

# A comparative *in vitro* study of salt tolerance in cultivated tomato and related wild species

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**Abstract** Salinity stress is a major abiotic stress for plants worldwide. This study was carried out to determine the variation in salt tolerance for 12 different genotypes belonging to three different tomato species: *Solanum lycopersicum* (L), *S. peruvianum* (L) and *S. pimpinellifolium* (L). Shoot apices and callus cultures were exposed to different levels of salinity stress ranging from no salt (control) to 100, 200 and 300 mmol L<sup>-1</sup> NaCl. All growth and physiological parameters were significantly affected by salt stress. Most shoot apices of *S. lycopersicum* did not develop roots when exposed to low NaCl levels, whereas apices of *S. peruvianum* and *S. pimpinellifolium* developed roots when exposed to all salt levels. This difference in salt tolerance was clearly shown on the basis of root fresh weights and root surface areas. Callus growth in response to increased salinity was much greater in *S. peruvianum* and *S. pimpinellifolium* than in *S. lycopersicum*. The Cl<sup>-</sup> and Na<sup>+</sup> concentrations increased significantly with increasing salt in the three species, although the *S. peruvianum* lines accumulated more ions compared with the others. As the salt concentration increased, less K<sup>+</sup> accumulated in *S. lycopersicum* compared to the related wild species. The results obtained in this study suggest that *S. peruvianum* line 0043-1 was the accession with the best salt tolerance. The most tolerant cultivated tomato (*S. lycopersicum*) cultivar was 'Rutgers.' Both *S. peruvianum* line 0043-1 and *S. lycopersicum* 'Rutgers' are good candidates for inclusion in tomato breeding programs for salt-tolerance.

**Key words:** cultivated tomato, physiological parameters, salinity stress, salinity tolerance index, wild species.

Environmental conditions that are not optimum for plant growth or production are defined as stress factors (Thornton 2002). Abiotic stresses such as salinity, drought, temperature, and flooding are some of the main causes of crop yield loss worldwide. Abiotic stresses can reduce the average yields of most major crops by more than 50% (Bray et al. 2000; Flowers and Yeo 1995; Flowers et al. 2000). In particular, high salinity is a serious threat to crop production. As the world population continues to grow, food production needs to be increased by increases in cultivated land and/or by increases in crop productivity. According to the FAO Land and Nutrition Management Service (2008), over 6% of the world's land, more than 800 million ha, is affected by either salinity or sodicity. The percentage of cultivated land affected by salt is even greater with 23% of the cultivated land being saline and 20% of the irrigated land suffering from secondary salinization (Flowers 2004; Flowers and Yeo 1995; Ghassemi et al. 1995). Population growth and land degradation by salinization have led plant researchers to the concept of developing salt-tolerant crops by different approaches (Cuartero et

al. 2006; Munns 2005; Munns et al. 2006; Yamaguchi and Blumwald 2005). The physiological, biochemical and molecular mechanisms of salt tolerance in plants are not sufficiently understood; hence, progress in developing salt-tolerant crops has been slow.

Salinity stress affects plants through morphological, physiological and metabolic changes in their organs (Amini and Ehsanpour 2005; Zhang et al. 2004). Perhaps the best investigated of the traits related to salt tolerance are those associated with the ion content of plants grown in the presence of salt stress (Abel 1969; Tal and Shannon, 1983). Essential mechanism of tolerance involves the ability to reduce the ionic stress on the plant by minimizing the amount of Na<sup>+</sup> that accumulates in the cytosol of cells, particularly those in transpiring leaves. In the majority of plant species grown under saline conditions, Na<sup>+</sup> appears to reach a toxic concentration before Cl<sup>-</sup> does, and so most studies have concentrated on Na<sup>+</sup> exclusion and the control of Na<sup>+</sup> transport within the plant (Munns 2002). The mechanism of Na<sup>+</sup> exclusion allows the plant to avoid or postpone the problem related to ion toxicity, but if Na<sup>+</sup> exclusion is

not compensated for by the uptake of  $K^+$ , there becomes a greater demand for organic solutes for osmotic adjustment. The synthesis of organic solutes jeopardizes the energy balance of the plant. Thus, the plant must cope with ion toxicity on the one hand, and turgor loss on the other (Bernstein, 1963; Munns and Tester 2008; Phillips et al. 1979).

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide. Tomato is a member of the Solanaceae family that includes several other economically important crops such as potato, pepper, and eggplant. In terms of human health, tomato fruit is a major component of daily meals in many countries and constitutes an important source of minerals, vitamins, and antioxidant compounds (Naika et al. 2005). Nevertheless, areas providing optimal growing conditions for tomatoes are becoming more restricted around the world because of salt affected soils. The growth and yield of tomato is significantly reduced by high salinity (Feigin et al. 1987; Shalhevet and Hsiao 1986; Smith et al. 1992). The response of tomato to salinity is variable depending upon the line or cultivar (Shannon et al. 1987). Compared with Arabidopsis, there is much less information on breeding for salt tolerant tomato cultivars (Cuartero et al. 2006).

Salinity tolerance by plants depends primarily on the genotype that determines such processes as uptake and transport of salt by roots in combination with metabolic and physiological events (Foolad and Lin 1997; Winicov 1993). Classic screening methods for the selection of salt tolerant variants are difficult and time consuming. *In vitro* techniques make it possible to screen the required number of genotypes rapidly since *in vitro* plant cultures, even at different stages of development, may exhibit their capacity to withstand the stress (Tewary et al. 2000; Zaki et al. 2016). Several researchers have proposed using *in vitro* screening to identify tomato germplasm with salinity tolerance (Amini and Ehsanpour 2005; Cano et al. 1996; Cano et al. 1995; Cuartero et al. 2006; Cuartero et al. 1992; Emilio et al. 1998; Garcia-Reina 1988). Fewer of these studies have examined the salinity tolerance in cultivated tomatoes and their wild species. Cuartero et al. (1992) attempted to enhance the salt-tolerance of the cultivated tomato using the tolerance of wild species of *Lycopersicon*, suggesting that *in vitro* screening of large numbers of wild species for salinity tolerance is needed. Emilio et al. (1998) also found the possibility of using *in vitro* shoot apex culture to evaluate salt tolerance of cultivated (*Lycopersicon esculentum* Mill.) and wild (*Lycopersicon pennellii* (Correll) D'Arcy) tomato species and reported relation to the response obtained by callus culture.

Therefore, it is very important to screen the available cultivated and wild species for their salt tolerance in order to recommend cultivars that can be cultivated

in high saline soil conditions or to use salt-tolerant genotypes in breeding programs. The objective of this study was to determine the extent of genetic variability for salinity tolerance among different cultivated and wild tomato genotypes using tissue culture techniques. Shoot apex and callus growth and physiological characteristics were determined for twelve genotypes in the absence and presence of NaCl to evaluate the varying degrees of salt tolerance among the genotypes.

## Materials and methods

Some parts of this study were conducted at the Laboratory of Plant Breeding, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan during the period of 26 November 2013 to 25 May 2014.

### Plant materials

Salt responses were studied in shoots and callus tissues of twelve tomato genotypes. The species used in this study included six tomato cultivars *Solanum lycopersicum* (L) ('Moneymaker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa') and two accessions of the wild species *Solanum peruvianum* (L) (lines 0043-1 and 0046) and four accessions of the wild species *Solanum pimpinellifolium* (L) (lines 0041w1, 0043, 0048 and 0049-w1). Seeds of all genotypes were obtained from the Tomato National Bioresource Project (NBRP), Japan.

### Plant micropropagation

Tomato seeds were surface sterilized for 20 s with 90% (v/v) ethanol and for 30 min with 1% (w/v) sodium hypochlorite. Thereafter, the seeds were rinsed in sterile deionized water three times. Seeds were then germinated *in vitro* on Phytigel-solidified (4.5–5 gL<sup>-1</sup>) Murashige and Skoog (MS) basic medium (Murashige and Skoog 1962). The medium pH was adjusted to 5.7±0.1 before the addition of Phytigel and subsequent autoclaving at 121°C and 15 psi for 20 min. All cultured seeds were maintained under a 16 h photoperiod, 25±2°C and white cool fluorescent bulbs providing approximately 90 μmol m<sup>-2</sup>s<sup>-1</sup> PPFD. When the plantlets had developed two true leaves (2 weeks after germination) explants were collected.

### Shoot apex and callus culture under salinity treatments

#### Shoot apex culture

Shoot apices 1 cm in length were cut and inserted upright into Magenta GA-7 plant culture boxes (Sigma-Aldrich Co. LLC.) containing 20 ml of medium (Cano et al. 1995). The medium consisted of ½-strength MS mineral salts (Murashige and Skoog 1962), 100 mgL<sup>-1</sup> myo-inositol, 1 mgL<sup>-1</sup> thiamine HCl, 0.1 mgL<sup>-1</sup> indoleacetic acid, 10 gL<sup>-1</sup> sucrose, and 4.5–5 gL<sup>-1</sup> Phytigel. To compare the genotype tolerance or sensitivity to salinity stress, shoot apices were grown on media supplemented with three different concentrations of NaCl (100, 200 or

300 mmol L<sup>-1</sup>) and compared with the control (no NaCl). All media were adjusted to pH 5.7±0.1 before autoclaving at 121°C and 15 psi for 20 min. The cultures were maintained under a 16h photoperiod, 25±2°C and white cool fluorescent bulbs providing approximately 90 μmol m<sup>-2</sup>s<sup>-1</sup> PPFD. After four weeks, plantlets were removed from the culture boxes; plantlet fresh weight, shoot and root fresh weight were measured manually, and root characteristics (total root length, root surface area, and average root diameter) were analyzed by WinRHIZO Basic 2009 image analysis software (Regent Instruments Canada, Inc.). Ten plantlets per genotype were evaluated for each of the salinity treatments and the experiment was repeated at approximately monthly intervals three times.

### Callus induction

Callus cultures were initiated from leaves of the same plantlets used for shoot apices culture on MS medium containing macro and micronutrients and supplemented with myo-inositol (100 mg L<sup>-1</sup>), sucrose (30 g L<sup>-1</sup>) and solidified with Phytigel (4.5–5 g L<sup>-1</sup>). Plant growth regulators were 0.4 mg L<sup>-1</sup> NAA and 2 mg L<sup>-1</sup> kinetin. Three different NaCl concentrations (100, 200 or 300 mmol L<sup>-1</sup>) were compared with the control (no NaCl) to test the genotypes' capacities for callus induction under salinity stress. The pH of the medium was adjusted to 5.7±0.1 before autoclaving at 121°C for 20 min. The cultures were maintained in the dark for the first two weeks and subsequently transferred to light conditions for another two weeks. After four weeks, each explant was evaluated for callus fresh weight, percent of explants forming callus and percentage of explants forming roots. Ten calli per genotype were evaluated for each of the salinity treatments and the experiment was repeated three times.

### Ion content analysis

Two hundred mg of shoot tissues were transferred to 100 cm<sup>3</sup> digestion flasks containing 50 ml 0.1 N HNO<sub>3</sub>. After 30 min of shaking, the extracts were filtered (Whatman qualitative filter paper, Grade 1) Na<sup>+</sup> and K<sup>+</sup> were determined by atomic absorption spectrophotometry, whereas Cl<sup>-</sup> was determined by potentiometric titration with 0.01 N AgNO<sub>3</sub>. Ions of chloride, sodium and potassium were determined according to the method of Emilio et al. (1998).

### Salinity tolerance index (STI)

The Salinity Tolerance Index (STI) for each genotype was calculated following the method of Reddy and Vaidyanath 1982. STI was calculated as the ratio of the trait performance at 100, 200, or 300 mmol L<sup>-1</sup> to the trait performance at 0 mmol L<sup>-1</sup> NaCl according to the following formula:

$$STI = \frac{T_s}{T_p}$$

Where  $T_s$  is the trait of genotype under stress,  $T_p$  the trait of genotype under normal conditions.

All STI variables were analyzed and for ease of discussion,

the best linear unbiased predictors (BLUPs) illustrated in the Figures are referred to as means and standard errors in the discussion.

### Statistical analyses

The experimental design was a 12×4 (genotypes×NaCl concentrations) factorial experiment in a randomized complete block with three replications ( $n=10$ ). Data obtained from this study were subjected to analysis using MSTATC software version 4 (1996). Differences among tomato genotypes were tested by an analysis of variance (ANOVA), and mean significant differences were tested by the Least Significant Difference (LSD) test at the 0.05 level of significance.

## Results

### Effects of salinity on plantlet growth

The effects of salinity stress on the fresh weights of plantlets, shoots and roots grown *in vitro* from shoot apices are shown in Figure 1. All fresh weights decreased as the salinity stress level increased with reductions generally greater in tomato cultivars than in the wild species. In general, most of the *S. lycopersicum* cultivars showed more plantlet fresh weight and shoot fresh weight at the control level (no NaCl) than any of the other species, although *S. peruvianum* lines 00-43-1 and 00-46 and *S. pimpinellifolium* line 00-43 did not differ significantly from the performance of *S. lycopersicum*. The two *S. peruvianum* lines had higher plantlet fresh weights (Figure 1A and A') and shoot fresh weights (Figure 1B and B') when grown in media with elevated salt concentrations.

Root fresh weight declined as a result of salt stress in all *S. lycopersicum* cultivars (Figure 1C and C'), an expected result since changes in root characteristics are one of the primary responses of plants to salinity stress; however there were clear differences in root growth at the lower NaCl concentrations among the cultivars. With the lowest salt concentration (100 mmol L<sup>-1</sup> NaCl), 'Aichi-first' did not develop roots at all; however, other cultivars for example, 'Moneymaker', 'Ailsa Craig', 'M82' and 'Ponderosa' did develop roots. 'Rutgers' even had roots when subjected to the highest level of salt stress (300 mM L<sup>-1</sup> NaCl) as clear in Figure 1C. On the other hand, root fresh weight increased with increasing salt concentrations in *S. peruvianum* (lines 0043-1 and 00-46) and *S. pimpinellifolium* (lines 00-48 and 0049-w1). Statistical analysis indicated that two accessions of *S. peruvianum* (lines 0043-1 and 00-46) had significantly heavier root fresh weights when grown in media with high NaCl concentrations than all other accessions and were closely followed by two accessions of *S. pimpinellifolium* (lines 00-48 and 0049-w1). *S. pimpinellifolium* line 0041-w1 had the lowest root fresh weight at the highest level of salt stress, closely followed

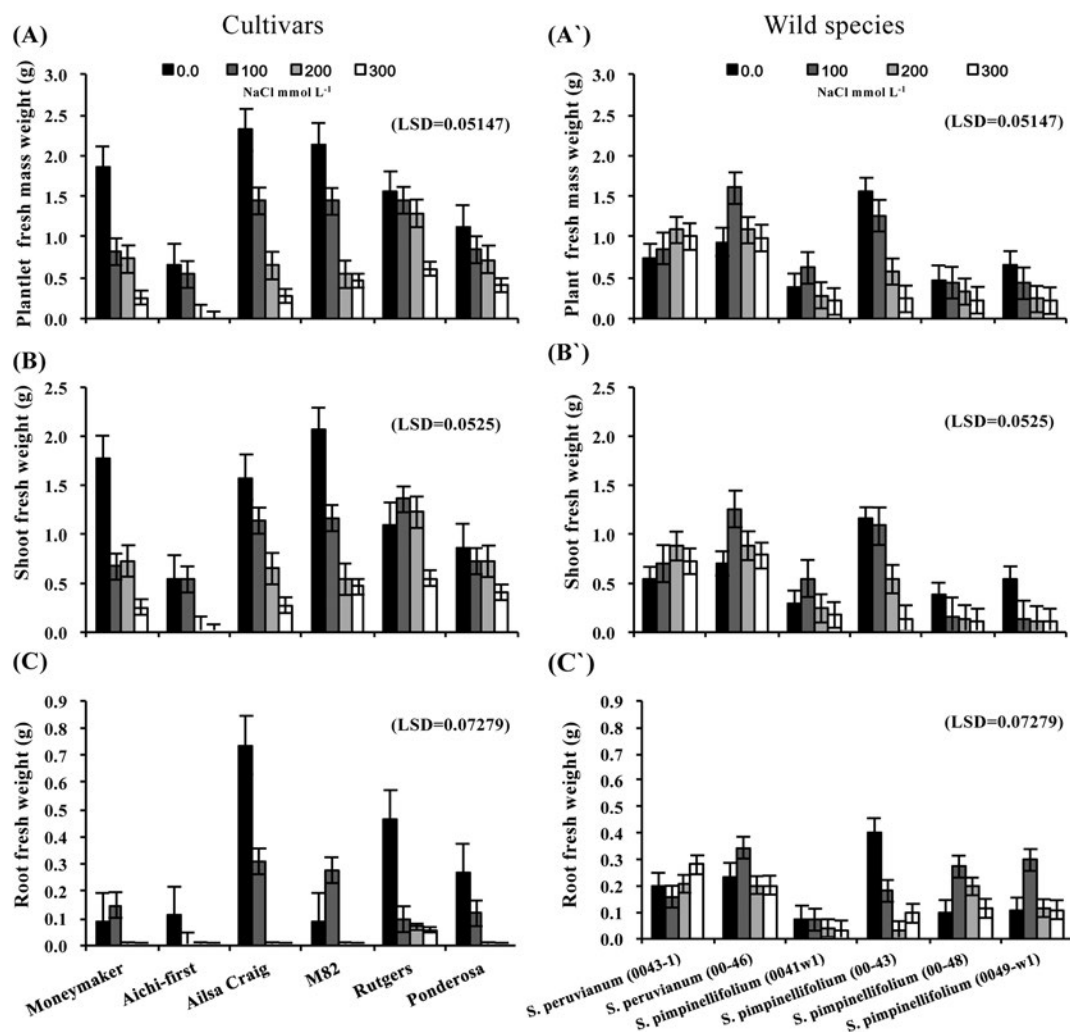


Figure 1. Plantlet fresh weight (A and A'), shoot fresh weight (B and B') and root fresh weight (C and C') of *in vitro*-cultured shoot apices of six tomato *S. lycopersicum* cultivars ('Moneymaker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa') and two accessions of the wild species of *S. peruvianum* (lines 0043-1 and 0046) and four accessions of *S. pimpinellifolium* (0041w1, 0043, 0048 and 0049-w1). Shoot apices were grown on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol L<sup>-1</sup>) and plantlet growth parameters were compared with the control (no NaCl). Values are means  $\pm$  SE, ( $n=10$ ) and the experiment was repeated three times.

by *S. lycopersicum* 'Rutgers' (Figure 1C').

Total root length, root surface area and the average root diameters of tomato cultivars were compared with those of the related *Solanum* wild species (Figure 2). Overall comparisons of the 12 tested genotypes indicated significant differences among cultivated tomato and wild species in these root properties at all tested NaCl concentrations and the no NaCl control. Total root length and mean root area in most of the genotypes were significantly affected by salinity stress. Several of the *S. lycopersicum* cultivars had significantly longer roots than the *S. peruvianum* and *S. pimpinellifolium* accessions at the lowest level of salinity (100 mmol L<sup>-1</sup> NaCl) as shown in (Figure 2A); however, the wild tomato species formed longer roots under high salinity stress than the tomato cultivars. Statistical analysis indicated that *S. peruvianum* (lines 0043-1 and 00-46) had significantly longer roots under high salinity conditions than accessions of the two

other species examined in this study (Figure 2A').

The mean root surface area for most of the examined genotypes differed significantly under high salt levels (Figure 2B and B'). Only *S. lycopersicum* 'Rutgers' had significantly more root area than the other tomato cultivars grown on media containing 300 mmol L<sup>-1</sup> NaCl (Figure 2B). On the other hand, wild tomato species had much less root surface area under salt stress. The root surface area of *S. peruvianum* lines increased with increasing salinity levels, but root surface areas remained lower than the control (Figure 2B'). Overall, the mean root surface area ranged from 0.0 to 2.49 cm<sup>2</sup> among the 12 accessions, an approximately 2.5-fold difference.

In contrast, mean root diameter increased with increasing salt concentration in all tested tomato cultivars and most of the wild species accessions. Interestingly, the two *S. peruvianum* lines formed thinner roots under the highest level of stress used; however, this

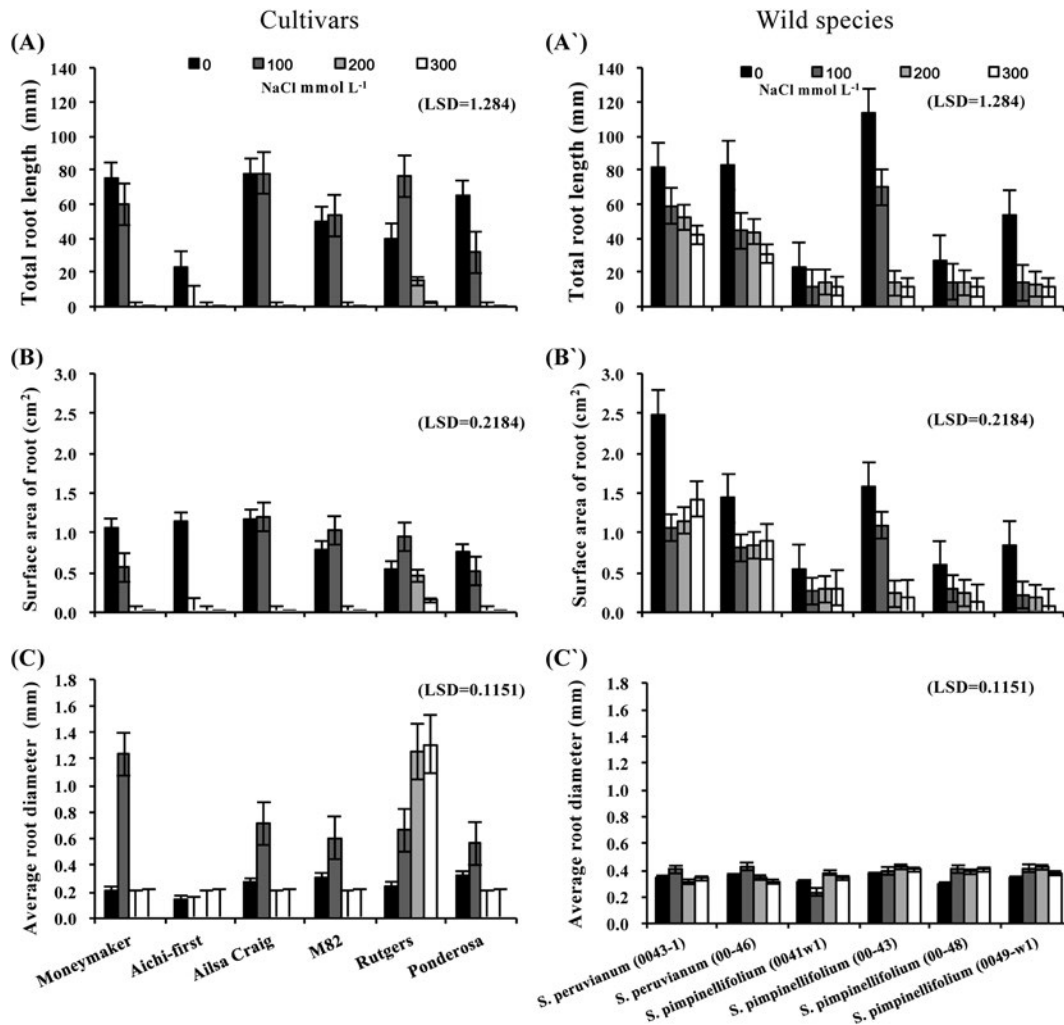


Figure 2. Total root length (A and A'), root surface area (B and B') and average root diameter (C and C') of *in vitro*-cultured shoot apices from six *S. lycopersicum* cultivars ('Moneymaker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa') and two accessions of the wild species of *S. peruvianum* (lines 0043-1 and 0046) and four accessions of *S. pimpinellifolium* (lines 0041w1, 0043, 0048 and 0049-w1). Shoot apices were grown on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol L<sup>-1</sup>) and root growth parameters were compared with the control (no NaCl). Values are means  $\pm$  SE, ( $n=10$ ) and the experiment was repeated three times.

difference in root diameter was not significant (Figure 2C and C').

Salt tolerance, as expressed by the salt tolerance index (STI) is shown in Figure 3. The STIs of root fresh weight (Figure 3A and A'), total root length (Figure 3B and B') and root surface area (Figure 3C and C') revealed a greater tolerance to salt in the wild species accessions compared with tomato cultivars. The STIs decreased with increasing salinity in all examined cultivars. Variations in the STI among cultivars were noticed from low to high salinity; however, the STI decreased with increasing salinity stress. In contrast, wild species accessions were more tolerant than the tomato cultivars under high salt stress conditions (Figure 3A', B' and C'). Two accessions of *S. peruvianum*, lines 0043-1 and 00-46, and one line of *S. pimpinellifolium*, 0041-w1, had better STI values across all saline levels. Two lines of *S. pimpinellifolium*, lines 00-48 and 0049-w1, did not show remarkable changes

across all saline levels, and line 00-43 preferred slightly or moderately saline conditions. Thus, more accessions were rated salt-tolerant by root fresh weight and root surface area STI values (Figure 3A' and C', respectively) than by total root length STI values (Figure 3B').

In general, 'Rutgers' remained the most salt-tolerant cultivar among the *S. lycopersicum* cultivars (Figure 3). 'Rutgers' plantlets under different salinity treatments are shown in Figure 4A. *S. peruvianum* line (0043-1) was identified as the accession with superior root fresh weight STIs when subjected to the highest level of NaCl treatment (Figure 3). Plantlets of line (0043-1) under different salinity treatments are shown in Figure 4B. Several other species accessions were superior at lower levels of NaCl treatment.

#### Effects of salinity on callus growth

The effects of salinity on callus fresh weight, % of

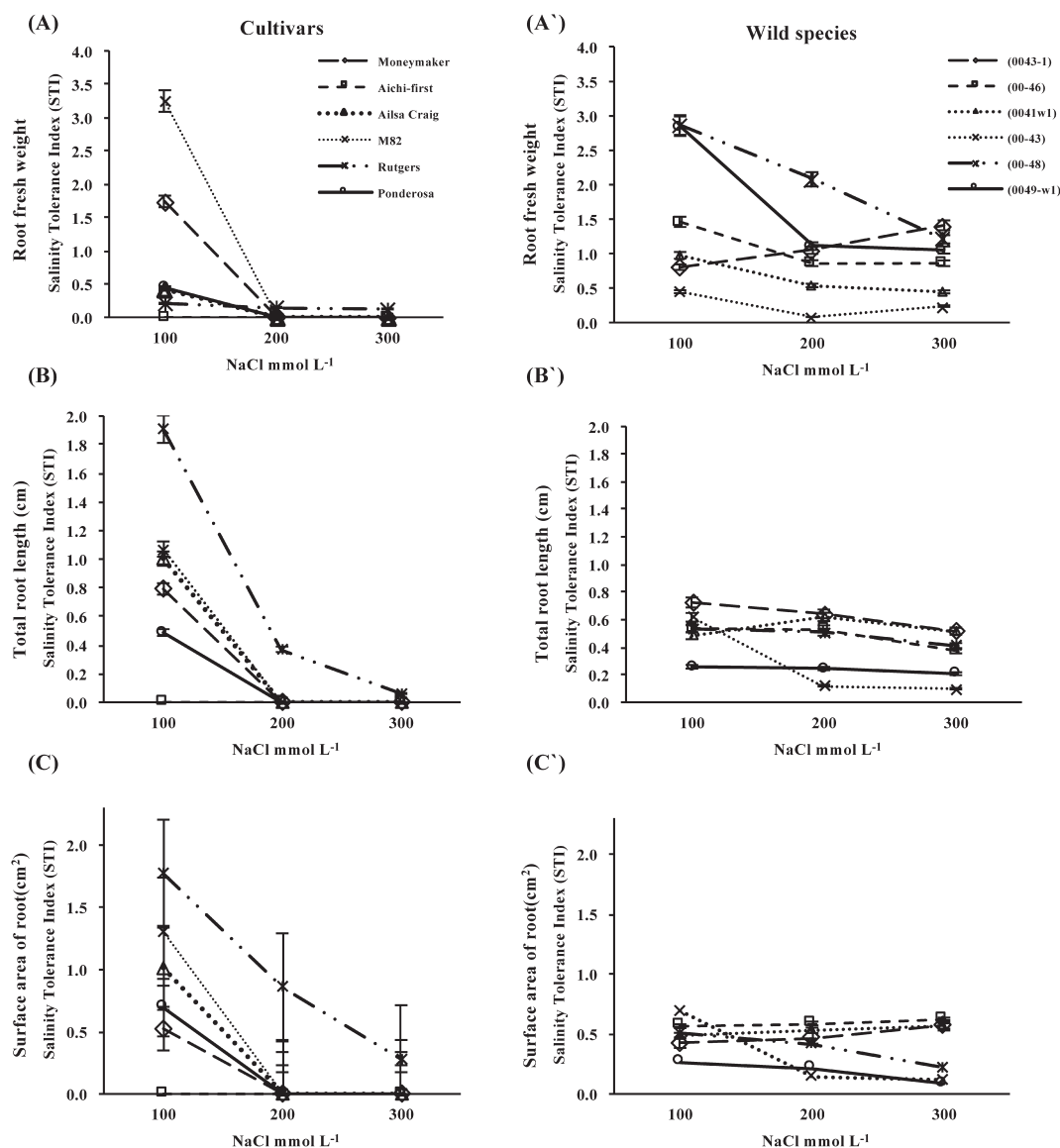


Figure 3. Salt tolerance, as expressed by the salt tolerance index (STI) of root fresh weight (A and A'), total root length (B and B') and root surface area (C and C') of *in vitro*-cultured shoot apices from six *S. lycopersicum* cultivars ('Money maker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa') and two accessions of the wild species of *S. peruvianum* (lines 0043-1 and 0046) and four accessions of *S. pimpinellifolium* (lines 0041w1, 0043, 0048 and 0049-w1). Values are means  $\pm$  SE, ( $n=10$ ) and the experiment was repeated three times.

explants forming callus and % of explants forming roots after four weeks are shown in Figure 5. Callus fresh weight was reduced significantly at every salt level for the *S. lycopersicum* cultivars and *S. pimpinellifolium* lines 0041-w1, 00-43 and 0049-w1, whereas callus growth was stimulated in the *S. peruvianum* lines for the lower levels of salt treatment and only reduced at the highest level of salinity stress (Figure 5A and A'). *S. pimpinellifolium* line 00-48 performed relatively consistently across all saline levels. For the % of explants forming callus, all evaluated species could form callus under different levels of salinity; however, the percentage decreased with increasing salt concentration in all tomato cultivars. Among the cultivars, only 'Rutgers' was consistent in its ability to form callus across all salt stress levels. In

contrast, most of the wild tomato species formed the highest percentages of callus under the different levels of salt stress (Figure 5B and B'). The percentage of callus that developed roots was significantly affected by salinity stress (Figure 5C and C'). The greater effect was observed on cultivars of the *S. lycopersicum*; however, 'Rutgers' had remained to show the considerable ability forming roots. Accessions from the wild species showed great salt tolerance with 58.3–93.0% of all explants forming roots under high salt stress.

Salt tolerance, as expressed by the salt tolerance index (STI) is shown in Figure 6. The STI for callus fresh weight (Figure 6A and A') and % of explants forming callus (Figure 6B and B') decreased with increasing salinity stress. Although reductions in the % of explants forming

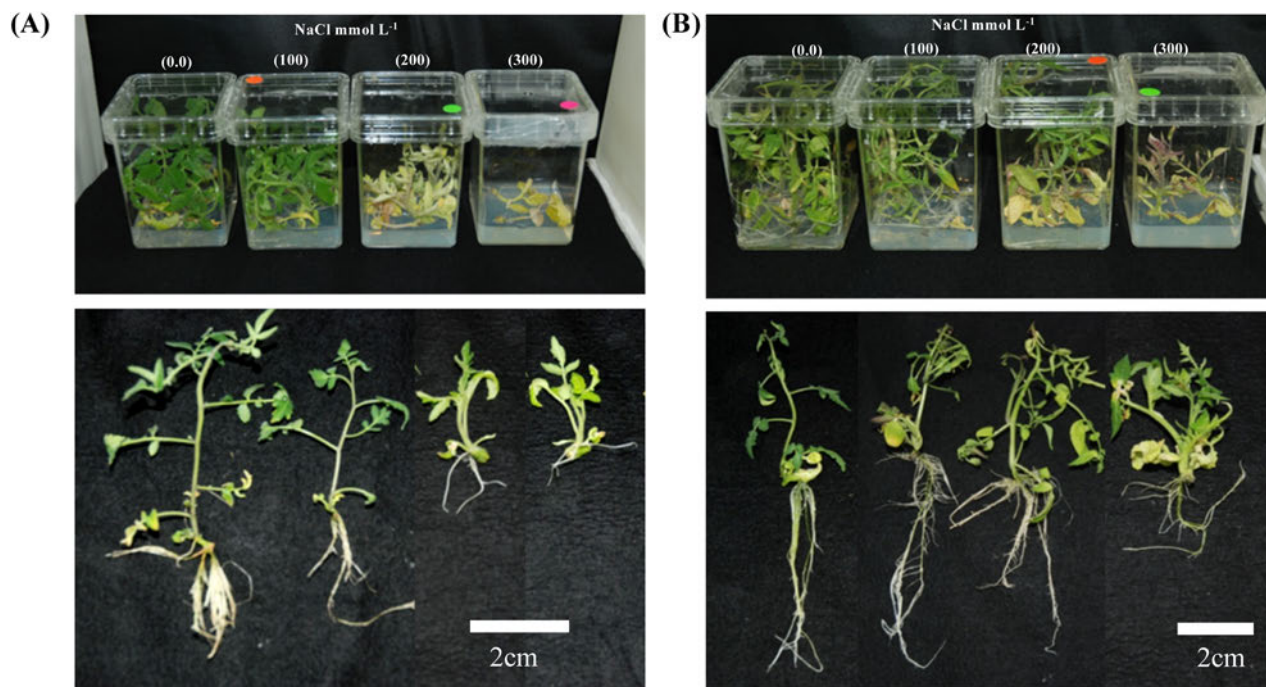


Figure 4. Plantlets from the most tolerant *S. lycopersicum* 'Rutgers' (A) and a wild accession *S. peruvianum* line 0043-1(B). Shoots were cultured on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol L<sup>-1</sup>) and compared with the control (no NaCl).

callus were much greater in tomato cultivars than for the wild species accessions, the trends for the cultivars and accessions did not differ significantly. A significantly higher % of callus formed roots when the callus was initiated from the wild species than the cultivated tomato accessions (Figure 6C and C').

#### Effects of salinity on the ion content of shoots

The Cl<sup>-</sup> and Na<sup>+</sup> concentrations (mmol kg<sup>-1</sup> FW) in shoots increased significantly with exposure to increasing salt levels in all three species (Figure 7). Large variations in shoot Cl<sup>-</sup> (Figure 7A and A') and Na<sup>+</sup> (Figure 7B and B') accumulation by plantlets derived from the wild species were found, although there was a trend toward higher accumulation in the *S. peruvianum* lines compared with those from *S. pimpinellifolium* at the highest salt levels. Accumulation of saline ions was greater in 'Rutgers' than other cultivars, especially at the higher salt levels. At 100 mmol L<sup>-1</sup> NaCl, shoots of *S. lycopersicum* either accumulated similar levels or more of Cl<sup>-</sup> and Na<sup>+</sup> than those of *S. peruvianum* and *S. pimpinellifolium*, whereas the opposite trend was found at the higher salt levels.

Treatment with increasing salt levels resulted in a significant decrease in K<sup>+</sup> in the shoots of all three species, although the K<sup>+</sup> reduction was greater for *S. lycopersicum* than for *S. peruvianum* and *S. pimpinellifolium* (Figure 7C and C'). K<sup>+</sup> was reduced significantly by salt stress in the shoots of *S. lycopersicum* started from the treatment using the lowest concentration of NaCl however, 'Rutgers' did not

show significantly reduction across salinity levels. In all accessions of the wild species, a significant reduction in K<sup>+</sup> was observed in the 100 mmol L<sup>-1</sup> NaCl treatment, although the decrease was not statistically significant at the highest level of salinity stress.

The change in Na<sup>+</sup>/K<sup>+</sup> ratio was calculated from the shoots sodium and potassium concentrations as shown in Figure 8. The greater Na<sup>+</sup>/K<sup>+</sup> ratio was observed on cultivars of the *S. lycopersicum*; however, 'Rutgers' had the lowest ratio under the highest level of salinity (Figure 8A). In wild species; *S. peruvianum* lines exhibited the lowest Na<sup>+</sup>/K<sup>+</sup> ratio compared with those from *S. pimpinellifolium* at the highest salt levels (Figure 8A'). *S. peruvianum* line (0043-1) was identified as the accession with superior tolerance to salt as Na<sup>+</sup>/K<sup>+</sup> ratio was the lowest among all tested genotypes.

#### Discussion and conclusions

Tomato (*Solanum lycopersicum*) is one of the most widely grown and commercially important vegetables throughout the world (Naika et al. 2005). Sources of salt tolerance have been identified among related wild species and primitive cultivars of tomato (Cuartero et al. 1992; Flowers 2004; Jones 1986). Genetic variation in the wild species has already been used to improve salt tolerance in some modern crop cultivars (Zaki et al. 2016). In this work, we determined the tolerance of the tomato cultivars and wild species of *Solanum* under three different levels of salinity stress *in vitro*. The wild species were chosen for their expected high salt tolerance at the

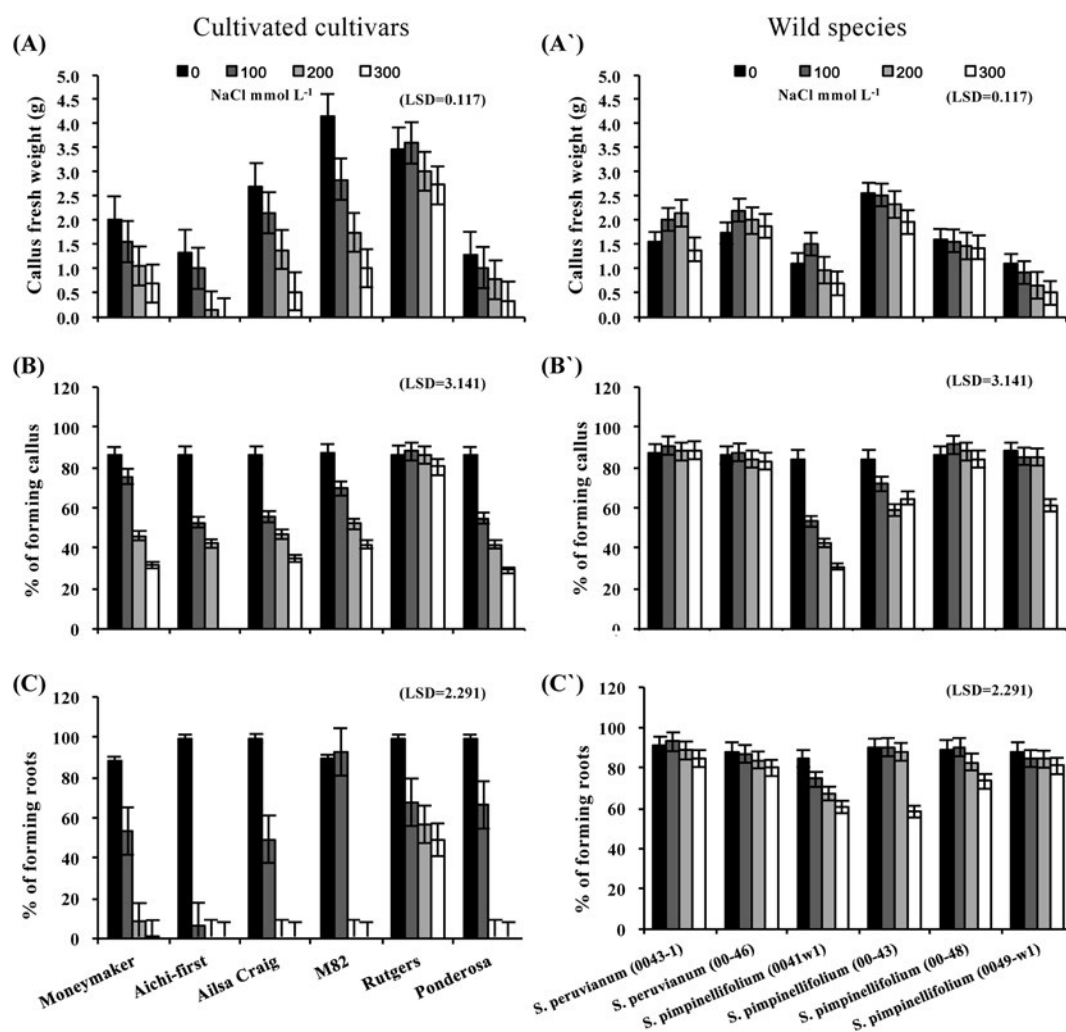


Figure 5. Callus fresh weight (A and A'), % of explants forming callus (B and B') and % of explants forming roots (C and C') of callus cultures initiated from leaves of six *S. lycopersicum* cultivars ('Moneymaker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa') and two accessions of the wild species of *S. peruvianum* (lines 0043-1 and 0046) and four accessions of *S. pimpinellifolium* (lines 0041w1, 0043, 0048 and 0049-w1). Callus growth was initiated on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol L<sup>-1</sup>) and compared with the control (no NaCl). Values are means  $\pm$  SE, ( $n=10$ ) and the experiment was repeated three times.

whole plant level, but the level of tolerance is different among tomato genotypes and/or within genotypes (Bolarin et al. 1991).

*In vitro* culture, besides its use as a tool for obtaining salt tolerant plants, may offer the potential for quick evaluation of germplasm for salt stress tolerance (Tewary et al. 2000; Zaki et al. 2016). In tomato cultivars, *Solanum lycopersicum*, a positive correlation between growth of calli and whole plants in saline conditions has been reported (Perez-Alfocea et al. 1994). Screening methods involving *in vitro* shoot apex culture could provide an efficient protocol for testing and selecting tomato germplasm for salt tolerance (Cano et al. 1996). Callus culture has the advantage that, given the genetic stability of shoots multiplied by apices or auxiliary buds (Hu and Wang 1983), presumably new variability is generated during selection.

In this study, apices and calli were exposed to different

levels of salinity stress *in vitro* ranging from 100 to 300 mmol L<sup>-1</sup> NaCl, and growth was compared to control conditions (no NaCl). Other investigators have reported the use of NaCl for *in vitro* salinity screening in other species of plants (Vijayan et al. 2003; Zhao et al. 2009), but the concentrations used have not been as high as those used in this study. All growth and physiological parameters were significantly affected by salt stress. Root growth is more adversely affected than shoot growth as the salt concentration increases (Cano et al. 1996; Sweby et al. 1994). Similar results were obtained in this study; although both root and shoot growth were inhibited by salt, the effects were more pronounced on root growth, mainly in *S. lycopersicum* rather than wild species. Only, 'Rutgers' developed roots under the highest level of salt stress (Figures 1 and 2). On the other hand, root fresh weight increased with increasing salt concentrations in one accession of *S. peruvianum* (lines 0043-1) and one



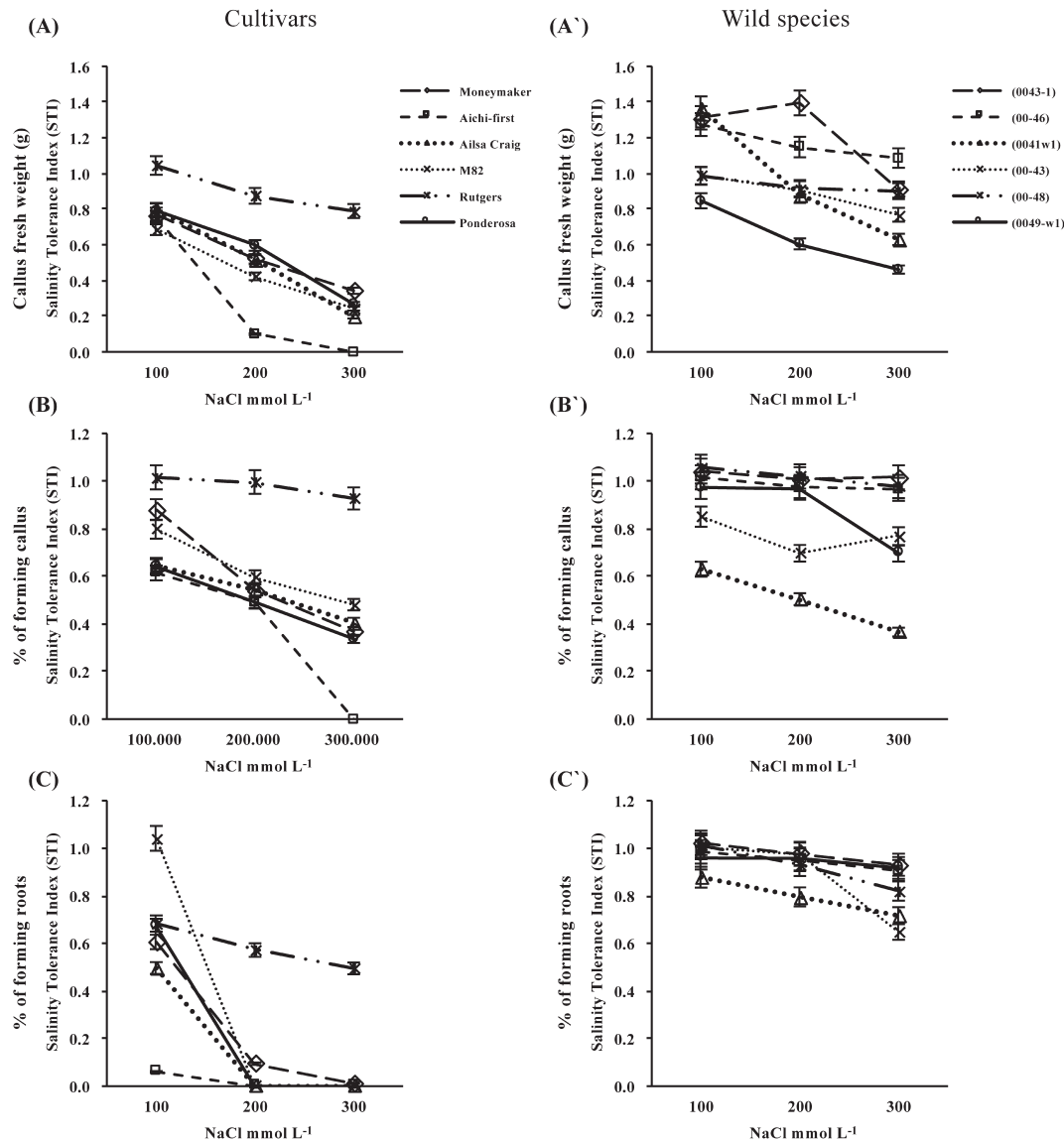


Figure 6. Salt tolerance, as expressed by the salt tolerance index (STI) of callus fresh weight (A and A'), % of explants forming callus (B and B') and % of explants forming roots (C and C') of callus cultures initiated from leaves of six *S. lycopersicum* cultivars ('Moneymaker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa') and two accessions of the wild species of *S. peruvianum* (lines 0043-1 and 0046) and four accessions of *S. pimpinellifolium* (lines 0041w1, 0043, 0048 and 0049-w1). Callus growth was initiated on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol L<sup>-1</sup>) and compared with the control (no NaCl). Values are means  $\pm$  SE, ( $n=10$ ) and the experiment was repeated three times.

accession of *S. pimpinellifolium* (lines 00-48). These results all suggest that there is considerable variation in response to salt stress among species and within species (Figure 1).

A higher salt tolerance index (STI) was previously reported for wild tomato species compared with that for cultivars of the *S. lycopersicum*, both at the whole plant level and in callus culture (Perez-Alfocea et al. 1994). In our study, the higher STI of *S. peruvianum* and *S. pimpinellifolium* compared with that of *S. lycopersicum* was clearly shown by the root fresh weight, total root length and root surface area in the 200 and 300 mmol L<sup>-1</sup> NaCl conditions (Figure 3); however the STI decreased with increasing salinity stress and 'Rutgers' was the most

tolerant cultivar among the cultivars. Two lines of *S. peruvianum* (lines 0043-1 and 00-46) and one line of *S. pimpinellifolium* (0041-w1) grew better across all saline levels (Figure 3). Thus, on the basis of reduced shoot fresh weight with increasing salinity, the salt tolerance of *S. lycopersicum* was lower than that of the other two wild species; although no differences were observed as great as those found for the root growth parameters. Thus, root growth, especially root fresh weight and root surface area are good characteristics than shoot growth for evaluating the salt tolerance of tomato genotypes through *in vitro* shoot apex culture.

Higher salt tolerance has been reported for wild tomato species than for cultivars of the *S. lycopersicum*

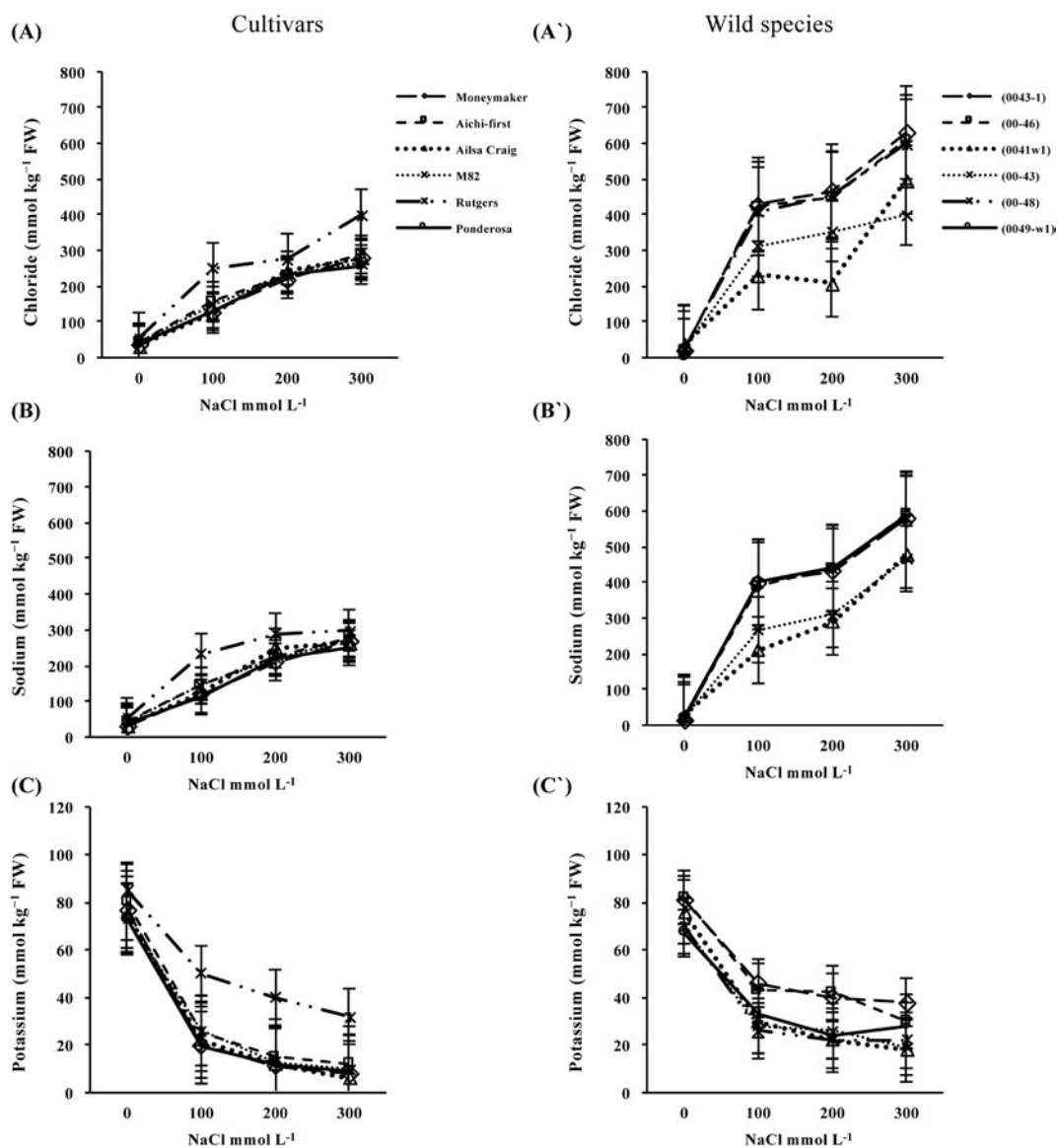


Figure 7. Chloride (A and A'), sodium (B and B') and potassium (C and C') ion concentrations ( $\text{mmol kg}^{-1}$  FW) of shoots from six *S. lycopersicum* cultivars ('Moneymaker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa') and two accessions of the wild species of *S. peruvianum* (lines 0043-1 and 0046) and four accessions of *S. pimpinellifolium* (lines 0041w1, 0043, 0048 and 0049-w1). Plants were grown on media supplemented with three different concentrations of NaCl (100, 200 or  $300 \text{ mmol L}^{-1}$ ) and ion concentrations were compared with those of the control (no NaCl). Values are means  $\pm$  SE, ( $n=10$ ) and the experiment was repeated three times.

in callus culture (Emilio et al. 1998; Perez-Alfocea et al. 1994). The higher salt tolerance of *S. peruvianum* and *S. pimpinellifolium* than that of *S. lycopersicum* was clearly shown by the callus fresh weight, % of explants forming callus and % of explants forming roots in cultures grown from shoot apices after four weeks as shown in Figure 5. The trend for wild species to be more salt tolerant than *S. lycopersicum* is similar to that previously reported by Cano et al. (1996); however, our study used accessions of different wild species.

$\text{Na}^+$  and  $\text{Cl}^-$  accumulations were higher in the wild species than in cultivars of the *S. lycopersicum*. Accumulation of these ions increased with increasing salinity stress in all species (Figure 7). This fact suggests

that the predominant salt tolerance mechanism involves ion accumulation. The interpretation of this data suggests that accessions of wild species were more salt-tolerant, not because they are more capable of restricting  $\text{Cl}^-$  and  $\text{Na}^+$  uptake at high NaCl levels than cultivars, but because they have a superior ability to tolerate high levels of  $\text{Cl}^-$  and  $\text{Na}^+$  in their tissues as reported by Bernstein (1963). It is also plausible that high ion accumulation is a necessary consequence of the ability to survive. Chloride and  $\text{Na}^+$  accumulations in wild species have been reported by other researchers (Dehan and Tal 1978; Phills et al. 1979; Tal and Shannon 1983), suggesting that  $\text{Na}^+$  leaf concentrations could be used as a key characteristic in evaluation of germplasm for salt-tolerance breeding

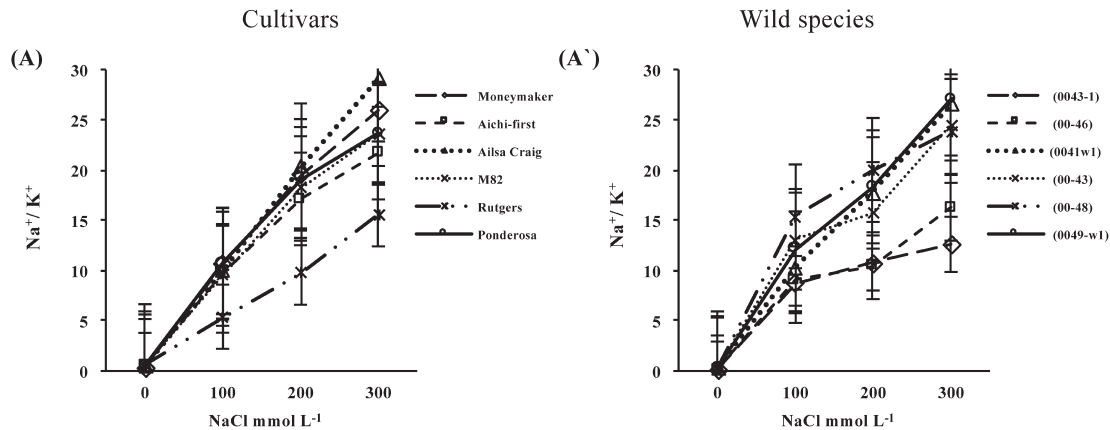


Figure 8. Sodium to potassium ratio over three different concentrations of NaCl (100, 200 or 300 mmol L<sup>-1</sup>) in shoots of (A) six *S. lycopersicum* cultivars 'Moneymaker', 'Aichi-first', 'Ailsa Craig', 'M82', 'Rutgers' and 'Ponderosa' and (A') two accessions of the wild species of *S. peruvianum* (lines 0043-1 and 0046) and four accessions of *S. pimpinellifolium* (lines 0041w1, 0043, 0048 and 0049-w1). Values are means  $\pm$  SE, ( $n=10$ ) and the experiment was repeated three times.

programs of cultivated tomato (Rush and Epstein 1981). On the other hand, there were small, but significant, differences in the K<sup>+</sup> concentrations within shoots treated with the different NaCl levels (Figure 7C and C'). Generally, treatments with higher salt levels resulted in a significant decrease in shoot K<sup>+</sup> levels, although the reduction was greater for *S. lycopersicum* than for *S. peruvianum* and *S. pimpinellifolium*. The ability to maintain potassium uptake in the presence of high concentrations of external sodium may be a desirable trait, as suggested by Yeo and Flowers (1989). Ion transport could be essentially influenced by salt stress in a cell-specific manner. Under salinity the increase in cytoplasmic Na<sup>+</sup> and reduction of K<sup>+</sup> result in changes of membrane potential, osmotic pressure (Munns 2002). Lower Na<sup>+</sup>/K<sup>+</sup> ratio under salt treatment were also found in roots of halophyte compared to glycophyte (Zahran et al. 2007). The lowest Na<sup>+</sup>/K<sup>+</sup> ratio was recorded for *S. peruvianum* accessions (lines 0043-1 and 00-46), followed by *S. lycopersicum* 'Rutgers' under higher salt level (Figure 8), the genotypes that showed the greatest tolerance to salt as judged by other parameters. Meanwhile, there was overall correlation between growth under saline conditions and the Na<sup>+</sup>/K<sup>+</sup> ratio, these genotypes might be valuable germplasm for breeding.

The results obtained in this study suggest that shoot apex and callus culture may be very useful for rapidly screening and evaluating tomato genotypes. Root characteristics, root fresh weight, surface area of root followed by total root length, are good selection criteria for salt tolerance in tomato. Also, the potassium content and Na<sup>+</sup>/K<sup>+</sup> ratio are important traits. These traits are clearly recognizable and might be transferred to *S. lycopersicum* during breeding. *S. peruvianum* line 0043-1 was the accession showing the best salt tolerance overall. 'Rutgers' was the most tolerant cultivar among the tested *S. lycopersicum* cultivars. Both *S. peruvianum* line 0043-

1 and *S. lycopersicum* 'Rutgers' are good candidates for inclusion in breeding programs for salt tolerance.

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