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Effect of Salinity Stress on Slow- and Fast-Growing *Festuca* Grass Species

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IMPROVING tolerance to salinity stress is a major challenge in many regions worldwide. In this study, the effect of salinity stress on slow- and fast-growing Festuca species was examined. Plants were exposed to 0, 50, 100 or 200 mM of NaCl for two weeks in a greenhouse using a hydroponic culture system. Shoot dry mass, water status, membrane stability were monitored as well as contents of proline, sodium, potassium, calcium, magnesium, nitrogen and phosphorus. Salinity stress had negative effects on shoot dry mass, water status, and membrane stability. Although fast-growing species had higher shoot dry mass, the slope of decreases were much higher in the fast-growing species. Slow-growing species showed greater accumulation of Na^+, greater increases in ion leakage and Mg content and greater decreases in proline content. The results suggested that the interspecific differences among species in resistance to salinity stress associated mainly with tolerance ability to salinity stress rather than avoidance ability. The difference is due mainly to growth habits which is associated mainly with relative growth rate and leaf properties. Also, there was interference of Mg, but not Ca, on Na^+ uptake by plant shoot, in addition to the important role of proline content in tolerance mechanism.

Keywords: *Festuca* grass, Interspecific difference, Na^+, Proline, Salinity stress

Salinity stress is one of the major environmental stress limiting growth and productivity of plants. One-third of the irrigated land suffers from salinity, especially in the arid and semiarid regions (Taiz and Zeiger, 2002 & FAO, 2011). The main sources of the accumulated salts in arable soils are seawater and the irrigation water that contains sodium chloride (NaCl) (Flowers & Yeo, 1995 and Tester & Davenport, 2003).

Plants differ greatly in their tolerances to salinity stress either among species or populations within the same species (Munns & Tester, 2008, Witzel *et al*.,

2009 and Amjad *et al*., 2014). Plants are damaged by salinity stress in several ways including early occurring osmotic stress, ionic stress, oxidative stress, alteration in metabolic processes, nutritional disorders, membrane disorganization, reduction of cell division and expansion and/or genotoxicity (Munns, 2002, Munns & Tester, 2008 and Carillo *et al*., 2011). Ionic damage occurs when salts accumulate in plant tissues at toxic level. Accumulation of Na^+ ion in plant tissues at excessive levels is one of the major factors causing salinity damage (Flowers & Hajibagheri, 2001 and Mitsuya *et al*., 2003). Increasing the concentration of salt such as NaCl in the soil reduces the ability of plants to uptake the water. The accumulation of ionic Na^+ in plant tissue impairs the metabolic processes and decreases the photosynthetic efficiency which in turn negatively effect on the plant growth (Flowers & Yeo, 1995 and Mäser *et al*., 2002). Although many studies have explored plants response and resistance to salinity stress, many challenges still lie ahead for understanding the key traits that confer such tolerance (Vinocur & Altman, 2005, Bartels & Sunkar, 2005 and Deinlein *et al*., 2014).

In this study, comparison experiment was conducted among four *Festuca* grass species differed in their growth habit, two slow-growing and two fast-growing species, under different salinity levels using hydroponic system to clarify how species differs in their responses to salinity stress. The objectives of this study were (1) to clarify the interspecific differences in salinity tolerance among species, (2) to illustrate if species growth properties influence tolerance trait, and (3) to ascertain whether response to salinity stress interference with ions uptake and proline content.

Materials and Methods

Plant materials and growth conditions

Four species belonging to *Festuca* genus were used in this study, fast-growing species (*Festuca arundinacea* Schreb., *F. pratensis* Huds.), and slow-growing species (*F. ovina* L., *F. rubra* L). This study was conducted in a greenhouse using a hydroponic culture system during June-July, 2014. Plants were grown in plastic nursery trays placed on 25-L containers, with half-strength modified Hogland and Arnon No. 2 nutrient solution (Sugiyama and Nikara, 2004). The full-strength modified Hogland and Arnon No. 2 nutrient solution contains macronutrients in mM, N 15.0, P 1.0, K 6.0, Ca 4.0 and Mg 2.0, along with micronutrients in μM, B 3.0, Mn 0.5, Cu 0.2, Zn 0.4, Mn 0.05, and Fe-EDTA 20.0. After 40 days, the plants were exposed to salinity stress using NaCl with concentrations of 0, 50, 100, and 200 mM for two weeks. The pH was adjusted daily at 5.5 using 1 N H_2SO_4 and/or NaOH. The nutrient solution was renewed every two weeks. Aeration was supplied at a rate of 2 L/min using a mini pump throughout the experiment. The experiment was set up as a randomized block layout.

Physiological measurement and chemical analysis

After two weeks of salinity treatments, plants were harvested and divided into two sets (each set contain four replicates). A set of plants was used to measure cell membrane stability and leaf water status. Cell membrane stability was measured by ion leakage (IL) from leaf tissues using the methods described by Jiang and Huang (2002). Leaf water status was measured by relative water content (RWC) according to Loutfy *et al*. (2012) as,

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

where *FW* is the fresh weight, *DW* is the dried weight and *TW* is the turgid weight of tissue after being soaked in water for 12 h at room temperature.

Another set of plants were cut below the stem base and were separated into shoots and roots. Shoots were dried at 70°C for 48 h in a forced-air oven, then the dry weights were recorded. Free proline was determined according to Bates *et al*. (1973). Briefly, dried shoots after grinding (0.2 g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid for 10 min followed by filtration. Two milliliters of the filtrate were mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin, and the mixed solution was heated in water path for 1 h. The developed color was extracted in 4 ml toluene and measured colourimetrically at 520 nm against toluene. A standard curve with proline was used for calculate the final concentrations. For chemical analysis, dried plant shoots (0.2 g) were wet-degisted with concentrated H_2SO_4:H_2O_2 (1:1, v/v) using a heating digester (DK, Velp Scientific srl, Italy). The extracts were used for chemical analysis. Nitrogen content was measured using TOC analyzer (TOC-L, Shimadzu Corporation, Japan). Phosphorous (P) content was measured colourimetrically using UV-VIS Spectrophotometer. Sodium (Na^+), potassium (K^+), calcium (Ca^{+2}) and magnesium (Mg^{+2}) were analyzed using Polarized atomic absorption spectrophotometer (Z-2000, Hitachi Ltd., Tokyo, Japan).

Statistical analysis

The statistical analysis was carried out using JMP (versions 4.0, SAS Institute Inc., USA). The statistical difference among salinity treatments was tested by analysis of variance (ANOVA) for each species. The experiment was set up in a randomized block layout incorporating four replications for each set.

Results

Under control conditions, the four species differed greatly in shoot dry mass and contents of proline, nitrogen and phosphorus. The other traits showed no significant differences under control conditions. One-way ANOVA revealed significant changes in shoot dry mass, water status as expressed by relative water

content (RWC), membrane stability as expressed by ion leakage (IL), and chemical components under salinity stress in different *Festuca* grass species (Table 1). Fig. 1 showed significant decreases in shoot dry mass, RWC, and nitrogen content, while sodium (Na^+) and phosphorus (P) contents increased significantly in the four species with increasing NaCl concentration in culture solution. The slow-growing species, *F. ovina* and *F. rubra*, showed significant decreases in proline content and significant increases in IL and Mg content under salinity stress. On the other hand, fast-growing species, *F. arundinacea* and *F. pratensis*, showed no significant changes in IL, Mg, and proline content under stress. Also, no significant changes were shown in potassium (K) and calcium (Ca) contents in both fast-growing and slow-growing species.

The grass *F. pratensis* had the highest shoot dry mass followed by *F. arundinacea*, *F. rubra*, and *F. ovina* at all levels of salinity stress, however the slope of decreases was much higher in *F. pratensis* compared to other species (Fig. 1). Proline content was significantly higher in the slow-growing species compared to fast-growing species under control conditions. In contrast, proline content was significantly higher in the fast-growing species under salinity stress. The decreases in RWC were much higher in *F. arundinacea* and *F. rubra*. On the other hand, *F. ovina* showed the highest ion leakage under stress associated with the highest accumulation of Na^+ compared to other species. Nitrogen content differed significantly among species even under control. The highest nitrogen content was shown in *F. arundinacea* and the lowest in *F. ovina*. Similar results were shown in term of phosphorus content.

TABLE 1. Effect (*F* value) of salinity stress on shoot dry mass (DM), relative water content (RWC), ion leakage(IL), Proline, sodium (NA), potassium (K), calcium (Ca), magnesium (Mg), nitrogen (N), and phosphorus (P) in different *Festuca* grass species

	***F* value**									
	DM	**RWC**	**IL**	**Proline**	**Na**	**K**	**Ca**	**Mg**	**N**	**P**
F. arundinacea	7.97**	29.5***	1.78	1.27	71.7***	0.89	0.16	0.66	54.1***	9.78**
F. ovina	13.3***	7.07**	14.1***	128.1***	62.4***	3.38	0.40	17.9***	20.3***	10.4**
F. pratensis	10.4**	7.45**	3.28	2.08	181.4***	0.42	0.72	2.66	297.8***	6.96**
F. rubra	12.3***	18.6***	3.94*	103.1***	697.8***	4.83*	0.88	5.14*	30.1***	13.3***

*, **, *** Significant at probability of 0.05, 0.01 and 0.001, respectively.

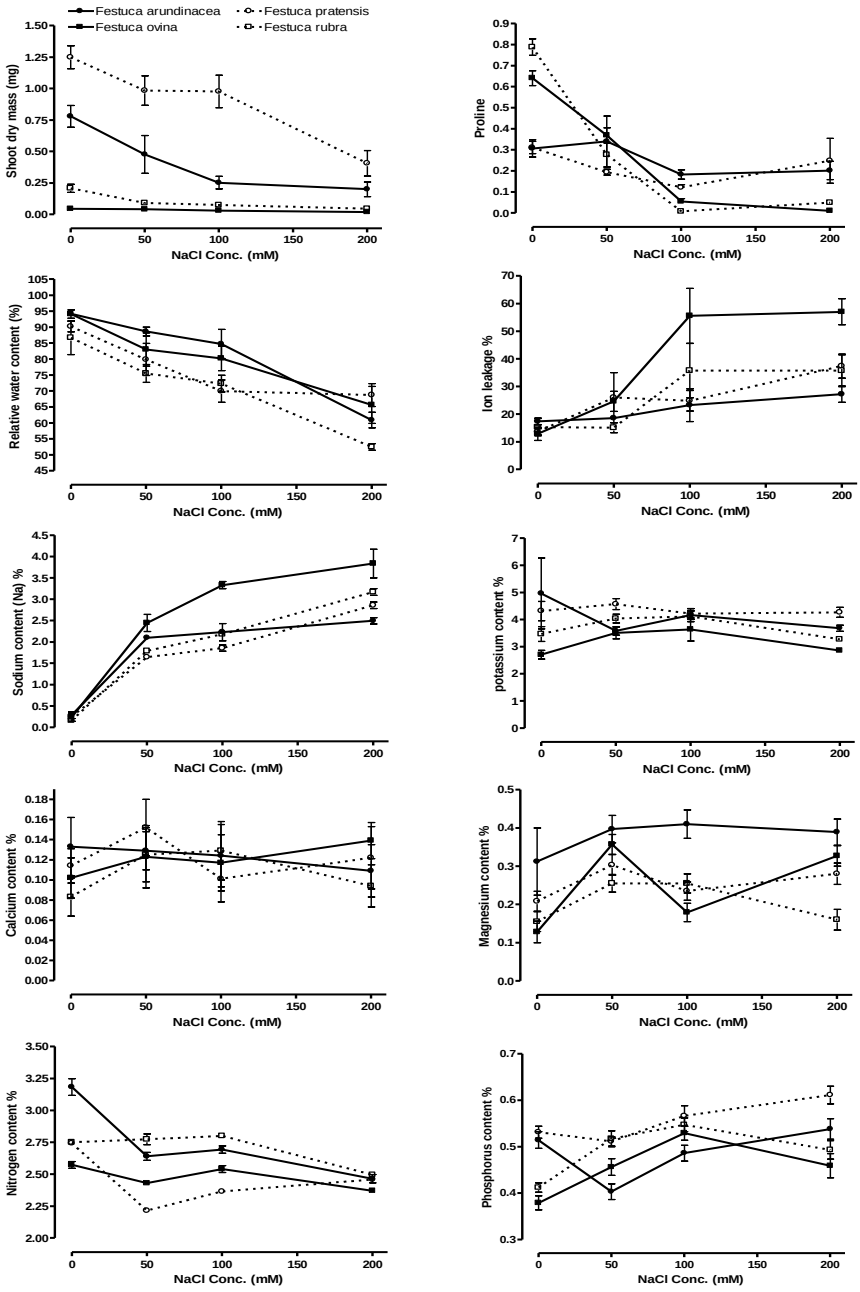

Fig.1. Responses of shoot dry mass, proline content, relative water content, ion leakage, and sodium, potassium, calcium, magnesium, nitrogen, and phosphorus contents in four Festuca grass species at different levels of salinity stress.

Discussion

In previous study under controlled conditions, Sugiyama (2005) revealed an interspecific difference in shoot relative growth rate (RGR) among grass species including Festuca species, which is closely associated with variation in specific leaf area (SLA). Sabreen and Sugiyama (2008) found trade-off between RGR and leaf structural properties under optimum conditions with resistance and tolerance to Cd stress. The slow-growing species included *F. ovina* and *F. rubra* with low RGR and SLA showed higher resistance to stress, while the fast-growing species include *F. arundinacea* and *F. pratensis* with high RGR and SLA showed lower resistance (Sugiyama, 2005). The crop yield is the indicator of plant tolerance to stress. It is difficult to evaluate the effect of salinity under field conditions because of the variability within fields and interactions with other environmental stresses. This study was conducted to evaluate the response of slow- and fast-growing *Festuca* grass species to different levels of salinity stress. The species showed great differences in shoot dry mass even under controlled conditions as a result of the difference among species in growth habit. Shoot dry mass decreased significantly under salinity stress condition. Slopes of linear regression of shoot dry mass against NaCl treatments were calculated to evaluate the difference in resistance ability among species. *F. ovina* had the highest resistance ability (greatest slope: –0.0001), followed by *F. rubra* (slope: -0.0007), *F. arundinacea* (slope: -0.0028), and finally *F. pratensis* (least slope: –0.004). These results clearly demonstrated that slow-growing species had much higher resistance ability to salinity stress compared to fast-growing species. This result revealed the important role of growth habit and leaf properties in success of individual plants under stress conditions.

Plant resistance to environmental stress such as salinity represents the ability of plant to reduce the negative impact of stress, which is based on two components: avoidance and tolerance (Munns & Tester 2008 and Carillo *et al*., 2011). Avoidance is the ability of plant to escape from the stress conditions, while tolerance is the ability of plant to withstand the imposed stress (Levitt, 1972 and Pierce *et al*., 2005). In this study, Na^+ accumulation in shoots showed great variation among species (Fig. 1). The slow-growing species showed higher Na^+ accumulation rather than fast-growing species under salinity stress. These results revealed that slow-grown species had lower ability to reduce Na^+ accumulation in shoots, and also greater ability to withstand the accumulated Na^+ ion. These suggested that resistance to ionic toxicity caused by salinity stress is associated mainly with the ability to tolerate the accumulated Na^+ ions rather than avoidance ability. This is consistent with resistance to cadmium (Cd) stress (Zha *et al*., 2004 and Sabreen & Sugiyama, 2008).

Salinity had negative impact on physiological process such as water relations (Maeda and Nakazawa, 2008), nutritional imbalance (Yang *et al*., 2008) and membrane stability (Dogan *et al*., 2010). Sodium sequestration and K^+ retention are crucial factors in salinity tolerance (Adem *et al*., 2014). Plants acclimate to salt stress by preventing K^+ leakage and Na^+ accumulation suggesting that salt

tolerance is associated mainly with ion-specific component rather than osmotic component of stress (Pandolfi *et al*., 2012). In this study, salinity stress had negative effects on water status (measured by relative water content, RWC) and membrane stability of species (measured by ion leakage, IL). *F. pratensis* showed the highest RWC, while *F, rubra* showed the lowest RWC under salinity stress (Fig.1). No clear role of RWC in differences between slow- and fast-growing species was evident. The slow-growing species showed significant increases in IL, while the fast-growing species showed no significant changes under stress. *F. ovina* showed the greatest IL compared to other species, in addition to the greatest Na^+ accumulation. Magnesium (Mg) content increased significantly under salinity stress for slow-growing species, but no significant changes in fast-growing species. Also, *F.* ovina showed the greatest increase in Mg content under stress compared to other species. These results indicate occurrence of membrane damage as a result of Na^+ ion toxicity, Mg may play importance role in tolerance mechanism.

Compatible osmolytes, such as proline, are low molecular weight, highly soluble organic compounds synthesis and accumulating in varied amounts depending on plant species. The major functions for these osmolytes are protecting the structure and maintaining osmotic balance within the cell through different course, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Bohnert & Jensen, 1996 and Hasegawa *et al*., 2000). Proline acts as a component of signal transduction pathways that regulate stress responsive genes by protecting the protein turnover machinery against stress-damage and up-regulating stress protective proteins (Khedr *et al*., 2003). Proline functions as an osmolyte for the intracellular osmotic adjustment and plays a critical role in protecting photosynthetic activity under salt stress (Silva-Ortega *et al*., 2008). Also, proline decreases the level of reactive oxygen species and lipid peroxidation as well as improves membrane integrity by increasing antioxidant gene providing a protection against NaCl-induced cell death (Banu *et al*., 2009). The exogenous proline mitigated the detrimental effects of salt stress by increasing antioxidant enzyme activities (Hoque *et al*., 2007). In this study, the species showed great differences in proline content even under control conditions. Table 1 and Fig. 1 showed that slow-growing species, *F. ovina* and *F. rubra*, had higher proline content under control conditions compared to fast-growing species, and the proline content decreased significantly under salinity stress. The fast-growing species showed no significant changes in proline content under salinity stress. These results suggest that slow-growing species had higher ability to use proline for reducing the negative impacts of salinity stress. The protective role of proline may be induced as a result of damage in membrane stability.

Salinity stress can have effects on plant growth and development in different ways including osmotic stress, ionic stress, and oxidative stress. Resistance of salinity stress is a complex trait. It is important to understand the tolerance mechanism to improve plant growth and productivity under stress. The results of

these study suggested that the ionic stress is the main cause of damage rather than osmotic stress. The resistance to salinity stress is due to the ability of species to tolerate the ionic stress rather than the ability to avoid the accumulation of toxic ions. The great variation among species in their response to salinity stress is due mainly to the difference in growth properties. The results suggest also the important role of proline in salinity stress tolerance.

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تأثير الإجهاد الملحي علي أنواع نجيل الفستوكا بطيئة وسريعة النمو

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و **معمل ايكولوجيا النبات- كلية الزراعة وعلوم الحياة – جامعة هيروساكي– اليابان.

تحسين قدرة النباتات على تحمل الملوحة يشكل تحديا كبيراً في العديد من المناطق في جميع أنحاء العالم. في هذه الدراسة، تم دراسة تأثير الملوحة على أنواع نجيل الفستوكا البطيئة والسريعة النمو. وقد تم تعريض النباتات إلى مستويات ملوحة ٠، ٥٠، ١٠٠ أو ٢٠٠ ميلليمول من كلوريد الصوديوم لمدة أسبوعين، وتمت التجربة داخل صوبة زجاجية باستخدام تقنية الزراعة المائية (الهيدروبونيك). تم رصد الوزن الجاف والوضع المائي وثبات الأغشية فضلاً عن محتويات البرولين والصوديوم والبوتاسيوم والكالسيوم والمغنيسيوم والنيتروجين والفوسفور. الملوحة أظهرت تأثيراً سلبياً على الوزن الجاف والوضع المائي وثبات الأغشية. على الرغم من أن الأنواع سريعة النمو كانت أعلى في الوزن الجاف، الا أن معدل الانحدار كان أعلى بكثير في الأنواع سريعة النمو. وأظهرت الأنواع بطيئة النمو تراكم أكبر من الصوديوم، وزيادات أكبر في تسرب الأيون ومحتوى المغنيسيوم، وانخفاض أكبر في محتوى البرولين بالمقارنة مع الأنواع سريعة النمو. وأشارت نتائج هذه الدراسة إلى أن الاختلافات بين الأنواع في صفة المقاومة للإجهاد الملحي مرتبطة أساساً بقدرتها علي تحمل الإجهاد وليس بقدرتها علي تجنب الإجهاد. ويرجع ذلك أساساً إلى طبيعة النمو والتي ترتبط بشكل رئيسي بمعدل النمو النسبي وخصائص الأوراق، وقد أوضحت النتائج أيضاً الي التداخل بين المغنيسيوم، وليس الكالسيوم علي امتصاص الصوديوم، بالإضافة إلى الدور الهام لمحتوى البرولين في آلية المقاومة.