SEED GERMINATION AND EARLY SEEDLING DEVELOPMENT OF TOMATO INTROGRESSION LINES UNDER SALINITY

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Abstract: Salinity affects productivity of many crops including tomato. On tomato, the severity of the stress is more on seedling stage than the growth stages thereafter. As a result, knowledge on the effect of salinity on seed germination and seedling establishment is therefore important to understand salt tolerance in tomato better. Hence, this study was conducted on a set of 76 tomato introgression lines and M82. Twenty seeds from each line were germinated in plates. Tap water containing 3 mM CaCl₂ and 3 mM + 120 mM NaCl were used for control and salinity treatment, respectively. Ten uniformly germinated seedlings were transferred to pots filled with coarse quartz sand. Half-strength Hoagland solution, with or without 120 mM NaCl, was used for treatment and control, respectively, to culture the seedlings for 3 weeks under growth chamber. The result showed that salt stress affected negatively almost all of the growth parameters measured; and the effect varied depending on genotypes. No single line performed best in all parameters measured under all conditions considered. IL6-4 and IL8-3 showed superiority for germination and root growth under salinity. The rest of ILs, described as better performing ones, however, were better only in single parameter: for example IL7-2 and IL7-4-1 had very good germination under salinity but their seedling establishment (shoot and root length) was average. These introgressed segments of S. pennellii accession LA-716 genes in ILs showing tolerance to salinity are good candidate for the improvement programs of tomato for salt tolerance.

Keywords: tomato, Solanum pennellii, introgression lines, salinity, germination, tolerance.

1. INTRODUCTION

Salinity is one of the major challenges in the irrigated agricultural lands of arid and semi arid regions. The main cause of salinity in these regions is secondary salinization brought about by low precipitation, high surface evaporation, weathering of native rocks, clearance of forest, poor cultural practices, and irrigation with saline water (Flowers and Flowers, 2005; Foolad, 2004). Aquifers in the region, which are the main source of irrigation water, have high level of dissolved salt (Mizrahi et al., 1988); NaCl, CaSO₄, MgSO₄, and NaHCO₃ being the most common salts in irrigation water (Grattan, 2002). When these salts get dissolved, it results in above threshold availability of cations and anions for which most cultivated crops are sensitive.

A crop exposed to salinity stress may suffer reduced growth, necrosis and eventual death (Xiong and Zhu, 2002). These changes results because salinity causes osmotic stress and creates ion imbalance, thereby disrupting cellular homeostasis (Munns and Tester, 2008; Flowers et al., 2015; Julkowska and Testerink, 2015). Subsequently failure in the maintenance of turgour pressure, altered mineral distribution, membrane instability, and oxidative stress ensues in the stress episode (Cramer, 2002; Fricke and Peters, 2002). Oxidative stress is actually a secondary effect of salinity caused by stomatal
closure. Stomatal conductance is compromised during osmotic stress leading to photoreduction of excess oxygen, causing the accumulation of reactive oxygen species (Xiong and Zhu, 2002). Excess mount of reactive oxygen species disrupt protein, lipids and nucleic acid of stressed plant cells (Mittler, 2002; Neill et al., 2002).

Cultivated tomato, *Solanum lycopersicum* L., is characterized as moderately sensitive to sensitive for salinity stress (Maas, 1986; Foolad, 1996). The crop is more susceptible to salinity during germination and seedling development stages than during flowering and fruiting stage (Elshourbagy and Ahmed, 1975). Salinity impairs seed water uptake and affects jasmonic acid and abscisic acid biosynthesis (Groot and Karssen, 1992; Kim and Park, 2008), an important phytohormones for endosperm weakening and embryo development, resulting in reduced germination rate and extended time for germination (Katembe et al., 1998; Cuartero and Frenandez-Munoz, 1999; Jamil et al., 2006). Despite this, there existed salt tolerant wild relatives of tomato such as *S. cheesmaniae, S. chmielewskii, S. habrochaites, S. pennellii, S. peruvianum* and *S. pimpinellifolium* (Tal and Shannon, 1983; Hassan et al., 1990; Saranga et al., 1991), with which most cultivated tomato readily crosses, to produce introgression lines (ILs).

Such ILs are very important to understand the genetic bases of salt tolerance. This is because salt tolerance is a quantitative, multigenic trait and the epistatic and environmental interactions result in composite response, making the understanding of tolerance very complex (Xiong and Zhu, 2002). In this regard, interspecific segregating populations and ILs derived from salt-sensitive and salt-tolerant genotypes are helpful (Saranga, et al., 1992; Zamir, 2001; Gur and Zamir, 2004; Lippman et al., 2007). The introgressed segments in an IL can give a better knowledge of its underlying function with regard to the stress. Thus, an IL library, produced from *S. pennellii* accession LA-716 in the background of *S. lycopersicum* (cv. M82), was used to understand salinity stress on germination and seedling development.

### 2. MATERIALS AND METHODS

#### 2.1. Plant Materials and Experimental Setup:

A set of 76 introgression lines along with their parental cultivar, M82, were used. Twenty seeds from each line were germinated in a Petri dish with filter paper, and supplemented with 1.75 mg tap water containing 3 mM CaCl$_2$ and 3 mM + 120 mM NaCl for control and salinity treatment, respectively. The Petri dishes were wrapped with aluminum foil to avoid illumination during germination. Germination data was taken daily after the first 48 hours. Ten uniformly germinated seedlings, having about 5 mm radical length, were transferred from each dish to pots filled with 170 g course quartz sand. Half-strength modified Hoagland solution with or without 120 mM NaCl was used for treatment and control, respectively. Pots were watered every day. Both the Petri dishes and the pots were kept in a growth chamber having relative humidity of 70%, a temperature of 25°C, and 11 hours light. The experiment was replicated 5 times.

#### 2.2. Germination Indices, Growth Parameters, and Data Analysis:

Germination indices were calculated as:

$$G \% = \left(\frac{N_G}{N_T}\right) \times 100$$

Where $G \%$, germination percentage; $N_G$, number of germinated seeds in the treatment; $N_T$, total number of seeds used in the treatment.

$$AS = \left[N_1/N + N_2/2 + N_3/3 \ldots + N_n/n\right]$$

Where AS, speed of accumulated; $N_1, N_2, N_3, N_n$: Cumulative number of seeds which germinate on time 1, 2, 3, ..., n.

$$CGR = \frac{((N_1 + N_2 + N_3 + \ldots + N_n) / ((N_1 \times T_1) + (N_2 \times T_2) + (N_3 \times T_3) + \ldots (N_n \times T_n))) \times 100}{T}$$

Where CGR, coefficient of the rate of germination; $N_1$: number of germinated seeds at time $T_1$; $N_2$: number of germinated seeds at time $T_2$; $N_n$: number of germinated seeds at time $T_n$.

$T =$ Time to first observed germinant

Seedlings were uprooted 3 weeks after being transferred to pots and shoot and root length measurements were taken using ruler with mm scale.

Percentage performance under stress condition was computed as the percentage of individual’s performance to that of M82 under salinity. Student’s *t*-test was used to compare treatment means between the parental line and ILs. For germination percentage, arcsine transformation was employed to stabilize variance.
3. RESULTS

3.1. Effect of Salinity on Germination Indices:

Salt stress the seeds were subjected affected germination indices measured: total germination, time required to germinate, and rate of germination both in cultivar M82 and the 76 ILs (Figure 1). Generally, there observed up to 60 % reduction in total germination (G) and about 3 days delay in time to first observed germinant (T) due to salinity. Similar negative effect was also noticed on rate of germination, i.e. speed of accumulated germination (AS) and coefficient of the rate of germination (CRG). Six times slower speed of germination per day was caused when seeds were subjected to water containing 120 mM NaCl as compared to when they were incubated with tap water alone, 0 mM NaCl.

![Figure 1: Effect of salinity on germination and allied parameters on tomato cv. M82 and its introgression lines, ILs. G: accumulated germination percentage; T: time to first observed germinant; AS: speed of accumulated germination; and CRG: coefficient of the rate of germination. Indices value for ILs is mean of the 76 ILs.](image)

3.2. Germination Rate of Introgression Lines and Salinity:

Considering the performance of individual ILs and their parental line cv. M82, at control condition, the germination percentage of 21 ILs was significantly lower than M82 (Figure 2); IL12-3 being with the lowest germination percentage, 64 %, followed by the 3 introgressions of the third chromosome, IL3-2, IL3-3, and IL3-4 (66, 69 and 69 % in respective order). In the case of saline water treatment, 30 ILs germinated significantly lower than M82, with germination percentage ranging from 2 % (IL9-3-2) and 20 % (IL6-2-2). Thirteen of these ILs, i.e. IL1-3, IL3-2, IL3-3, IL3-4, IL6-2, IL6-3, IL7-4, IL9-3, IL9-3-1, IL9-3-2, IL11-1, and IL11-3, had also a significantly lower germination under control condition. What is interesting to notice here is that ILs such as IL1-1-3, IL2-3, IL5-4, IL5-5, IL7-5-5, IL10-1, IL10-1-1, IL10-2-2, IL10-3, and IL11-4, which showed a shocking germination under salinity, had germinated more than 95 % under control condition, implying these introgressions made M82 more sensitive to salinity (Figure 2).

On the other hand 9 ILs: IL1-4 (87 %), IL9-1-3 (86 %), IL6-4 (85 %), IL7-4-1 (81 %), IL7-2 (79 %), IL8-3 (63 %), IL4-4 (63 %), IL9-2-5 (62 %), and IL8-1-3 (61 %) exhibited a significantly higher germination than M82 (43 %); and their germination under non-saline condition was above 90 % (Figure 2).

As shown in Supplemental Table 1, there existed an intrinsic variability in time required for germination and rate of germination among ILs themselves and between ILs and M82 as well. Up to 2 days lag behind M82 was observed in 14 ILs under control condition; nevertheless, there was no single IL germinated significantly quicker than M82 (Table 1). In saline condition, however, IL7-4-1 and IL9-1-3 were quicker while IL2-6, IL7-4, and IL7-5-5 were slower than their parent to initiate germination (Table 1). Similarly, genotype and salinity stress affected rate of germination (Table 1 and Supplemental Table 1). As expected, those ILs that took longer time to initiate germination scored poor rate of germination (Table 1).
Figure 2: Germination performance of tomato cv. M82 and its 76 introgression lines. ctrl, 0 mM NaCl; trt, 120 mM NaCl. Red solid line and broken line show the performance of M82 at Ctrl and Trt conditions, respectively. Error bar represents the standard error of the mean of five replicates. Asterisks, *, ** & *** are significant levels where α is equal to 0.05, 0.01 & 0.001, respectively, between individual IL and M82, using unpaired heteroscedastic Student’s t-test.
Table 1: Rate of germination of selected ILs that showed high differences compared to M82 (Full list is provided in Supplemental Table 1)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Time to first observed germinant (T)</th>
<th>Speed of germination (AS)</th>
<th>Accumulated germination</th>
<th>Coefficient of the rate of germination (CRG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mM NaCl</td>
<td>120 mM NaCl</td>
<td>0 mM NaCl</td>
<td>120 mM NaCl</td>
</tr>
<tr>
<td>M82</td>
<td>3.2 (± 0.20)</td>
<td>6.4 (± 0.68)</td>
<td>14 (± 1.6)</td>
<td>2.7 (± 0.5)</td>
</tr>
<tr>
<td>IL1-1-3</td>
<td>4.7 (± 0.33)*</td>
<td>7.5 (± 1.26)</td>
<td>6 (± 2.2)</td>
<td>0.6 (± 0.5)</td>
</tr>
<tr>
<td>IL1-3</td>
<td>4.7 (± 0.33)*</td>
<td>6.8 (± 1.03)</td>
<td>7 (± 0.6)</td>
<td>0.7 (± 0.2)</td>
</tr>
<tr>
<td>IL1-4</td>
<td>3.2 (± 0.20)</td>
<td>5.0 (± 0.55)</td>
<td>17 (± 2.1)</td>
<td>7.3 (± 1.0)</td>
</tr>
<tr>
<td>IL2-5</td>
<td>4.7 (± 0.33)*</td>
<td>6.7 (± 0.88)</td>
<td>6 (± 0.6)</td>
<td>0.2 (± 0.1)</td>
</tr>
<tr>
<td>IL2-6</td>
<td>3.6 (± 0.40)</td>
<td>8.5 (± 0.65)*</td>
<td>9 (± 0.9)</td>
<td>1.0 (± 0.4)</td>
</tr>
<tr>
<td>IL3-3</td>
<td>5.0 (± 1.00)*</td>
<td>5.3 (± 0.33)</td>
<td>5 (± 0.5)</td>
<td>0.2 (± 0.1)</td>
</tr>
<tr>
<td>IL3-4</td>
<td>5.0 (± 0.58)*</td>
<td>7.3 (± 0.33)</td>
<td>5 (± 0.4)</td>
<td>0.3 (± 0.2)</td>
</tr>
<tr>
<td>IL4-3</td>
<td>3.8 (± 0.58)</td>
<td>5.2 (± 0.73)</td>
<td>9 (± 0.8)</td>
<td>5.0 (± 2.4)</td>
</tr>
<tr>
<td>IL5-4</td>
<td>4.0 (± 0.63)*</td>
<td>7.2 (± 0.80)</td>
<td>11 (± 1.3)</td>
<td>0.8 (± 0.4)</td>
</tr>
<tr>
<td>IL5-5</td>
<td>4.7 (± 0.33)*</td>
<td>7.3 (± 1.31)</td>
<td>8 (± 2.3)</td>
<td>1.4 (± 0.8)</td>
</tr>
<tr>
<td>IL6-2</td>
<td>4.7 (± 0.33)*</td>
<td>5.7 (± 0.67)</td>
<td>6 (± 1.4)</td>
<td>0.2 (± 0.1)</td>
</tr>
<tr>
<td>IL6-3</td>
<td>4.7 (± 0.88)*</td>
<td>6.7 (± 1.20)</td>
<td>7 (± 1.8)</td>
<td>0.6 (± 0.3)</td>
</tr>
<tr>
<td>IL7-4</td>
<td>3.3 (± 0.33)</td>
<td>8.4 (± 0.68)*</td>
<td>13 (± 1.6)</td>
<td>0.6 (± 0.2)</td>
</tr>
<tr>
<td>IL7-4-1</td>
<td>3.0 (± 0.00)</td>
<td>4.6 (± 0.68)*</td>
<td>15 (± 1.8)</td>
<td>8.1 (± 1.1)</td>
</tr>
<tr>
<td>IL7-5-5</td>
<td>5.0 (± 1.15)*</td>
<td>8.3 (± 0.88)*</td>
<td>8 (± 1.0)</td>
<td>0.1 (± 0.0)</td>
</tr>
<tr>
<td>IL8-1-1</td>
<td>4.0 (± 0.63)*</td>
<td>6.6 (± 0.24)</td>
<td>10 (± 1.9)</td>
<td>1.6 (± 0.3)</td>
</tr>
<tr>
<td>IL9-1-3</td>
<td>3.2 (± 0.20)</td>
<td>4.6 (± 0.68)*</td>
<td>15 (± 1.1)</td>
<td>9.1 (± 1.9)</td>
</tr>
<tr>
<td>IL9-2-5</td>
<td>3.2 (± 0.20)</td>
<td>5.2 (± 0.80)</td>
<td>10 (± 1.7)</td>
<td>5.5 (± 1.8)</td>
</tr>
<tr>
<td>IL9-3-2</td>
<td>3.3 (± 0.33)</td>
<td>7.0 (± 1.00)</td>
<td>11 (± 0.2)</td>
<td>0.1 (± 0.0)</td>
</tr>
<tr>
<td>IL10-1</td>
<td>4.7 (± 0.88)*</td>
<td>5.4 (± 0.68)</td>
<td>7 (± 1.0)</td>
<td>1.4 (± 0.6)</td>
</tr>
<tr>
<td>IL10-2-2</td>
<td>4.7 (± 0.33)*</td>
<td>7.0 (± 1.00)</td>
<td>8 (± 0.6)</td>
<td>0.4 (± 0.2)</td>
</tr>
<tr>
<td>IL12-2</td>
<td>4.0 (± 0.55)*</td>
<td>6.8 (± 0.97)</td>
<td>9 (± 1.7)</td>
<td>2.1 (± 0.8)</td>
</tr>
</tbody>
</table>

Value in parenthesis shows standard error. * is significant level at $\alpha \leq 0.05$ between individual IL and M82, using unpaired heteroscedastic Student’s t-test.

3.3. Early Seedling Growth of Introgression Lines under Salinity Stress:

Considering the growth of individual genotypes at control condition, shoot growth was affected much than root growths by the salinity stress (Figure 3). Surprising enough that the salinity stress reduced shoot length of ILs by about half, compared to non-saline condition, despite the fact that they got introgressions from salt resistant parent (Figure 3). Unlike to shoot growth, the response of genotypes in root growth for the salinity stress varied greatly. The root growth of IL2-1-1 and IL4-1-1 was severely affected, whereas those of IL2-6, IL6-4, IL7-3, IL8-1-3, IL8-3, IL9-2-6, and IL12-1-1 were affected least or not.

Taking the performance of M82 as a bench mark, under control condition, no much difference in shoot length was observed was observed between M82 and the ILs, only up to 10 % differences (Figure 3). Under saline condition, the shoot lengths of IL2-1-1, IL4-1, IL8-1-3, IL12-2, and IL12-3-1 were, however, up to 20 % shorter, and that of ILB-3-1 15 % longer than M82. The trend of root growth under control condition was similar to that of shoot growth, where most of the ILs’ root length was not much different to that of M82, except for IL4-4, where its root grew 42 % longer than M82 (Figure 3). Nonetheless, compared to M82, there existed high variability under salinity in the potential of ILs for root growth; IL2-1-1 and IL12-1-1 being with shortest and longest root length under salinity, respectively.
A set of 76 lines from the IL library of *S. pennellii* accession LA-716 in background of *S. lycopersicum* (cv. M82) were used along with M82 to investigate the effect of salinity (120 mM NaCl) on germination and early seedling growth. Generally, salinity negatively affected germination and seedling growth (Figure 1-3; and Supplemental Table 1). Reduced and delayed germination, and high hypocotyl mortality rate due to salinity stress was observed. Similar observations were made in several vegetable crops including tomato (Miyamoto et al., 1985; Jamil et al., 2006; Kaymaknova, 2009). The main reasons are: salinity affects seed water imbibition and water uptake (Cuartero and Fernandez-Munoz, 1999); it disturbs metabolic processes leading to increase phenolic compounds that affect respiratory enzymes during germination (Muscolo et al., 2001); and creates ion imbalance of certain mineral nutrients to cause toxicity at early stage (Jamil et al., 2006). In addition, the displacement of Ca$^{2+}$ bound to the external surface of the cell membrane by metal cations, due to the salt toxicity effect, may impair the integrity and permeability of the membrane to complicate germination (Kent and Läuchli, 1985; Lynch et al., 1987).

Despite the negative effects of salinity stress, certain ILs were superior to M82 in germination and early seedling growth. Very high germination was observed in IL1-4 (87 %), IL9-1-3 (86 %), IL6-4 (85 %), and IL7-4-1 (81 %), as compared to M82. The introgressed regions of the ILs cover more or less the loci which were identified for better seed germination under salinity in various segregating population of *S. pennellii* accession LA-716 (Foolad, 2004). Interestingly, these better-germinated ILs also required shorter time to germinate and also had good germination speed than M82. Genotypes which do not take longer period to germinate under salinity are advantageous since they can escape the crust formation on soil surface, a common phenomenon occurring due to saline water irrigation, which affects seedling emergence.

Introgressions in the 3$^{rd}$ chromosome were all inferior in germination: IL3-1, IL3-2, IL3-3, and IL3-4 had poor germination under non-saline condition while IL3-5 under salinity. On the other hand, introgressions in the 5$^{th}$ chromosome germinated more than 95 % under control but performed very poor under salinity, implying the introgressions may not involve in germination under stress condition *S. pennellii* accession LA-716.
Salinity generally slows tomato seedling growth. Shoot and root lengths were less under salinity than normal condition. These growth parameters provide important clue to the responses of genotypes to salt stress. About 50% and 15% reduction in shoot and root length, respectively, was caused by salinity stress to most of the ILs; implying that the salinity effect was more on shoot than root, despite the fact that the root system was directly exposed to the saline environment. Similar result was obtained by Snapp and Shennan (1992) on hydroponically-grown tomato seedlings. The inhibition of shoot and root growth can be due to the effects of Na⁺ and Cl⁻ ions on cell turgor pressure and membrane fluidity and selectivity (Munns, 2002). Contrary to this, IL6-4, IL8-3, IL8-3-1, and IL12-1-1 had higher shoot or root growth under salinity stress than M82; and the opposite happened for IL2-1-1 and IL12-3-1. It is expected that tolerance to salinity can be achieved in more than one way: some lines express their tolerance by reducing shoot growth while maintaining root mass or the other way round. Other lines may exhibit tolerance as the ability to continue shoot and root growth despite salinity stress. At seedling stage, however, tolerance to salinity stress is also associated with reduced number of stomata, thickened leaf cuticle, and higher water soluble antioxidant activity, to absorb solution of low osmotic potential (Munns, 2002; Frary et al., 2010). The availability of sufficient water in plant system, during stress, helps plant to avoid the accumulation of Na⁺ and Cl⁻ to toxic level, via compartmentalizing into vacuoles (Cuartero et al., 2006; Shabala, 2013). For the question which mechanisms these ILs use to tolerate salt stress remains for future work.

In conclusion, with few exceptions, ILs response varied for the various salt-tolerance parameters measured. IL6-4 and IL8-3 showed superiority for germination and root growth under salinity. Unlike to these two ILs, the rest of ILs, described as better performing ones were better only in single parameter: for example IL7-2 and IL7-4-1 had very good germination under salinity but their seedling establishment (shoot and root length) was average. This might suggest that pooling the introgressed segments with different potential in one genotype theoretically would help understanding salinity tolerance during germination and early seedling establishment better. Since tomato is more sensitive to salinity stress at seedling stage, genotypes performing well in the earlier establishment stage under salinity could perform well in the later growth stages, too. As a result, the introgressed segments of S. pennellii accession LA-716 genes in ILs showing tolerance to salinity are good candidate for the improvement program of tomato for salt tolerance.

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