maximum ER (1.76 g h^{-1}) was recorded without NaCl treatment with 2 mM K^+. With the increase of K^+ levels the ER rate was decreased in both the varieties without NaCl treatment. The minimum ER (0.02 g h^{-1}) was found in both the varieties with 8 mM K^+ under 50 mM NaCl treatment.

In AGS313 leaves, K^+ content tended to increase at 50 mM NaCl treatment regardless of K^+ concentration (Table 1). The Na^+ content decreased with 8 mM K^+ regardless

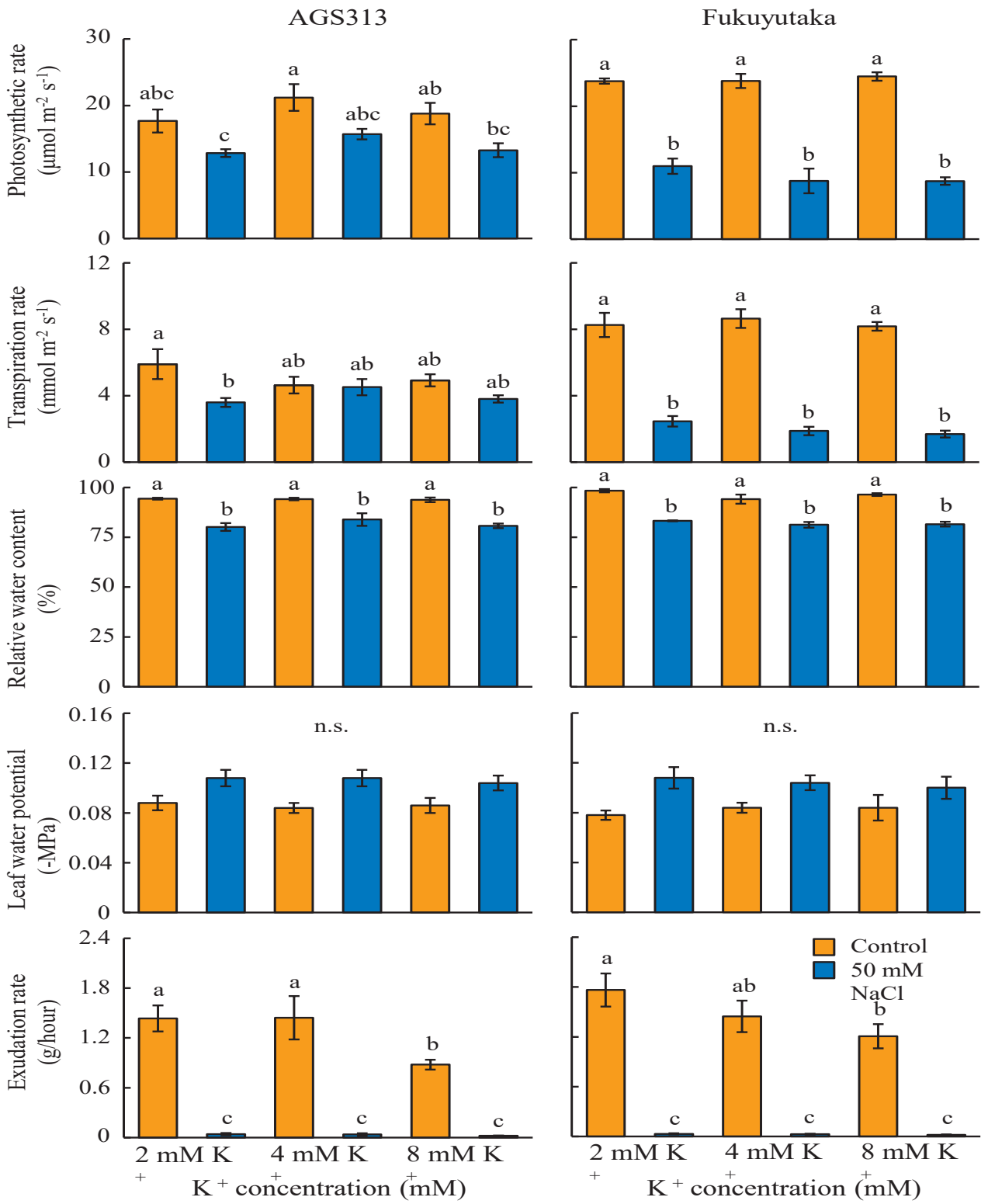


Figure 1. The effects of K+ application on photosynthetic rate, transpiration rate, relative water content, leaf water potential, and exudation rate under short-term 50 mM NaCl treatment for 15 days on AGS313 (left) and Fukuyutaka (right). Different letters indicate statistical differences at $p < 0.05$ by Tukey's multiple comparison test. n.s. indicates not significant.

Table 1. The effects of K^+ application on leaves Na^+ and K^+ contents under short-term 50 mM NaCl treatment for 15 days on AGS313 (left) and Fukuyutaka (right)

	Treatment		Na^+ (%)		K^+ (%)	
	NaCl (mM)	K^+ (mM)	AGS313	Fukuyutaka	AGS313	Fukuyutaka
	0	2	0.043	0.037	1.267	0.947
		4	0.049	0.045	1.397	1.064
		8	0.029	0.035	1.174	0.900
	50	2	0.042	0.041	1.436	0.944
		4	0.045	0.043	1.469	1.005
		8	0.036	0.039	1.567	1.413
Average	0		0.041	0.039	1.287	0.970
	50		0.041	0.041	1.491	1.121
		2	0.043	0.039	1.352	0.945
		4	0.047	0.044	1.433	1.035
		8	0.033	0.037	1.392	1.156
Significance	NaCl		n.s.	n.s.	n.s. (p = 0.07)	n.s.
	K^+		*	n.s.	n.s.	n.s.
	NaCl× K^+		n.s.	n.s.	n.s.	n.s. (p = 0.06)

Data are means of n = 5.

* and n.s. indicate significant difference at $p < 0.05$ and not significant, respectively.

of NaCl application. These results of K^+ and Na^+ contents indicated that K^+ can easily be taken up into leaves without K^+ application. In Fukuyutaka leaves, K^+ content tended to increase at 50 mM NaCl treatment with 8 mM K^+. The Na^+ content was not affected by K^+ concentration and NaCl application. Fukuyutaka was likely to concentrate K^+ into the leaves by K^+ application. Both the varieties are likely to have the function of Na^+ elimination under the condition of 50 mM NaCl treatment for 15 days. If they have the function of Na^+ elimination, K^+-Na^+ competitive mechanism presumably works a little.

Chloride (Cl^-) was excessively accumulated in Fukuyutaka leaves under saline condition compared to AGS313 leaves (Fujii and Higuchi, 2019). They measured ion concentrations in the leaves, stems, and roots of AGS313 and Fukuyutaka. The results showed that both the varieties accumulated a large concentration of Na^+ in roots, relatively lower in stems and a lesser concentration in leaves. Consequently, Na^+ transport was limited for both the varieties. This little transport indicated that the toxicity of Cl^- rather than the toxicity of Na^+ affected the growth negatively. They also noted that the Cl^- transport was accompanied by K^+. The application of K^+ was suggested to enhance K^+ uptake and also Cl^- uptake accompanied by K^+. It can be assumed that K^+ application to Fukuyutaka, which causes Cl^- transport into the leaves, would rather increase ion stress as a result.

The effects of NaCl and K^+ on grain yield and yield contributing characters are presented in Table 2. The results indicated that short-term NaCl had a significant effect on number of pods per plant in only AGS313 and not in Fukuyutaka. In AGS313 the maximum number of pods (122 plant^{-1}) was recorded with 2 mM K^+ followed by that with 4 mM K^+ without NaCl treatment. The minimum number of pods per plant (74 plant^{-1}) was recorded under 50 mM NaCl treatment with 4 mM K^+. In Fukuyutaka the maximum number of pods per plant (113 plant^{-1}) was recorded with 4 mM K^+ without NaCl treatment, while the minimum with 2 and 8 mM K^+ under 50 mM NaCl treatment. 50 mM NaCl treatment affected significantly the seed number per pod only in Fukuyutaka and not in AGS313. The maximum seed number per pod (1.68) in Fukuyutaka was recorded with 2 mM K^+ and 8 mM K^+ without NaCl treatment, while the minimum (1.60) with 4 mM K^+ under 50 mM NaCl treatment. The 100-grain weight was not affected by 50 mM NaCl treatment and K^+ levels in both varieties while the grain yield was affected in both AGS313 and Fukuyutaka by the 50 mM NaCl treatment. The maximum grain yield (49.8 g plant^{-1}) in AGS313 was recorded with 4 mM K^+ without

Table 2. The effects of K^+ application on number of pods, seed number per pod, 100-seed weight, and grain yield under short-term 50 mM NaCl treatment for 15 days on

	Treatment		Number of pods plant^{-1}		Seed number pod^{-1}		100-seed dry weight (g)		Grain yield (g plant^{-1})	
	NaCl (mM)	K^+ (mM)	AGS313	Fukuyutaka	AGS313	Fukuyutaka	AGS313	Fukuyutaka	AGS313	Fukuyutaka
	0	2	122 a	112	2.00	1.68	23.7	38.2	46.5 a	54.9
		4	117 a	113	2.01	1.66	25.0	38.5	49.8 a	56.9
		8	103 ab	102	1.96	1.68	25.2	38.8	42.2 ab	52.7
	50	2	79 c	104	1.94	1.61	23.7	37.2	32.7 bc	52.1
		4	74 c	111	1.99	1.60	22.2	36.4	27.9 c	49.2
		8	90 bc	104	2.05	1.65	23.2	36.3	34.8 bc	48.6
Average	0		114	109	1.99	1.67	24.6	38.4	46.2	54.9
	50		81	105	1.99	1.62	23.0	36.0	31.8	48.2
		2	101	106	1.97	1.65	23.7	37.0	39.6	52.2
		4	96	112	2.00	1.62	23.6	37.1	38.9	53.4
		8	97	102	2.00	1.67	24.2	37.9	38.5	49.7
Significance	NaCl		**	n.s.	n.s.	*	n.s	n.s.	**	*
	K^+		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	NaCl × K^+		**	n.s.	n,s.	n.s.	n.s.	n.s.	*	n.s.

Data are means of n = 8. **,*, and n.s. indicate significant differences at $p < 0.01$, $p < 0.05$, and not significant, respectively.
Different letters within the same column indicate statistical differences at $p < 0.05$ by Tukey's multiple comparison test.

NaCl treatment, while the minimum (27.9 g plant^{-1}) was recorded with 4 mM K^+ under 50 mM NaCl treatment. Similarly, in Fukuyutaka the maximum grain yield per plant (56.9 g plant^{-1}) was recorded with 4 mM K^+ without NaCl treatment and the minimum (48.6 g plant^{-1}) was recorded with 8 mM K^+ under 50 mM NaCl treatment.

The grain yield of AGS313 tended to increase when 8 mM K^+ was applied under the 50 mM NaCl treatment, and the yield increase was due to the increase in the number of pods. On the other hand, in Fukuyutaka 8 mM K^+ was not affected to the grain yield under 50 mM NaCl treatment, and the yield decrease was due to the decrease of the number of seeds per pod. Significant effect of K^+ was not observed in both the varieties. Thus, it is considered that the effect of K^+ application on reducing saline damage was limited for those varieties that can control the Na^+ transport into the leaves.

Conclusion

The effect of K^+ application was not obvious under short-term salinity for 15 days in soybean. It can be assumed that the K^+ application only works remarkably on the varieties those have the mechanism that restrict Cl^- transport and allow Na^+ transport into the leaves. This is because the K^+ application enhances both effects of the antagonistic relationship of K^+ to Na^+ and a synergistic relationship of K^+ and Cl^-. K^+ application will not be effective for some reasons such as Fukuyutaka can easily allow Cl^- to transport into the leaves. In this experiment, Na^+ transport into the leaves was little in both the varieties. This indicated that ionic stress of Na^+ was limited in both the varieties under short-term, 15 days, salinity when they are at the vegetative stage.

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