Heritability and gene effects for salinity tolerance in cucumber (Cucumis sativus L.) estimated by generation mean analysis

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1. Introduction

Salinity is one of the most important abiotic factors limiting growth and productivity of crops worldwide. About 7% (830 million ha) of the total global land is salt affected (FAO, 2009). It is estimated that 1/3 of world food production is obtained from irrigated agriculture unfortunately 20% (45 million ha) of the irrigated area is salt affected resulting in reduced yields (Negrão et al., 2011). The ever increasing world population and decreasing arable land due to factors such as land degradation poses a serious threat to food security (Davies et al., 2009).

Efforts to grow crops in salt affected soils as well as irrigating with saline waters have been employed through expensive technical reclamation methods (Davies et al., 2009). Other researches have shown that cucumber grafted on salt tolerant rootstocks had improved growth and yield (El-Shraiy et al., 2011). However, the main limitation of this approach is high labor requirement and bulkiness. The most feasible alternative to growing crops under salinity prone environments is through genetic improvement (Tiwari et al., 2011). Unfortunately, salt tolerant cucumber is yet to be developed. Recently, Munns et al. (2012) developed tolerant wheat through conventional breeding. This provides hope for genetic improvement provides hope of salt tolerant cucumber if accurate selection and screening method is identified. Moreover, seeds of salt tolerant crop cultivars could be produced through conventional breeding can be readily availed to farmers unlike the transgenic materials, which suffer stringent legal restrictions.

Cucumber is a popular fruit vegetable consumed worldwide and it has moderate sensitivity to salinity meaning that growth and productivity is restricted by high saline conditions (Mather and Jink, 1982). Genotypic variation salt tolerance in cucumber has already been confirmed (Malik et al., 2010; Tiwari et al., 2011). However, the genetics of salt tolerance in cucumber is poorly understood (Tiwari et al., 2011) due to the complexity of salt tolerance (Maas and Poss, 1989; Munns and Tester, 2008). Therefore it is necessary to investigate viable selection traits that can predict salinity tolerance of cucumber at the seedling stage. The

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potential for genetic improvement of salt tolerance in cucumber is feasible if the gene action of superior parents is fully understood and a suitable breeding program selected (Dashki et al., 2012).

Several screening criteria for salt tolerance have been proposed for glycophytes. Na+ exclusion is widely accepted as an efficient salt selection criterion for cereals (Munns et al., 2012; Munns and Tester, 2008). However, the utilization of this trait in routine breeding is limited. Munns and Tester (2008) pointed out that the Na+ exclusion by the roots is only feasible under mild salinity for a short time. Malik et al. (2010) reported lower leaf Na+ content in salt tolerant cucumber. In the contrary, the results of our previous study showed that both salt-tolerant cucumber genotypes, 11411S (salt tolerant) and salt-sensitive, 11439S did not have significant differences in shoot Na+ under salinity (Supplementary Table S1). The conflicting results might be explained by differences in stress period and screening system and method of inducing stress. A parallel study comparing salt stress and salt induced osmotic shock showed differential gene expression profile (Shavrukov, 2012). This underscores the importance of inducing salinity stress to assess salt tolerance in plants. In the present study, we applied NaCl gradually to simulate natural osmotic stress experienced under soil conditions.

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Salinity can be simply be assessed by simple measurements of growth parameters under stressed condition especially when using large number of germplasm (Tavakkoli et al., 2012). Stunting, reduced emergence and expansion of new leaves are generally accepted as adaptive mechanisms of plants exposed to osmotic stress due to salinity (Vaario et al., 2011). However, development of simple, replicable and reliable criteria for screening cucumber under salinity has remained elusive. Leaf and other growth parameters may serve as useful selection criterion for salt tolerance in cucumber at seedling stage if the inheritance pattern is known.

Generation mean analysis is a powerful tool that has been used to study various quantitative traits in crops such as yield (Rashwan, 2010), sucrose content (Tchiagam et al., 2011), sugar content (Audilakshmi et al., 2010), drought tolerance (Golparvar, 2011; Naveed et al., 2009) and aluminum tolerance (Spehar and Galway, 1995), salt tolerance (Dashki et al., 2012; Samineni et al., 2011) and waterlogging (Yeboah et al., 2008) in various crops. How-
were estimated by weighted least square method using reciprocals of the variance of respective mean weight (Kearsey and Pooni, 1998). The estimates of dominance ratio, narrow sense heritability, heterosis (mid-parent), heterosis (better parent) and potence ratio were calculated using the following equation (Kearsey and Pooni, 1998):

Environmental variance, \( V_E = \frac{1}{4} \left( s^2 P_1 + s^2 P_2 + 2 s^2 F_1 \right) \); 

Additive variance, \( V_A = \left[ s^2 F_2 - (s^2 B_1 + s^2 B_2) \right] \); 

Variance of dominance in \( F_2 \), \( V_D = \left[ (s^2 B_1 + s^2 B_2) - s^2 F_2 - V_E \right] \); 

Mid-parent heterosis \( (H\%) = \left[ \frac{F_1 - MP}{MP} \right] \times 100 \); 

Heterobeltiosis \( (HB\%) = \left[ \frac{F_1 - BP}{BP} \right] \times 100 \), 

where \( s^2 \), MP and BP represent the variance, mid-parent and better parent means, respectively.

Dominance ratio = \( \left( \frac{4V_D}{2V_A} \right)^{1/2} \)

Narrow sense heritability, \( h^2_E = \frac{2s^2F_2 - (s^2B_1 + s^2B_2)}{s^2F_2} \)

Phenotypic correlations were estimated in \( F_2 \) individuals using MINITAB statistical software. Genotypic correlation \( (r_g) \) between two traits \((X, Y)\) was estimated using the formula (Ehdaie et al., 2001):

\[ r_g = \frac{COVg(X, Y)}{[(Vg(X) \cdot Vg(Y)]^{1/2}} \]

where \( COVg = (X, Y)F_2 - (1/4)[COV(X, Y)P_1 + COV(X, Y)P_2 + 2COV(X, Y)F_1] \)

COVg, \((X, Y)F_2\), COV\((X, Y)P_1\), COV\((X, Y)P_2\), COV\((X, Y)F_1\) are covariances of traits \(X\) and \(Y\) with genetic and non-allelic effects and \(Vg(X)\) and \(Vg(Y)\) are genetic variances of \(X\) and \(Y\), respectively.

### 3. Results

The mean values for TOL and RLN14 in \( F_1 \) was close to the average of the two parental lines while the means for VL and LA in \( F_1 \) was greater than in the mean values of the two parents (Table 1). The mean values for all the traits in \( F_2 \) were lower than \( P_1 \) but similar to \( P_2 \) except for RLN14 (Table 1). Although \( P_1 \) and \( B_1 \) had significantly higher TOL than \( P_2 \) and \( F_2 \), the TOL values were not significantly different among \( P_1 \), \( F_1 \) and \( B_1 \) at 80 mM NaCl (Table 1). At 80 mM NaCl, the RLN14 for \( P_1 \) and \( P_2 \) were highest and lowest \((P<0.05\), respectively (Table 1)). Although, there were no significant differences between the parental lines with respect to LA and VL, \( F_1 \) individuals exhibited the highest values for the two traits (Table 1). For LA, \( B_1 \) had higher mean values than the salt tolerant parent, whereas no differences were observed between \( P_1 \), \( P_2 \) and \( B_1 \) with regards to VL (Table 1). The \( B_1 \) mean performance was significantly higher than \( B_2 \) for all the traits except TOL (Table 1). Mean performance of \( F_2 \) individuals was lower than \( F_1 \) progeny for all traits except TOL indicating inbreeding depression. The \( F_2 \) individuals showed wide transgressive segregation in all traits studied (Fig. 1).

The A, B, C scaling test was non-significant for all the traits (data not presented). However, all the traits had significant \( X^2 \) estimates for 3-parameter model under saline conditions indicating that the inadequacy of additive-dominance model (Table 2). Component ‘\( m’ \) was significant for all the traits (Table 2) indicating that they are inherited quantitatively. Additive gene effect was predominant for TOL and RLN14, whereas LA and VL were predominantly influenced by dominance \( [h] \) and additive \( x \) additive \( [i] \) epistatic gene effects (Table 2). For TOL and RLN14 and VL, the best model was \( m, [d], [h] \) and \( [i] \), whereas the best model fit for LA was \( m, [d], [b], [i] \) and \( [j] \) (Table 2).

Estimates of additive, dominance, environmental components of variance, dominance ratio, mid-parent heterosis, better parent heterosis, potence ratio and narrow sense heritability are shown in Table 3. Dominance ratio for all the traits was lower than 1 except for RLN14. TOL and RLN14 had very low mid-parent heterosis, whereas LA and VL showed higher mid-parent heterosis (Table 3). The better parent heterosis was negative for both RLN14 and TOL, whereas the values for VL and LA were high and positive (Table 3). For TOL and RLN14, narrow sense heritability was moderate (0.57) and low (0.25), respectively, while LA and VL had high narrow sense heritability (0.66 and 0.74 for LA and VL, respectively).

Relationships of TOL, RLN14, LA and VL were estimated in the \( F_2 \) population under salinity stress. A moderately significant positive phenotypic correlation \((0.58, R^2 = 0.31, P = 0.001) \) was observed between RLN14 and TOL while a weak significant negative phenotypic correlation \((-0.39, R^2 = 0.12, P = 0.03) \) was observed between TOL and VL (Table 4). Similarly a highly significant negative phenotypic correlation \((-0.54, R^2 = 0.28, P = 0.002) \) was recorded between RLN14 and VL. There was no correlation between LA and other traits in cucumber subjected to salinity stress. Genotypic correlation between TOL and RLN14 was positive while it was negative for TOL versus LA or VL (Table 4). Genotypic correlation of RLN14 with other traits also followed a similar trend while VL and LA had positive genotypic relationship (Table 4).

### 4. Discussion

The findings of this study reveal that the salt tolerant parent as well tolerant segregants had higher relative leaf number than the sensitive parent at 80 mM NaCl. Our findings agree with the report
Fig. 1. Frequency distribution of TOL (A), RLN14, LA (C) and VL of F2 individuals from a salt tolerant, 11411S (P1) crossed with salt sensitive, 11439S (P2) cucumber genotypes under 80 mM NaCl at seedling stage. The arrows indicate relative positions parental means.

Table 2
Estimates of genetic components based on generation means in a cucumber cross, 11411S × 11439S.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Parameters</th>
<th>m</th>
<th>[d]</th>
<th>[h]</th>
<th>[i]</th>
<th>[j]</th>
<th>[l]</th>
<th>X²(df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOL</td>
<td>2.78 ± 0.39&quot;</td>
<td>0.56 ± 0.14&quot;</td>
<td>0.52 ± 0.54</td>
<td>0.32 ± 0.43</td>
<td>0.64 ± 0.51</td>
<td>–</td>
<td>2.20 (1)</td>
<td></td>
</tr>
<tr>
<td>RLN14</td>
<td>1.57 ± 0.20&quot;</td>
<td>0.29 ± 0.05&quot;</td>
<td>0.29 ± 0.36</td>
<td>0.17 ± 0.21</td>
<td>–17 ± 0.21</td>
<td>–</td>
<td>0.23 (1)</td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>2.53 ± 0.53&quot;</td>
<td>–0.17 ± 0.24&quot;</td>
<td>6.58 ± 0.60*</td>
<td>3.1 ± 0.61*</td>
<td>–4.43 ± 0.83</td>
<td>–</td>
<td>3.57 (1)</td>
<td></td>
</tr>
<tr>
<td>VL</td>
<td>41.14 ± 5.35&quot;</td>
<td>–1.66 ± 1.51</td>
<td>20.65 ± 6.89&quot;</td>
<td>3.11 ± 5.80</td>
<td>17.29 ± 7.17</td>
<td>–</td>
<td>0.13 (1)</td>
<td></td>
</tr>
</tbody>
</table>

m, [d], [h], [i], [j], [l] and X² denote mean, additive, dominance, additive × additive, additive × dominance, chi square test, respectively. Values in brackets represent level of degree of freedom.

* Significant t-test at 5% level.
** Significant t-test at 1% level.

by Frary et al. (2010) who demonstrated that salt stress reduces formation of new leaves and further supports us our hypothesis that a high relative number of leaves in cucumber growing under salinity stress indicates salt tolerance. The importance of high rate of formation of new leaves is linked to sustained photosynthetic activity under salinity (Asgari et al., 2012; Prudent et al., 2009; Vaario et al., 2011). The high rate of development and maintenance of a higher number of leaves under salinity stress may dilute deleterious

Table 3
Variance components, dominance ratio, heterosis, heterobeltiosis, potency ratio and narrow-sense heritability estimates of some traits studied under 80 mM NaCl stress in cucumber under salt stress.

<table>
<thead>
<tr>
<th>Trait</th>
<th>V_A</th>
<th>V_D</th>
<th>V_AD</th>
<th>V_E</th>
<th>Dominance ratio</th>
<th>H%</th>
<th>HB%</th>
<th>Pot</th>
<th>h²_n</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOL</td>
<td>0.62</td>
<td>0.08</td>
<td>–0.30</td>
<td>0.39</td>
<td>0.29</td>
<td>4.92</td>
<td>–11.11</td>
<td>0.27</td>
<td>0.57</td>
</tr>
<tr>
<td>RLN14</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
<td>1.41</td>
<td>3.85</td>
<td>–7.41</td>
<td>0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>LA</td>
<td>1.66</td>
<td>0.15</td>
<td>–0.07</td>
<td>0.7</td>
<td>0.43</td>
<td>42.90</td>
<td>39.24</td>
<td>16.32</td>
<td>0.66</td>
</tr>
<tr>
<td>VL</td>
<td>181.06</td>
<td>19.85</td>
<td>47.23</td>
<td>64.96</td>
<td>0.14</td>
<td>39.73</td>
<td>34.73</td>
<td>5.36</td>
<td>0.74</td>
</tr>
</tbody>
</table>

V_A, additive variance; V_D, dominance variance; V_AD, additive dominance variance; V_E, environmental variance; H, heterosis of mid-parent; HB, heterosis better parent; pot, potency ratio; h²_n, narrow-sense heritability.
effect of Na\(^+\) and Cl\(^-\) while sustaining higher photosynthetic capacity (Vaario et al., 2011). In the current study, VL was inversely proportional to TOL or RLN14. This agrees previous reports that decreases of salinity tolerance (DeRose-Wilson and Gaut, 2011).

Table 4

<table>
<thead>
<tr>
<th>Phenotypic correlation</th>
<th>TOL</th>
<th>RLN14</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNL14</td>
<td>0.575</td>
<td>0.001 (R(^2) = 0.31)</td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>−0.096</td>
<td>−0.800</td>
<td></td>
</tr>
<tr>
<td>0.606</td>
<td>0.670</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL</td>
<td>−0.385</td>
<td>−0.544</td>
<td>0.255</td>
</tr>
<tr>
<td>0.03 (R(^2) = 0.12)</td>
<td>0.002 (R(^2) = 0.28)</td>
<td>0.166</td>
<td></td>
</tr>
</tbody>
</table>

The upper and lower numbers represent Pearson correlation and P-values, respectively. RNL14: relative leaf number at 14 days post salinity treatment; TOL: salt tolerance score; LA: area of second fully expanded leaf; VL: vine length.

The mean values for salinity score (TOL) and RLN14 of F1 in this cross were between mid-parent and tolerant parent values indicating that dominant genes controlled these traits. This dominance effect is further supported by the higher values for the two traits in B1 than B2. However, based on potential ratio this dominance was partial for the two traits. Since the phenotypic variation for RLN14 and TOL observed in F2 was larger than those of the parental lines, we suggest that salinity tolerance in this cross is polygenic with alleles from either parents contributing to increases and decreases of salinity tolerance (DeRose-Wilson and Gaut, 2011). The higher mean trait values observed in B1 compared to B2 may be attributed to differential contribution of increasing and decreasing alleles from either parents. Although the two parents did not differ in terms of VL and LA, the hybrid means were higher than both the parental and better parent means indicating heterotic response for these traits. The positive significant dominant gene action for these traits confirms our current finding.

Although A, B, C scaling test failed to detect non-allelic interactions for all the traits, the inadequacy of additive-dominance model suggests the presence non-allelic interactions. This is in agreement with observation made by Ray and Islam (2008). Mather and Jinks (1971) attributed this observation to canceling effects of the dispersed gene pair in the gene interactions as well as interaction involving two or more genes. Additive gene actions are important for selection of particular trait in breeding since they are stable fixable components of polygenic variation (Kearsey and Pooni, 1998). Significant positive additive gene and non-significant effects for RLN14 and TOL suggest the possibility of improving salinity tolerance of this cucumber cross by recurrent selfing and selection of advanced filial generations owing to low heritability of the traits. Improvement of LA and VL traits under salinity may be feasible at advanced filial generations owing to the presence of significant positive dominance and positive additive × additive epistatic gene components (Hussain et al., 2008). Although, non-significant, the negative additive × dominance gene effect observed in LA indicates that these effects are not fixable by selfing and selection (Farshadfar et al., 2001). Lack of correlation between LA and TOL in the current study suggests that the two traits are genetically inherited at least for this cross.

The positive phenotypic and genotypic correlations between RLN14 and TOL in this study suggest that indirect selection of photosynthetic enhancing traits such as relative leaf number may be employed to improve salt tolerance in cucumber. However, the feasibility of this approach should be surveyed a cross several crosses and generations of cucumber. Significant additive component coupled with low heritability observed in RLN14 necessitates advanced selection in order to minimize environmental effects and while enhancing additive gene effects. The unfavorable genotypic correlation between TOL and VL implies that selecting for one trait reduces the other. Thus segregants with higher, TOL, VL and RLN14 should be selected simultaneously for maximum salt tolerance performance. Selection of transgressive segregants via sib-mating of F1 is feasible for improving salt tolerance of cucumber from this cross.

In conclusion, the gene action estimated through generation mean analysis shows the importance of additive and dominance effects for the traits studied under salinity stress. The relative leaf number and salt tolerance are predominantly controlled by additive gene effect while leaf area and vine are mainly controlled by dominance and epistatic gene action. Our findings show that it is complicated to improve salinity tolerance of cucumber through simple selection procedures or pedigree breeding due to presence of dominance gene action. This coupled with low heritability of relative leaf numbers warrants delayed selection at an advanced stages followed by reciprocal recurrent selection in order to yield desirable segregants. To concentrate desirable alleles, inter-mating of superior segregants could also be pursued.

Acknowledgements

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