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EFFECTS OF SALT STRESS ON TILLERING NODES TO THE GROWTH OF WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

In monsoon climate regions, the tillering nodes of winter wheat can be stressed by high salt accumulation on the soil surface in spring, thereby leading to salt-induced damage. To understand whether tillering nodes could be stressed by salinity and to estimate its effects on the growth of winter wheat under salt stress, the tillering nodes of two wheat cultivars, H-4589 (salt-sensitive) and J-32 (salt-tolerant), were treated with salinity to investigate the physiological and biochemical changes in seedling growth. The results indicated that salt stress on tillering nodes significantly reduced plant height and shoot dry weight; increased Na^+ accumulation, soluble sugar and proline in both H-4589 and J-32; which demonstrated remarkable effects on the growth of winter wheatwhen the tillering nodes were under salt stress. Furthermore, equivalent Na^+ accumulations were discoveredin two cultivarswhen tillering nodes were under salt stress, while remarkably different Na^+ accumulations were discoveredin two cultivars when roots were under salt stress. Based on the results from anatomic analyses, we speculated that no anatomic differences in tillering nodes between two cultivars could give reason to the equivalent Na^+ accumulations in two cultivarswhen tillering nodes were under salt stress; and more lignified endodermis in primary roots as well as larger reduction of lateral root number in salt-tolerant cultivars which contributed to preventing Na^+ influx could explain the remarkably lower Na^+ accumulation in salt-tolerant cultivar when roots were under salt stress. All of these results indicated that the tillering nodes could mediate Na^+ influx from the environment leading to salt-induced damage to the growth of winter wheat.

Key word: Tillering nodes, Salt stress, Winter wheat, Na^+ accumulation, Soluble sugar, Proline, Anatomic analysis.

Introduction

Soil salinization has become increasingly serious, and the current global salinized area is growing (Munns, 2002). High levels of salts can cause ion toxicity, hyperosmotic stress, nutrient imbalances and oxidative damage (Genc *et al*., 2007); ultimately, this leads to plant growth and yield limitation (Munns & Tester, 2008). Therefore, soil salinity is responsible for significant losses of agriculture productivity(Xiong *et al*., 2002).

Wheat (*Triticum aestivum* L.) is a staple food crop cultivated in saline soil. The effective way to utilize saline soil is to breed salt-tolerant wheat cultivars. However, progress in developing salt-tolerant cultivars is limited by the genetic complexity of wheat. Hence, it is necessary to understand the salt-tolerant mechanisms in wheat.Many researchers have focused on exploring the mechanism of salt tolerance in wheat and have made many advances. As a glycophyte, wheat is a classic 'salt excluder', coping with salt stress by excluding $Na^+$from shoots as much as possible (Genc *et al*., 2007). For example, bread wheat is generally more tolerant than durum wheat under salinity because of better Na^+exclusion (Colmer *et al*., 2006); and the Na^+-exclusion capability of salt-tolerant wheat cultivars is stronger than salt-sensitive ones (Yang Hong-Bing, 2002). Meanwhile, wheat is moderately salt-tolerant. Under 100 mM NaCl, wheat will still grow with reduced yield, but will die after 250 mM NaCl treatment as the accumulation of high Na^+ concentration inhibits leaf function (Munns *et al*., 2006). Previous research has demonstrated that Na^+-exclusion localization differs with the variation of salt-tolerance in wheat. In salt-sensitive cultivars, Na^+-exclusion occurred mainly at the root-stem junction, whereas the Na^+-exclusion sites in salt-tolerant cultivars were roots(Hong *et al*., 2001). The Na^+-excluding mechanism included three processes(Min Chen, 2008). First, Na^+ absorption can be prevented with a Na^+/H^+ pump and apoplastic barriers, such as a Casparian strip in the roots. Secondly, the absorbed Na^+ can be retained in the roots to reduce the Na^+ content of the shoots, protecting them from Na^+ toxicity. Finally, the upward transportation of Na^+ is impeded by the xylem or phloem cells; then, the Na^+ is absorbed and secreted into the phloem, back to the roots. These processes occur in the stem but have little effect on Na^+-exclusion(Munns, 2007). Therefore, the key is to control the upward transportation of Na^+ from the roots to shoots. However, excluding Na^+ alone is not always sufficient for salt tolerance.In wheat, maintaining K^+ homeostasis is important for salt tolerance. It has been reported that a durum wheat mutant with salt tolerance is the result of a high ability to accumulate K^+ in the shoots (Rascio *et al*., 2001). High Na^+ concentrations could inhibit K^+absorption due to excessive Na^+ accumulation, which induces K^+ leakage in the cytosol (Shabala *et al*., 2006). Therefore, the cytosolic K^+/Na^+ratio falls dramatically. Maintenance of a high K^+/Na^+ ratio is thought to be a key feature of plant salt tolerance (Maathuis & Amtmann, 1999).

Most of the results on wheat salt tolerance in these studies were obtained under a treatment that only the roots were salt stressed. However, under field conditions, soil salt not only remains in root zones but also accumulates on the soil surface, especially during spring season in monsoon climate regions, such as the low plain around the Bohai sea saline area in China because of high

evaporation and low rainfall (Li *et al*., 2008). Therefore, we put forward a hypothesis that high salt levels in the topsoil around tillering nodes may generate high salt stress on tillering nodes and may lead to growth damage, thereby aggravating the salt injury in winter wheat during the reviving stage.

In this study, we exposed the roots or tillering nodes of winter wheat seedlings to salt stress and investigated the growth, ion absorption, soluble sugar and proline accumulation, as well as root and node anatomy of two wheat cultivars differing in their degree of salt tolerance to identify whether tillering nodes are involved in the salt tolerance mechanisms of winter wheat at the reviving stage.

Materials and methods

Plant growth conditions and treatments: Seeds of two winter wheat cultivars, cvs. H-4589 (salt-sensitive) and J-32 (salt-tolerant), were collected from Cangzhou City, in the Hebei province of northern China and were then stored at 4ºC until use. The seeds were first treated with 70% ethanol for 30 s, rinsed five times with sterile distilled water, and left to germinate on double-layer filter paper wetted with distilled water. The five-day-old wheat seedlings were transferred to 20 cm diameter pots containing vermiculite saturated with a half Hoagland nutrient solution. One week later, the tillering nodes of the wheat seedlings were treated with half Hoagland nutrient solution supplemented with 200 mM NaCl as the salt treatment (S), and a nutrient solution without salt served as the control (C) following the methods in Zhou *et al*., with modifications (Zhou *et al*., 2011). The detail treatment were showed in Fig. 1: given salt stress on tillering nodes was considered as TS treatment; given salt stress on roots was considered as RS treatment; neither salt stress on tillering nodes nor roots was considered as CK treatments. There were two plastic membranes, one was placed between the layers of roots and tillering nodes to prevent the movement of salt and water, the other was placed on the top of the pot to avoid soil water evaporation. The pots were maintained at field saturation capacity at a pH of 7.0 and irrigation was applied from the bottom of the pots when required. The plants were grown in a growth chamber at 23 ± 2ºC, with 80% relative humidity under 16 h light/8 h dark. The plant materials from three biological independent experiments were analyzed. The plants were harvested by washing the culture surface with sterile, distilled water on the 15th day of growth. The roots, tillering nodes and leaves were separately collected and used for experimental studies.

Determination of growth parameters: At the end of the treatment, the plant height of the seedlings, numbers of lateral roots, and length of primary and lateral roots were measured among twenty seedlings in each treatment. Twenty seedlings in each treatment were rinsed with distilled water twice. After absorbing water with filter paper, the seedlings were oven-dried at 105ºC for 15 min., followed by 65ºC for 48 h, and the dry weight (DW) was recorded to perform a biomass analysis per plant (Peng *et al*., 2009).

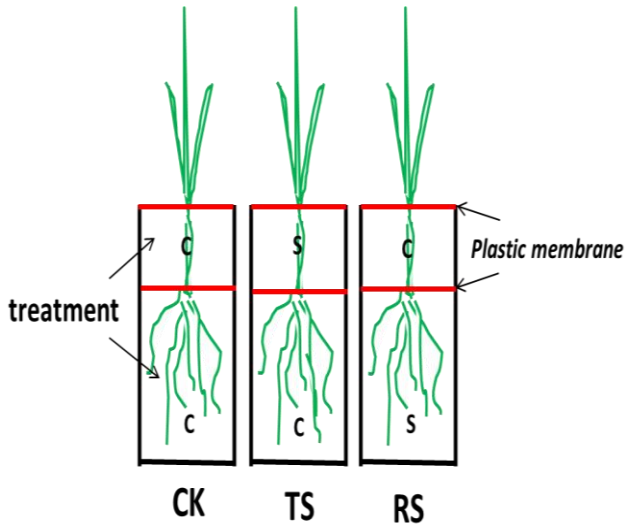

Fig. 1. The different treatments in this experiment.

Determination of K^+ and Na^+ concentrations: HCl was used to hydrolyze 0.5 g dried powder from the plant samples. Then, the supernatants of the K^+ and Na^+extracts were analyzed by atomic absorption spectrometry according to Peng *et al*. (2004).

Determination of soluble sugar and proline: The leaves (0.5 g) were homogenized with 2 ml of 80% ethanol solution using a mortar and a pestle. After heating the homogenate in a water bath at 75ºC for 10 min, the insoluble residue was removed by centrifuging at 5000 g for 10 min. The precipitate was re-extracted with 2 ml of 80% ethanol at 75ºC and re-centrifuged. The supernatants were pooled and dried under a stream of hot air, and the residue was re-suspended in 1 ml of water. The total soluble sugar were determined using the phenol–sulfuric acid method (Prado, Boero *et al*., 2000). The free proline content in the leaves was determined following the methods of Bates *et al*. (1973). Leaf samples (0.5 g) were homogenized in 5 ml of sulfosalicylic acid (3%) using mortar and pestle. Approximately 2 ml of extract was placed in a test tube and 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100ºC for 30 min. After the reaction mixture cooled, 6 ml of toluene was added and then transferred to a separating funnel. After through mixing, the chromophore containing toluene was separated and the absorbance was read at 520 nm in a spectrophotometer against a toluene blank. The concentration of proline was estimated by referring to a standard curve of proline.

Anatomic analyses of roots and tillering nodes: A series of fresh, free-hand cross sections of primary roots, lateral roots and tillering nodes were prepared at 1 mm intervals from the apex to the base. For lignin visualization in fluorescence microscopy, the free hand sections were stained with phloroglucinol–HCl(Lukačová *et al*., 2013). Auto-fluorescence of the lignin deposits was conducted using a microscope (Leica Digital Microscope DM5500B, Wetzlar, Germany).

Table 1. Comparison of plant height (A), shoot dry weight (B), primary root length (C), lateral root length (D), and number of lateral roots (E) under different treatments in two cultivars.

Cultivars	Treatments	Plant height (cm)	Shoot dry weight (mg)	Root length (cm)	Lateral root length (cm)	Number of lateral roots
J-32	CK	36.62 ± 1.35a	46.30 ± 0.69a	28.75 ± 2.75a	2.77 ± 0.61ab	38.80 ± 2.57b
	TS	32.15 ± 1.60b	32.32 ± 0.67c	29.00 ± 3.00a	2.55 ± 0.58c	36.35 ± 3.51c
	RS	19.75 ± 1.12e	16.20 ± 1.14e	13.60 ± 1.50c	1.09 ± 0.56d	20.35 ± 3.38e
H-4589	CK	31.35 ± 2.18c	41.70 ± 2.61b	23.00 ± 2.18b	2.97 ± 0.86a	41.15 ± 2.25a
	TS	25.42 ± 1.51d	29.22 ± 1.50d	21.75 ± 1.77b	2.55 ± 0.58c	37.00 ± 4.39bc
	RS	15.7 ± 1.17f	12.78 ± 1.24f	13.00 ± 3.31c	0.94 ± 0.40d	27.40 ± 4.00d

Means (n=3 per treatment ± SD) with at least one equivalent letter are not significantly different at $p<0.05$.

Statistical analysis: All of the data in the experiment were analyzed using one-way analyses of variance (ANOVA). Differences in the means were determined for significance using the Duncan test at $p<0.05$. Each data point was the mean of three biological replicates (n = 3) and comparisons with $p<0.05$ were considered significantly different. In all of the figures, the results were expressed as a mean and standard deviation of the mean.

Results

Growth performance under salt stress: The growth parameters collected in this experiment, such as shoot dry weight, plant height, primary root length, lateral root length and number of lateral roots, are listed in Table 1. Plant growth, such as plant height and shoot dry weight, decreased notably when the tillering nodes were exposed to salt stress. The decreases of plant height in J-32 and H-4589 were 12.22% and 18.90%, respectively; and the decreases of shoot dry weight in J-32 and H-4589 were 29.92% and 30.20%, respectively. However, when the roots were treated with salt stress, the decreases of plant height in J-32 and H-4589 were 46.07% and 49.92%, respectively; and the decreases of shoot dry weight in J-32 and H-4589 were 69.34% and 65.01%, respectively. Although the decreases of plant height and shoot dry weight in both J-32 and H-4589 caused by tillering nodes were lower than the decreases caused by roots under salt stress, the salinity imposed on tillering nodes could significantly inhibit the growth of winter wheat. Moreover, the reduction of plant heightand shoot dry weightin H-4589 was larger than J-32 when tillering nodes were under salt stress, suggesting that salt-induced inhibition caused by tillering nodes in J-32 was lower which was accordingto its salt tolerance. In addition, the primary root length, lateral root length and number of lateral roots were investigated when tillering nodes were exposed to salt stress. Different from the results that large reductions in primary root length, lateral root length and number of lateral roots were observed especially in J-32 when roots were under salt stress; primary root length, lateral root length and number of lateral roots exhibited little change when when tillering nodes were exposed to salt stress.

Contents of soluble sugar and proline: As shown in Figs. 3 and 4, when tillering nodes were subjected to salt stress, the contents of soluble sugar and proline in H-4589 and J-32 increased notably. Furthermore, the contents of soluble sugar and proline in J-32 were larger than the contents of soluble sugar and proline in H-4589, which was also observed after roots were exposed to salt stress. Compared to the treatment when roots were subjected to salt stress, soluble sugar and proline in both cultivars were less upregulated when tillering nodes were treated with salt stress. Although the upregulation of soluble sugar and proline caused by roots was larger than the tillering nodes in response to salt stress, the contents of soluble sugar and proline in winter wheat were remarkably affected when tillering nodes were under salt stress.

Na^+, K^+ concentrations and K^+/Na^+ ratio: Salt stress had significant effects on the concentrations of Na^+, K^+ and the K^+/Na^+ ratio in the roots, tillering nodes and leaves of both cultivars when tillering nodes were exposed to salt stress (Fig. 2). The remarkable increase of Na^+ contents was found in roots, tillering nodes and leaves when tillering nodes were exposed to salt stress. Similar results were also found in the treatment when roots were exposed to salt stress, but the increases caused by tillering nodes was lower compared to the increases caused by roots under salt stress. Furthermore, the increase in Na^+ contents caused by tillering nodes under salt stress presented no difference in the two cultivars, while the increase in Na^+ caused by roots was much higher in the salt-sensitive cultivar H-4589 than in the salt-tolerant cultivar J-32. This study also documented that the K^+ content of both cultivars significantly decreased when tillering nodes were under salt stress. The decrease in K^+ content in both cultivars caused by tillering nodes was less than that caused by roots. Salinity also caused a reduction in the K^+/Na^+ ratio in both cultivars when tillering nodes were under salt stress. The reductions in both cultivars caused by tillering nodes were less than those caused by roots under salt stress, which was consistent with the changes in K^+ contents.

Anatomic analysis of tillering nodes and roots: To explain the difference between equivalentNa^+ accumulation caused by tillering nodes and inequivalent Na^+ accumulation caused by roots in two cultivarsunder salt stress, freehand cross sections of the tillering nodes and roots in J-32 and H-4589 were observed with a fluorescence microscope to detect the lignification level. From Fig. 5A and 5B, it was determined that no difference existed in the structures and lignification of tillering nodes between the two cultivars, which may shed light on the equivalentNa^+ accumulation caused by tillering nodes in two cultivarsunder salt stress from an anatomical perspective. Moreover, high lignification of the outermost layer in the tillering nodes was detected in both cultivars (Fig. 5B) and the lignification in the tillering nodes was higher compared to the roots. According to the anatomical analysis of the roots, high and integrated lignification around the endodermis in well-developed roots was found in J-32, whereas a comparatively low and incomplete lignification around the endodermis was observed in H-4589 (Fig. 5C and 5D).

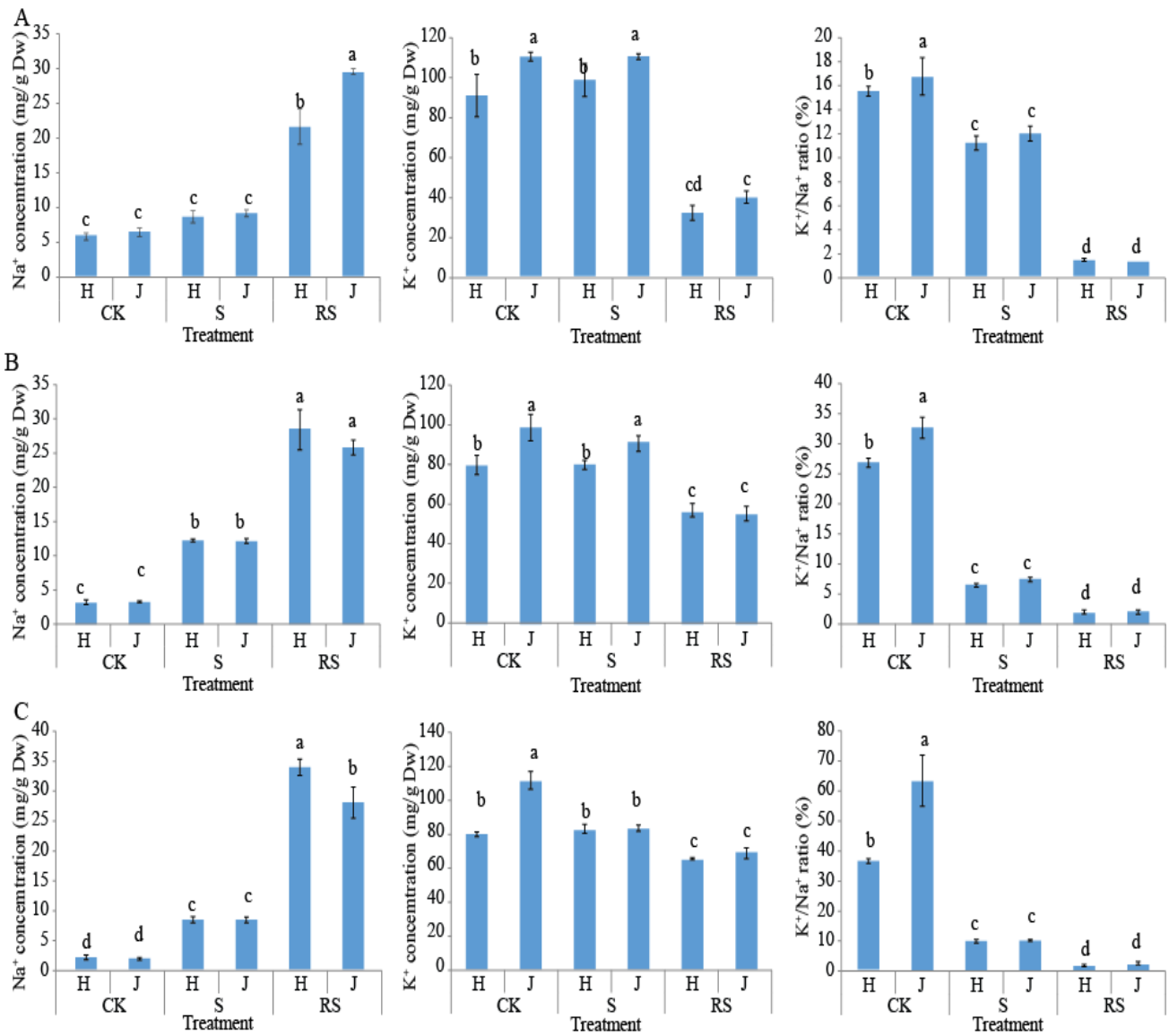

Fig. 2. Comparison of Na^+ concentration, K^+ concentration and the K^+/Na^+ ratio in roots (A), tillering nodes (B), and leaves (C) under different treatments in two cultivars. Means (n=3 per treatment ± SD) with at least one equivalent letter are not significantly different at $p<0.05$.

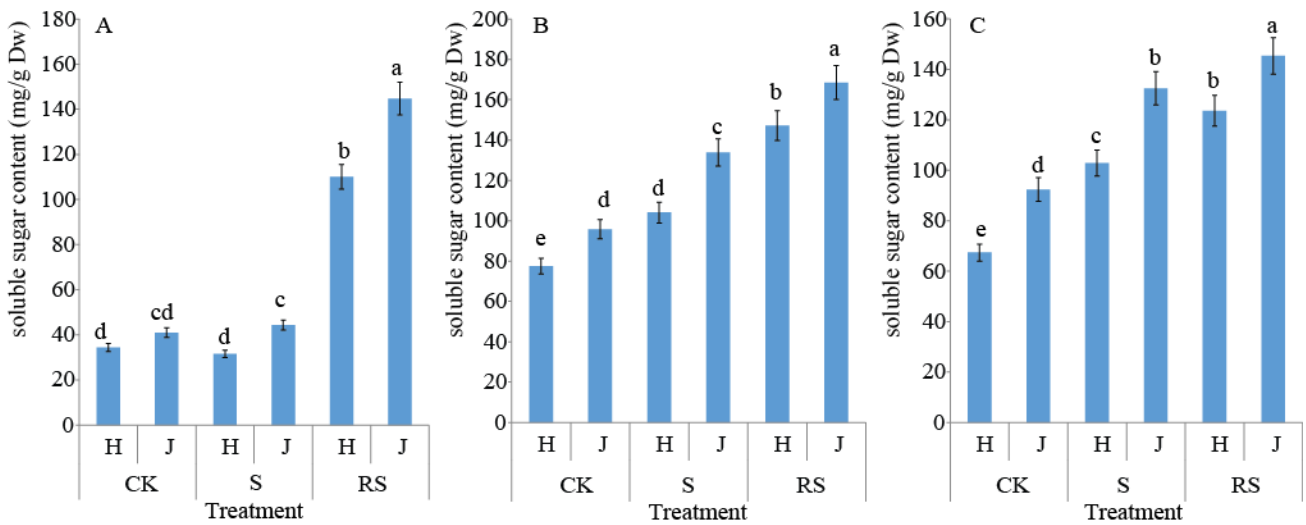

Fig. 3. Comparison of soluble sugar content in roots (A), tillering nodes (B), and leaves (C) under different treatments between H-4589 (H) and J-32 (J). Means (n=3 per treatment ± SD) with at least one equivalent letter are not significantly different at $p<0.05$.

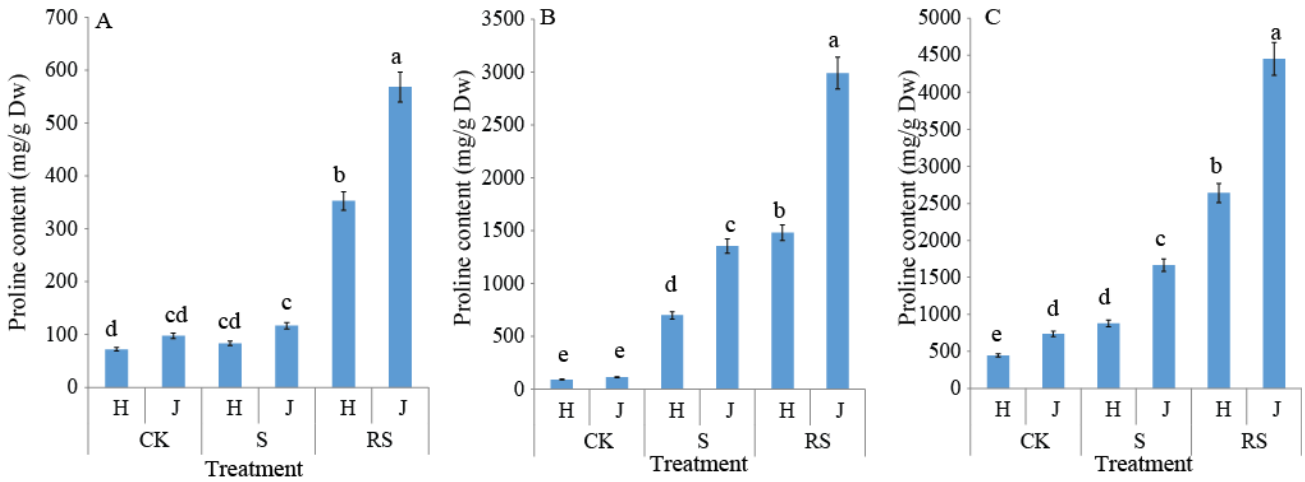

Fig. 4. Comparison of proline content in roots (A), tillering nodes (B), and leaves (C) under different treatments between H-4589 (H) and J-32 (J). Means (n=3 per treatment ± SD) with at least one equivalent letter are not significantly different at $p<0.05$.

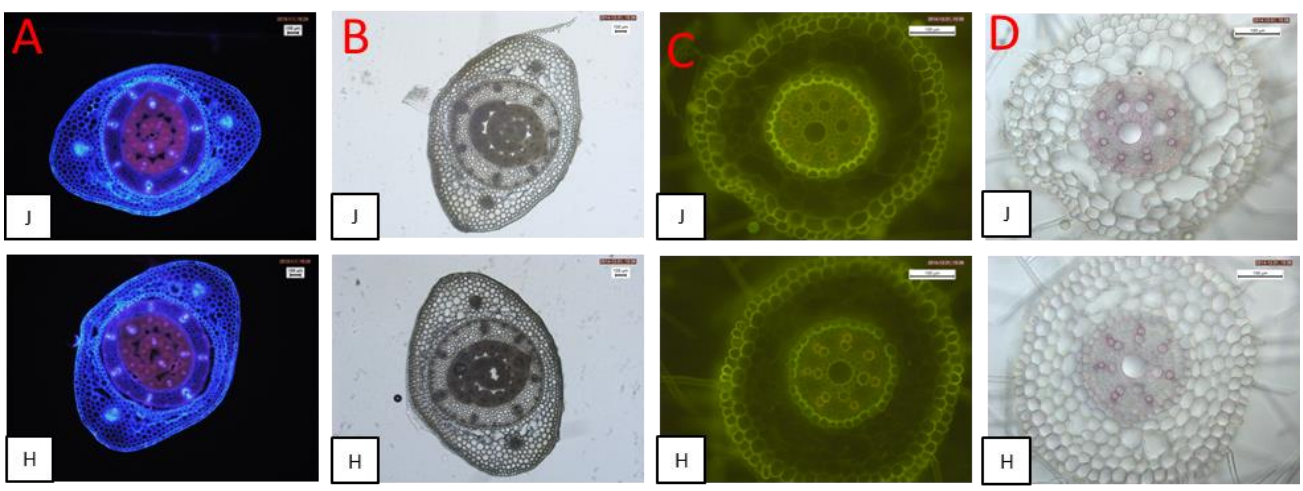

Fig. 5. Freehand cross-sections of wheat tissues were viewed under fluorescence and bright-field illumination. Cross-sections of tillering nodes in the position close to the root under fluorescence (A) and bright-field (B) illumination are shown; primary roots at a position of approximately 2/3 of the total length of the root under fluorescence (C) and bright-field (D) illumination are shown for J-32 (J) and H-4589 (H).

Discussion

Many studieshave reported the effects of salt stress on plants (Hernandez *et al*., 1995; Kasuga *et al*., 1999; Zhu, 2003; Chaves *et al*., 2009), includingion toxicity, ion imbalance,oxidative damage, nutrient deficiency, which then leads to photosynthesis inhibition and membrane damage, resulting in plant growth reductions, and even to death. It has been concluded that the greater the accumulation of toxic ions, the larger the damage from salt stress on plants. In our study, salt stress on tillering nodes significantly reduced the growth of winter wheat, such as biomass, plant height, and root length as well as root number. However, the reduction caused by tillering nodes exposed to salt stress was lower than that caused by roots, as the amount of Na^+ accumulation through tillering nodes was relatively lower compared to roots. From the growth parameter results, although the effects caused by tillering nodes is not as great as which caused by roots under salt stress, salt stress on tillering nodes is seen to affect the growth of winter wheat. Furthermore, a reduction in plant growthin the salt-sensitive winter wheat cultivar was larger than the salt-tolerant winter wheat cultivar when tillering nodes were exposed to salt stress, suggesting that the salt-induced inhibition was compatible with the salt tolerance in winter wheat.

Salinity on tillering nodes not only resulted in growth inhibition in winter wheat but also caused remarkable increases in the content of soluble sugar, proline and Na^+, notable decreases in K^+ content as well as the K^+/Na^+ ratio in both winter wheat cultivars. Soluble sugar and proline are the osmotic regulators in plant cell. The accumulation of proline and soluble sugar under salt stress could protect the cell from environmentaldamage (Sairam *et al*., 2002). When the tillering nodes were subjected to salt stress, the contents of soluble sugar and proline in both cultivars were notably upregulated. Furthermore, the contents of soluble sugar and proline in the salt-tolerant winter wheat cultivar were larger than in the salt-sensitive winter wheat cultivar, which was also observed after the roots were exposed to salt stress. Although the upregulation of soluble sugar and proline caused by roots was larger than the tillering nodes in response to salt stress, the contents of soluble sugar and

proline in winter wheat were remarkably affected when tillering nodes were under salt stress.From the Na^+ content results obtained under salt treatment, salinity caused significant increases in Na^+ content, especially in leaves. The increase in Na^+ content in leaves when tillering nodes were salt stressed indicated that Na^+ could enter through tillering nodes under salt stress, although the tillering node was not the normal organ for nutrient and water absorption. However, it was found that Na^+ accumulation through tillering nodes was smaller than through roots, demonstrating that the uptake of Na^+ through tillering nodes was inferior to roots. Because roots are the normal organ for nutrient and water absorption, it is reasonable that Na^+ influx through roots is larger than through tillering nodes. Moreover, the increase in Na^+ caused by tillering nodes under salt stress presented no difference between the two cultivars, whereas the increase in Na^+ contents caused by roots was much higher in the salt-sensitive cultivar H-4589 than in the salt-tolerant cultivar J-32. The equivalent Na^+ accumulation in two different cultivars suggested that the Na^+ entry through tillering nodes under salt stress exhibited no variation in cultivars, especially in the degree of salt tolerance. Much higher increases in Na^+ in the leaves of salt-sensitive cultivars under salt stress caused by roots are consistent with previous reports that the salt-tolerant cultivar is more efficient in excluding Na^+ than the salt-sensitive cultivar when roots are subjected to salinity (Gorham *et al*., 1990; Bilkis *et al*., 2016). K^+ is essential to balancing membrane potential, activating enzymes, regulating osmotic pressure, and stoma movement (Chérel, 2004). Moreover, maintaining a high K^+/Na^+ ratio is of great importance for the adjustment of cell osmoregulation, stomata function, enzyme activation, protein synthesis, oxidants metabolism and photosynthesis to increase plant salt tolerance (Maathuis & Amtmann, 1999; Shabala *et al*., 2003). The present study documented that the K^+ contents of both cultivars significantly decreased due to salt stress, which agrees with the results reported by Yanhui *et al.* (Yanhai *et al*., 2008). The decrease in K^+ contents in both cultivars caused by tillering nodes was less than that caused by roots. Salinity also caused a reduction in the K^+/Na^+ ratio in both cultivars. The reductions in both cultivars caused by salinity through tillering nodes was also less than roots, which was similar to the changes in the K^+ contents. The more accumulation of Na^+, the larger the decline of K^+ would be found. The salinity induced decreasing of K^+ accumulation possibly due to the competition between Na^+ and K^+ in K^+ transport system resulting in Na^+ influx and decrease of K^+ absorption(Peng *et al*., 2004); or it is also possible that the damage to the cell membrane was caused by salt stress leading to K^+ leakage .

In most plants, ions are absorbed mainly through symplast and apoplastic pathways. When subjected to unfavorable environmental conditions, plants can develop various anatomical and physiological strategies, such as enhancing selective absorption of ions through a symplast pathway (Cheeseman, 1988; Kronzucker *et al*., 2006; Munns & Tester, 2008) or increasing the apoplastic barriers to hamper the entry of ions (Gong *et al*., 2006; Redjala *et al*., 2011) to fight against stress. As most monocotyledonous crop species are glycophytes that are sensitive to salinity, they usually exclude external salt ions with specific mechanisms to reduce salt damage. Previous studies have demonstrated that the apoplastic pathway plays an important role in hampering the uptake of ions due to apoplastic barriers (Yeo *et al*., 1987; Schreiber *et al*., 1999) such as Casparian bands and suberin lamellae, or from other types of lignification in plants located in the endo- and exo-dermis(Perumalla & Peterson, 1986; Peterson & Lefcourt, 1990; Barnabas & Peterson, 1992; Enstone & Peterson, 1997; Schreiber *et al*., 1999; Roppolo *et al*., 2011). It is known that the development of apoplastic barriers is affected by environmental stresses. Increasing research has shown that earlier formation of Casparian bands and suberin lamellae along the root axes (Vaculík *et al*., 2009), increased deposition of suberin and lignin (Lee *et al*., 2009), and extensive development of apoplastic barriers to reduce Na^+ uptake (Krishnamurthy *et al*., 2011; Ranathunge *et al*., 2011) were involved in abiotic stress resistance. Furthermore, it has been found that suberin lamellae was more efficient than in Casparian bands in preventing the diffusion and transport of ions and compounds in spite of similar chemical compositions between them (Schreiber *et al*., 1999; Ranathunge *et al*., 2005; Franke & Schreiber, 2007). All of this evidence demonstrates the significant function of lignification in preventing ion uptake through nonselective apoplastic bypass. Through the analysis of anatomical structure in tillering nodes and roots, we revealed that no difference in tillering nodes was found between cultivars, but the endodermis of primary roots in a salt-tolerant cultivar was distinct from that of the salt-sensitive cultivar, which could explain why Na^+ accumulation only varied in the two wheat cultivars when Na^+ entered through roots. The lignin deposition of the endodermis was found to be thicker in the salt-tolerant cultivar compared to the salt-sensitive cultivar; and we also found that the lignification of tillering nodes was greater than roots, indicating that the higher the lignification, the less accumulation of Na^+ in plants. This corresponded with results indicating that the degree of lignification in plants determined the capacity for ion uptake in previous studies (Colmer & Bloom, 1998; Schreiber *et al*., 2005; Krishnamurthy *et al*., 2009; Krishnamurthy *et al*., 2011). Furthermore, the anatomical data indicates that the continuity of the endo- and exo- dermis was temporarily interrupted by the emergence of lateral roots at the primary root–lateral root junctions, and the lateral roots lacked impregnation with lignin.In monocots, the disruption of endo- and exo-dermis continuity by lateral roots could generate leakage of water and minerals into primary roots (Ma *et al*., 2001). Researchers have reported that lateral root development is down-regulated by salt stress in Arabidopsis (Burssens *et al*., 2000) and that most of the Na^+ enters into shoots through the so-called ‘open windows’ created by the initiation of lateral roots at the primary root–lateral root junctions(Ranathunge *et al*., 2004). Moreover, Zhou detected a significantly net Na^+ influx at the lateral root zone during initial development by using the scanning ion-selective electrode technique (Zhou, Wang *et al*., 2011). It has also been suggested that lateral roots may admit the entry of Na^+ as they lack lignin and suberin (Faiyue *et al*., 2010). Based on these anatomical results from previous studies, we could conclude that the initiation of lateral roots may allow significant influx of external Na^+ from the apoplastic barriers of the endo-/exo-dermis. Furthermore, the results

for lateral root growth under salt stress demonstrated a greater decrease in lateral root numbers developed from the primary roots in the salt-tolerant cultivar compared with the salt-sensitive cultivar, which could help to maintain a significantly lower Na^+ content in the salt-tolerant cultivar. These results suggested that the difference in Na^+ content entering the leaves through the roots was strongly influenced by the amount and continuity of apoplastic barriers in the roots.

Conclusions

In this study, when tillering nodes were treated with salt stress, plant growthand K^+ content as well as K^+/Na^+ ratio were significantly reduced; and Na^+ accumulation, soluble sugar and proline were remarkably increased in winter wheat. Although all of the salt-induced changes brought by tillering nodes were not as great as those from roots, the effects caused by tillering node on the growth of winter wheat when tillering nodes were under salt stress is notable. Our investigation also documented that a significant accumulation of Na^+ caused by salt stress through tillering nodes was observed despite that the lower accumulation of Na^+ caused by tillering nodes than roots under salt stress. Moreover, Na^+ accumulations caused by tillering nodes exhibited no difference between winter wheat cultivars with different salt-tolerances, whereas the Na^+ accumulations caused byroots were remarkably different. According to anatomical analyses, these may be due to no anatomical differences that exist in the tillering nodes in the cultivars, but more lignified endodermis of primary roots and reduction in lateral root numbers were observed in the salt-tolerant cultivar, which could contribute to the prevention of Na^+ influx. All of these results indicated that tillering nodes inferior to roots could mediate Na^+ influx from the environment and the Na^+ influx through tillering nodes exhibited no variation among wheat cultivars differing in salt tolerance, then leads to salt-induced damage to the growth of winter wheat. This implies that high salt accumulation on the soil surface could generate salt stress on tillering nodes, aggravating the damage of salinity on winter wheat in the reviving stage.

Acknowledgements

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