

## Interspecific variations in resistance, avoidance and tolerance to salinity stress among 12 C<sub>3</sub> grass species

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### ABSTRACT

Resistance mechanism to salinity stress is divided into two components; avoidance and tolerance. In this study, interspecific differences in resistance, avoidance and tolerance to salinity stress were examined among 12 C<sub>3</sub> grass species. In a greenhouse, plants were exposed to 50, 100 or 200 mM of NaCl for two weeks using hydroponics system. We found that interspecific differences in resistance to salinity stress associated mainly with tolerance ability plus significant contribution of avoidance ability. Contribution of the genetic potential in the interspecific variation for resistance and tolerance was higher than that of Na<sup>+</sup> concentration in the culture solution. Salinity stress had negative effects on shoot dry mass and water status. Relative water content correlated positively with resistance and tolerance, and negatively with avoidance. The species varied in proline content which associated positively with resistance and tolerance, and negatively with avoidance. Resistance and tolerance correlated negatively with relative growth rate and specific leaf area. These results suggested that the difference among species in resistance to salinity stress was due to both tolerance and avoidance. Proline content, relative growth rate and structural leaf properties may be used as indicators of future ability to tolerate salinity stress.

### INTRODUCTION

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 and Yeo 1995; Tester and Davenport 2003).

Plants differ greatly in their resistance to salinity stress either among species or populations within the same species (Munns and Tester 2008; Witzel *et al.* 2009; 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 and Tester 2008; Carillo *et al.* 2011). Ionic damage occurs when salts accumulate in plant tissues at toxic level. Accumulation of Na<sup>+</sup> ion in plant tissues at excessive levels is one of the major factors causing salinity damage (Flowers and Hajibagheri 2001; 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<sup>+</sup> in plant tissue impairs the metabolic processes and decreases the photosynthetic efficiency which in turn negatively effect on the plant growth (Flowers and Yeo 1995; 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 and Altman 2005; Bartels and Sunkar 2005; Deinlein *et al.* 2014).

Resistance to salinity stress is a complex trait that can be improved through integration between molecular geneticists and physiologists (Munns *et al.* 2006). To improve plant resistance to salinity stress, it is crucial to unravel the components of resistance mechanism. Plants can resist to abiotic stresses by two mechanisms; avoidance and tolerance. For salinity stress, plant can

resist stress by avoiding ionic stress by minimizing Na<sup>+</sup> accumulation in the cytosol of cells particularly of the transpiring leaves (avoidance) and/or by increasing the ability of leaves to survive in the presence of the accumulated ions (tolerance) (Munns and Tester 2008; Carillo *et al.* 2011).

In this study, comparison experiment was conducted among 12 C<sub>3</sub> grass species under different salinity levels using hydroponic system to clarify how avoidance and tolerance contribute the interspecific differences in the resistance to salinity stress. The main objectives of this study were to illustrate (1) how species properties such as productivity influence the resistance ability to salinity stress and (2) how resistance ability is determined by avoidance and tolerance abilities among the twelve species.

### MATERIALS AND METHODS

#### Plant materials and growth conditions

Twelve C<sub>3</sub> grass species were used in this study; *Agrostis alba* L., *A. tenuis* Sibth., *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *F. ovina* L., *F. pratensis* Huds., *F. rubra* L., *Lolium multiflorum* Lam., *L. perenne* L., *Phalaris arundinacea* L., *Phleum pratense* L., and *Poa pratensis* 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, with 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<sub>2</sub>SO<sub>4</sub> 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**

Plants were harvested after two weeks of salinity treatments by cutting below the stem base. 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.

Plants shoots were dried at 70°C for 48h 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 bath 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-digested with concentrated H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub> (10:4,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<sup>+</sup>), potassium (K<sup>+</sup>), calcium (Ca<sup>+2</sup>) and magnesium (Mg<sup>+2</sup>) were analyzed using Polarized atomic absorption spectrophotometer (Z-2000; Hitachi Ltd., Tokyo, Japan).

**Calculations for resistance, avoidance and tolerance**

Linear regression was used to calculate resistance, avoidance and tolerance of each species to salinity stress as described by Sabreen and Sugiyama (2008). Resistance was calculated by a linear regression of shoot dry mass against NaCl treatments, avoidance was calculated by a regression of shoot Na<sup>+</sup> concentration against NaCl treatments, and tolerance was calculated by a regression of shoot dry mass against shoot Na<sup>+</sup> concentration. The slopes of these linear

regressions represented the resistance, avoidance and tolerance to salinity stress, and a flat slope represents higher ability of resistance, avoidance and tolerance.

**Relative growth rate and specific leaf area**

The data of shoot relative growth rate (RGR) and specific leaf area (SLA) of the twelve species were obtained from previous studies (Sugiyama 2005). The RGR and SLA of plants were measured in a controlled growth chamber with 22°C/16°C day/night temperatures, photon flux of 250mol m<sup>-2</sup> s<sup>-1</sup>, 16-h photoperiod.

**Statistical analysis**

The statistical analysis was carried out using JMP (versions 4.0; SAS Institute Inc., USA). Whereas different species have different genetic background, linear regression was used to evaluate the species responses to salinity stress. Heterogeneity of slopes among the regression equations was used to test the interspecific differences in resistance, avoidance and tolerance (Sokal and Rohlf 1981). The relative contribution of plant species and common slope was assessed among 192 plants including species and NaCl treatments.

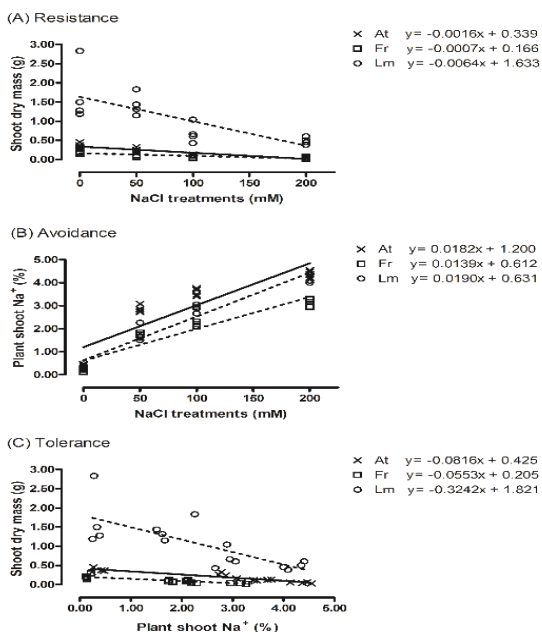
**RESULTS AND DISCUSSION**

Plant shoot dry mass showed negative linear regressions with both NaCl concentration in culture solution and shoot Na<sup>+</sup> concentration. In contrast, shoot Na<sup>+</sup> concentration correlated positively with Na<sup>+</sup> concentration in culture solution (Figure 1). The slope of the regression line, which represents the extent of response, was used as the index of resistance, avoidance and tolerance in each species (Table 1). The grass species differed significantly in resistance, avoidance and tolerance to salinity stress as shown by *F* values of 28.21, 2.49 and 29.45, respectively. *F. ovina* had the highest resistance ability (greatest slope: -0.0001), while *P. arundinacea* had the lowest resistance ability (least slope: -0.0097), which was clearly evident by yellowing most plant leaves. The contribution of plant species on resistance to salinity stress was 54.4% of sources of variation, which was greater than the contribution of common slope (31.4%).

**Table 1. Resistance, avoidance, and tolerance of species to salinity stress as represented by slopes of regressions and their coefficients of determinations (R<sup>2</sup>)**

Species	Resistance		Avoidance		Tolerance	
	Slope	R <sup>2</sup>	Slope	R <sup>2</sup>	Slope	R <sup>2</sup>
1. <i>Agrostis alba</i>	-0.0013	0.582	0.0158	0.927	-0.0849	0.683
2. <i>Agrostis tenuis</i>	-0.0016	0.823	0.0182	0.794	-0.0816	0.869
3. <i>Dactylis glomerata</i>	-0.0016	0.517	0.0196	0.970	-0.0747	0.457
4. <i>Festuca arundinacea</i>	-0.0028	0.528	0.0094	0.596	-0.2322	0.550
5. <i>Festuca ovina</i>	-0.0001	0.755	0.0165	0.736	-0.0058	0.536
6. <i>Festuca pratensis</i>	-0.0040	0.679	0.0120	0.881	-0.3035	0.623
7. <i>Festuca rubra</i>	-0.0007	0.543	0.0139	0.885	-0.0553	0.714
8. <i>Lolium multiflorum</i>	-0.0064	0.561	0.0190	0.946	-0.3242	0.558
9. <i>Lolium perenne</i>	-0.0014	0.553	0.0177	0.940	-0.0737	0.541
10. <i>Phalaris arundinacea</i>	-0.0097	0.646	0.0230	0.975	-0.4250	0.677
11. <i>Phleum pratense</i>	-0.0023	0.384	0.0208	0.833	-0.0960	0.337
12. <i>Poa pratensis</i>	-0.0009	0.538	0.0150	0.670	-0.0364	0.319
% of SS	Resistance		Avoidance		Tolerance	
Common regression	31.4		16.6		30.4	
Species-specific regression	54.4		7.1		54.9	
F value for species effect	28.21***		2.49**		29.45***	

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



**Figure 1. Linear regressions of resistance, avoidance and tolerance to NaCl stress in three representative species, *Agrostis tenius* (At), *Festuca rubra* (Fr) and *Lolium multiflorum* (Lm).**

For avoidance ability, *F. arundinacea* had the lowest Na<sup>+</sup> accumulation in plant shoots over salinity treatments and thus the greatest avoidance ability by shown the least slope (0.0094) in the regression of shoot Na<sup>+</sup> contents against salinity concentration in the environment (hydroponic culture). On the other hand, *P. arundinacea* had the highest shoot Na<sup>+</sup> contents and thus the least avoidance ability as shown by the greatest slope (0.0230) of the regression. The contribution of the common slope over 12 species to avoidance abilities terms of Na<sup>+</sup> accumulation was 16.6%, while the proportion explained by 12 species was 7.1% to the total variation. As for the tolerance ability, *F. ovina* showed the least decline of shoot dry mass to shoot Na<sup>+</sup> content and thus had the greatest tolerance to salinity stress as shown by the highest slope (−0.0058) in the regression of shoot mass against shoot Na<sup>+</sup> content. On the other hand, *P. arundinacea* had the least tolerance ability (the lowest slope: −0.4250). The contribution of species and common slopes to tolerance were 54.9 and 30.4%, respectively.

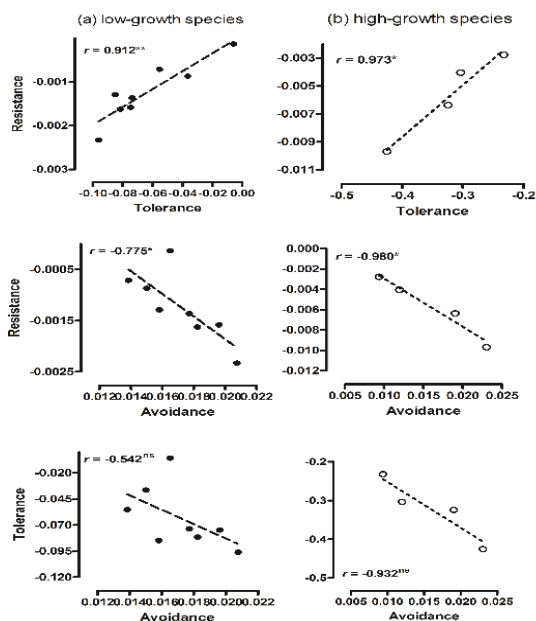
The twelve species differed greatly in their growth even under control conditions because of their wide genetic background. The shoot dry mass per plant under control ranged from 0.05 to 2.35 g, with average mean of 0.73 g. The species were divided into two groups in term of growth; low-growth (unproductive) species group which had dry mass < 0.73 g, and high-growth (productive) species group which had dry mass > 0.73 g per plant under control condition. The productive species group included *F. arundinacea*, *F. pratensis*, *L. morifolium*, and *P. arundinacea*, and the unproductive species group included the other species. Figure 2 showed the relationships among resistance,

avoidance and tolerance to salinity stress. Resistance was highly correlated with tolerance among 12 species ( $r = 0.947$ ), but correlation was not significant with avoidance ( $r = -0.417$ ). However, when 12 species was separated into two groups in terms of their growth, avoidance showed significant correlation with resistance ability. In contrast, the two groups showed no clear correlations between avoidance and tolerance abilities. These results suggest that the interspecific differences in resistance to salinity stress among species were associated mainly with tolerance to accumulated Na<sup>+</sup> in the plant tissue, but avoidance ability also contribute to resistance ability.

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 and Tester 2008; 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; Pierce *et al.* 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. In this greenhouse experiment, hydroponic system was used to evaluate resistance, avoidance and tolerance of 12 C<sub>3</sub> grass species against different levels of salinity stress. Resistance, avoidance and tolerance of species were evaluated using slopes of regression of shoot dry mass against NaCl treatments, Na<sup>+</sup> concentration in plants shoot against NaCl treatments, and shoot dry mass against Na<sup>+</sup> concentration in plants shoot, respectively. The species showed significant difference in their responses to salinity stress (Table 1). The resistance correlated significantly with tolerance and avoidance (Figure 2). These results indicate that there is great variation among species in their abilities to reduce Na<sup>+</sup> accumulation in shoots, and also great variation in shoots ability to withstand the accumulated Na<sup>+</sup> ion. In addition, resistance to ionic toxicity caused by salinity stress is associated mainly with the ability to tolerate the accumulated Na<sup>+</sup> ions in shoots ( $r = 0.912, 0.973$ ) as well as avoidance ability ( $r = -0.775, -0.980$ ) for low- and high-growth species, respectively. No significant relationship was found between tolerance and avoidance among the two groups, indicating that avoidance and tolerance contribute independently to total resistance to salinity stress. This is consistent with resistance to cadmium (Cd) stress (Zha *et al.* 2004; Sabreen and Sugiyama 2008). Contribution of the genetic potential in the interspecific variation for salinity resistance and tolerance (about 55%) was higher than that of Na<sup>+</sup> concentration in the culture solution (about 30%). In contrast, contribution of genetic potential for Na<sup>+</sup> avoidance was only 7% (Table 1). This may interpret why total resistance was related more significantly to Na<sup>+</sup> tolerance than Na<sup>+</sup> avoidance.

Relative water content (RWC) decreased significantly with increasing of NaCl concentration in the culture solution. The slope of regression of RWC showed significantly correlation with resistance,

avoidance and tolerance ( $r = 0.983$ ,  $-0.759$  and  $0.738$ , respectively) among the low-growth species, but no significant correlations were shown among the productive species (Figure 3). These results indicate that maintaining water status is associated with both tolerance and avoidance abilities.



**Figure 2.** Relationship among resistance, avoidance and tolerance to salinity stress among  $C_3$  species; (a) low-growth species group and (b) high-growth species group (productive species).

Table 2 showed that there were significant differences in nitrogen (N), phosphorous (P) and magnesium (Mg) content among species, but the differences were not significant in potassium (K) and calcium (Ca). The linear regression of nitrogen content correlated negatively with salinity concentrations in the culture solution for all species except *P. arundinacea* which showed increasing in nitrogen content under

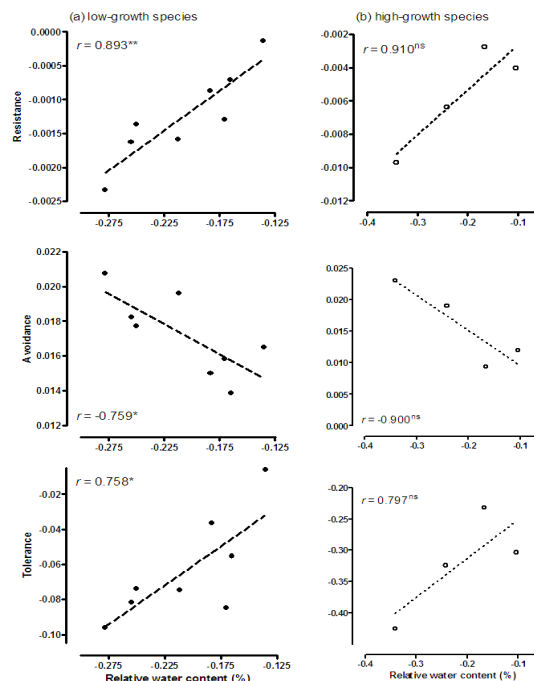
**Table 2.** One-Way ANOVA (*F* value) of chemical analysis among the twelve species under control as well as correlations with resistance, avoidance, and tolerance among both low and high-growth species groups

	<i>F</i> value	Resistance		Avoidance		Tolerance	
		Low-growth	High-growth	Low-growth	High-growth	Low-growth	High-growth
Proline	22.48***	0.74*	0.71	-0.84**	-0.84	0.73*	0.59
Nitrogen	78.74***	0.18	0.96*	-0.08	-0.94	0.14	0.99**
Phosphorus	7.05***	-0.52	0.35	0.46	-0.51	-0.31	0.17
Potassium	1.72	-0.56	0.94	0.47	-0.88	-0.42	0.99**
calcium	0.71	0.05	0.82	0.10	-0.70	-0.13	0.90
Magnesium	2.17*	-0.27	0.03	0.03	-0.02	-0.43	0.24

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

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). Pandolfi *et al.* (2012) found that plants acclimate to salt stress by preventing  $K^+$  leakage and  $Na^+$  accumulation suggesting that salt tolerance is associated mainly with ion-specific

salinity stress. By excluding the productive species, nitrogen content showed no clear correlation with resistance, avoidance and tolerance. On the other hand, nitrogen content correlated significantly with resistance and tolerance abilities, but not with avoidance among the productive species. P, K, Ca, and Mg showed no clear correlations with resistance, avoidance and tolerance.

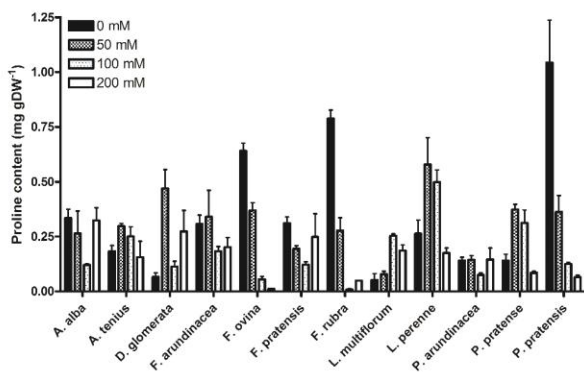


**Figure 3.** Relationships of resistance, Avoidance, and tolerance to salinity stress with regression slopes of relative water content (RWC) against NaCl concentrations among  $C_3$  species; (a) low-growth species group and (b) high-growth species group (productive species).

component rather than osmotic component of stress. In this study, salinity stress had negative effect on water status measured by relative water content (RWC). Species with high resistance, avoidance and tolerance abilities showed higher RWC (Figure 3). RWC showed significant correlation with both avoidance and tolerance. These results suggested that water status was associated with both avoidance of ionic  $Na^+$  accumulation and tolerance to ionic stress. No clear

contribution of P, K<sup>+</sup>, Ca<sup>+2</sup>, and Mg<sup>+2</sup> to resistance were shown. The most sensitive species, *P. arundinacea* showed increasing in nitrogen content under stress, while other species showed decreasing in nitrogen content. Nitrogen content correlated significantly with resistance and tolerance only among productive species (Table 2). These results indicated that accumulated Na<sup>+</sup> ions in plant tissues affected negatively on plant water status. The differences among species were due to the ability to tolerate the accumulated Na<sup>+</sup> ions, not to the ability to maintain water status.

The species showed significant differences in proline content over the stress treatments including the control (Figure 4). Among the low-growth species, proline content under control showed significant correlations with resistance, avoidance and tolerance (0.739, -0.837, and 0.731, respectively). The correlations were not significant among high-growth species group (Table 2). Most species showed no significant changes under stress. It is worth to mention that the highest resistant species; *F. ovina*, *F. rubra*, and *P. pratensis* had higher proline content under the control, and the proline content decreased significantly under stress for those species.



**Figure 4. Effect of salinity stress on proline content for twelve C<sub>3</sub> species.**

Compatible osmolytes, such as proline, glycine betaine, sugar, and polyols, 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 and Jensen 1996; 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 unstressed conditions. Table 2 showed that proline content had positive correlations with resistance, avoidance and tolerance abilities among the low-growth species, but not among productive species (high-growth). *F. ovina*, *F. rubra*, and *P. pratensis*, had high proline content under control conditions, and the proline content decreased significantly under stress. These results suggest that high-resistant species had higher ability to use proline for reducing the negative impacts of salinity stress. In contrary, the productive species had low proline content which in turn reflected low resistance ability. The great variation among species in their response to salinity stress is due to their wide genetic background. These results suggest the important role of proline in stress tolerance. The proline content may be used as indicator for further resistance ability to stress.

Both resistance and tolerance showed significant correlation with relative growth rate (RGR) and specific leaf area (SLA), whereas avoidance showed no significant correlation with these traits (Figure 5). Relative growth rate correlated negatively with resistance and tolerance among low-growth species (-0.737, -0.788, respectively), but the correlation were not significant among high-growth species. Also, SLA showed negative correlations with resistance (-0.801, -0.969) and tolerance (-0.716, -0.996) among low- and high-growth species, respectively. This reflected the role of leaf traits in resistance to salinity stress by contributing in tolerance ability.

In a previous study, Sabreen and Sugiyama (2008) found trade-off between relative growth rate (RGR) and leaf structural properties under optimum conditions with resistance and tolerance to Cd stress. In this study, RGR and specific leaf area (SLA) showed negative correlations with resistant and tolerance abilities to salinity stress (Figure 5). High SLA is a result of high water content and low leaf dry matter concentrations, which in turn cause implies high RGR (Sugiyama 2005). These results reflect the important roles of leaf structural properties in further resistance and tolerance to salinity stress.

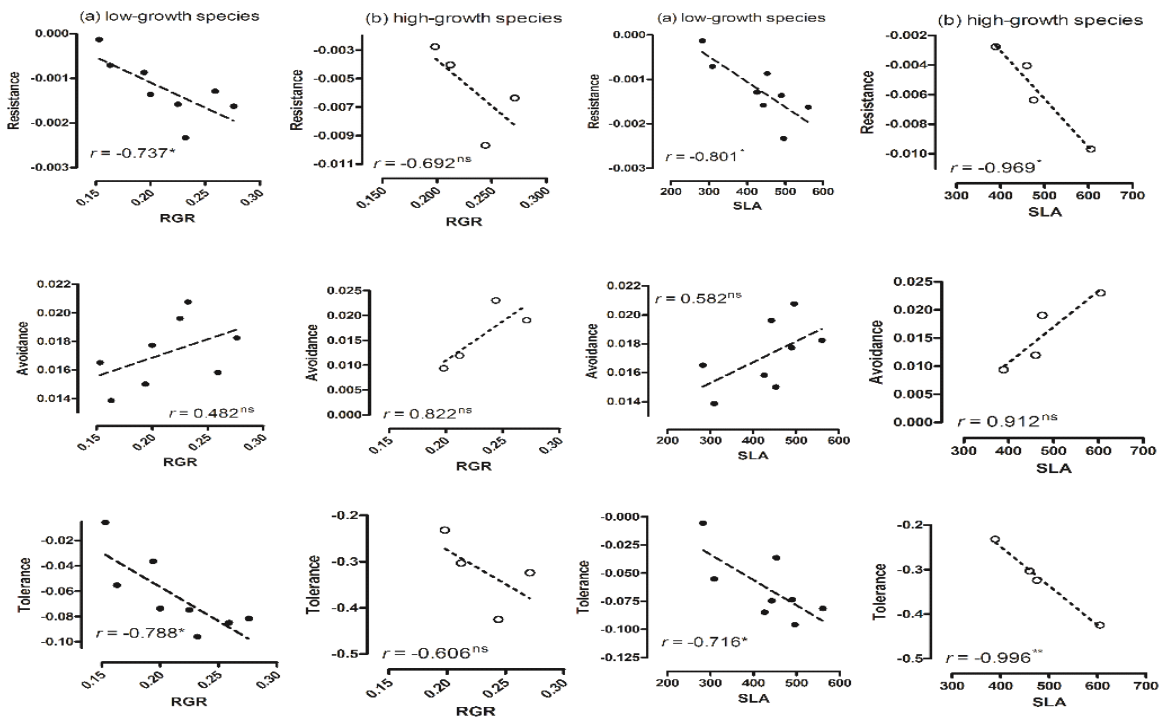
Salinity stress can have effects on plant growth and development in different ways including osmotic stress, ionic stress, and oxidative stress. Resistance of salinity stress is a complex trait. It is important to understand the components of resistance mechanism to improve plant tolerance. The results of these study suggested that the ionic stress is 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, as well as avoid the accumulation of toxic ions. The difference among species in leaf structural properties as well as proline content may contribute in their ability to tolerate future exposure to salinity stress.



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**Figure 5. Relationships of resistance, avoidance and tolerance to salinity stress with relative growth rate (RGR) and specific leaf area (SLA) among C<sub>3</sub> species; (a) low-growth species group and (b) high-growth species group (productive species).**

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## الاختلافات بين الأنواع في صفات المقاومة والتجنب والتحمل للإجهاد الملحي بين ١٢ نوع من حشائش الموسم البارد

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تنقسم آلية مقاومة الإجهاد الملحي إلى عنصرين هما آلية التجنب وآلية التحمل. في هذه الدراسة تم فحص الفروق بين ١٢ نوع من نباتات الموسم البارد في صفة المقاومة وصفة التجنب وصفة التحمل للإجهاد الملحي. تمت التجربة في صوبة زجاجية وباستخدام نظام الزراعة المائية (هيدروبونيك)، وقد عرضت النباتات لمدة أسبوعين لمستويات مختلفة من الإجهاد الملحي كالتالي ٥٠ أو ١٠٠ أو ٢٠٠ ميليمول من كلوريد الصوديوم بالإضافة الي الكنترول (بدون معاملة). ولقد جردنا أن الاختلافات بين الأنواع في صفة المقاومة للملوحة مرتبطة أساساً بقدرتها علي تحمل الإجهاد، وأيضاً الاختلافات بين الأنواع في صفة المقاومة للملوحة تساهم فيها قدرة الأنواع علي تجنب الإجهاد. بالنسبة لصفة المقاومة وصفة التحمل للإجهاد الملحي فإن الاختلافات بين الأنواع تأثرت بشكل أكبر بالاختلافات الجينية (الوراثية) بين الأنواع مقارنة بتأثير التركيزات المختلفة للصوديوم في المحلول المغذي. الملوحة كان لها آثار سلبية على الكتلة الجافة للنباتات والتوازن المائي داخل الأنسجة. محتوى الماء النسبي ارتبط معنوياً بشكل طردي مع صفة المقاومة وصفة التحمل وارتبط عكسياً مع صفة التجنب. ولقد وجدت اختلافات معنوية بين الأنواع في محتوى البرولين تحت ظروف الكنترول والتي اظهرت ارتباط إيجابي مع صفة المقاومة وصفة التحمل وارتباط سلبي مع صفة التجنب. أيضاً أظهرت صفتي المقاومة والتحمل تناسباً عكسياً مع المعدل النسبي للنمو والمساحة النوعية للورقة. وأشارت هذه النتائج إلى أن الفرق بين الأنواع النباتية في صفة المقاومة للإجهاد الملحي مرتبطة بأكملها من صفة التحمل وصفة التجنب للإجهاد. أيضاً فإنه يمكن استخدام البيانات الخاصة بمحتوى البرولين والمعدل النسبي للنمو وخصائص الورقة كمؤشرات أولية لتوقع قدرة النباتات على المقاومة في حال تعرضها للإجهاد.