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Original Research Article

Uncovering Genetic Variation and Co-Located QTL for Seed Germination under Salt and High Temperature Conditions in Recombinant Inbred Lines of Tomato

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Abstract: Abiotic stresses such as salt stress, high temperature and others can affect seed germination, germination speed, seedling growth in the field and final yield. But genetic variation exists among tomato germplasm response to adverse maternal environments. One hundred one Recombinant Inbred Line**s (**RILs) tomato along two parents were replicated twice to teste seed germination and detect QTLs under salt stress, high temperature and control conditions. Strong significant (P<0.001) difference observed among RILs for all germination parameters under three growth conditions, indicating that high genetic variation observed for seed germination. Germination was more delayed at -0.5 MPa of NaCl, salt stress than high temperature and control. Recombinant Inbred Line of 291 showed the highest germination rate and better performed for all germination traits under salt stress, high temperature and control conditions. QTL analyzed showed at least 1 to 4 significant QTLs per trait identified. Furthermore, four significant co-located QTLs were found on chromosomes 9 and 11, each of two controlling for early set and speed of germination traits, each ranging from 9.9 % to 13.2 % per traits under salt and high temperature stress conditions. This might be suggesting that the common base of genes control for multiple traits. The study demonstrated that some derived RILs showed resilience to germinate under abiotic stresses conditions. Further study is required on co-located QTL through fine mapping, validation and will provide valuable insights into the genetics base of seed tomato for germination under salt stress and high temperature conditions.

Keywords: Speed of germination, variation, QTLs, salt stress, high temperature, Tomato.

1. INTRODUCTION

Seeds play a major role in agriculture input for crop production, as ultimate products for human food, animal feed and others. Seed production and the whole value chain depend on seed quality which is composed of genetic, physical and physiological characteristics that enable germinate and produce normal seedlings under wide environmental conditions (Kazmi *et al*., 2012). This means that seed quality and its traits are controlled by genomic and environment interactions (Koornneef *et al*., 2002). This interaction can also influence seed germination and subsequently post-germination characteristics such as uniformity, seed rate and normal seedling in the field.

Adverse environmental conditions such as salt stress, high temperature and others may affect seeds germinate, establishment of seedling in the field and final tomato production. Tomato response to salt tolerance depends on its phenological growth stage. Among these stages, seed germination and early seedling stages are more susceptible to salinity stress (Ashraf and Foolad, 2005). Salinity stress delays the seed germination, elongates the time required to complete germination, decrease the rate and increase the dispersion of the germination process and afterwards could lead to poor performance of seedlings in the field which results in low yield (Cuartero and Fernandez-Munoz, 1998; Ashraf and Foolad, 2005). In tomatoes, most seeds germinate within 36–72 hour of imbibition under optimal conditions ranged from 20–25 °C (Foolad *et al.*, 1999). However, when temperature is raised to 35 ^{0}C, it delayed the onset of germination and reduce the speed of germination. However, these environmental effects depend on the

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intensity and duration of stress as well as its interaction with the genetic background of the crop.

In many crops including tomato, QTLs were detected for seed germination under cold stress, salt stress and non-stress condition (Foolad *et al*., 1996; Wang *et al*., 2011). Also, studies reported that germination and vigor traits are controlled by many genes and strongly affected by environmental interaction (Koornneef *et al*., 2002; Finch-Savage *et al*., 2010). However, very limited studies on detection of QTL for seed germination in RILs that developed from intraspecific crossed of cultivated with wild species.

Most of cultivated tomato are sensitive to environmental stresses during seed germination and seedling growth under field condition. Thus, wide range of genetic variation should be considered such as land races, cultivated tomato and wild related to coup under fluctuated environment. For example, intraspecific crossing cultivar with wild species were developed to broaden genetic variation and it is a novel choice to study seed germination under adverse environment conditions. Foolad *et al.,* (2007) reported that natural variation was used for the dissection of the genetic architecture of germination under various environmental stresses in tomato. In this study, Recombinant inbred lines (RILs) of tomato developed from two genetic background; *Solanum lycopersicum* (cultivated tomato named as Money maker) crossed with *Solanum pimpinellifolium* (wild relative, is called Pimp) used as source of genetic material for germination studies under salt stress, high temperature and control conditions. Therefore, the objective study was to evaluate the genetic variation of RILs tomato for seed germination under high temperature and salt stresses as well as to detect QTLs that regulate germination traits. We hypothesized that high genetic variation within RILs would be expected under stress compared to non-stressful conditions for different germination parameters. Also, the occurrence of more genetic variation would be expected to the more QTLs detection for different traits under stress.

2. MATERIALS AND METHODS

Plant Materials: The RILs population of F_8 were developed from crosses of cultivar, money maker (*Solanum lycopersicum)* and Pimp, wild relative (*Solamun pimpinellifolium*). One hundred one RILs along with two parents were used for seed germination experiment.

Experimental Arrangement

RILs along two parents were replicated twice in complete design for each environmental condition in separate experiments at Wageningen University and Research Center in Seed Lab of the Plant Physiology laboratory during 2015 in the Netherlands. Three sets of experiments were independently conducted 1) 15 ml of water was applied on each tray at room temperature for control condition 2) 15 ml (-0.5 MPa) salt solution was added to each germination tray and 3) high temperature stress condition was treated at 35 ^{0}C. The plastic bags were put in incubator at 25 ^{0}C under continuous light for salt and non-stress conditions whereas incubated at 35 ^{0}C for high temperature.

Data Collection

Tomato seed germination data was recorded when radical protrusion visible for three growth conditions for fourteen consecutive days during the germination period. After 14 days, the remaining seeds were scored as non-germinated seeds.

Statistical Analysis

Germination traits analyzed using fitting script of Germinator package (Joosen *et al*., 2010). Statistical output for five germination parameters includes; t10 (the time required in hours to reach 10 % germinated seed), t50 (the time needed in hours to reach 50 % germination of seeds), Gmax (%) the maximum germination capacity of the seed batches, U8416 (%) the uniformity of germination and AUC, the area under the germination curve which is the integration of the fitted curve between t= 0 and a user defined endpoint x in hour.

Linkage Analysis

The genetic linkage map was performed accordingly (Kazmi *et al*., 2012), The RILs tomato population genotyped using 5,529 SNPs. SNP markers that had identical values and low call rate were removed, leaving 2,150 polymorphic markers. Furthermore, co-segregating markers were also removed. The remaining 716 unique markers were used for generating the genetic linkage map, which contains 12 individual linkage groups corresponding to the 12 chromosomes of tomato and the average distance between markers was about 10 cM.

QTL Analysis

The mean of germination trait was used as input data for QTL analysis. QTL analysis was performed by using mapping software; MapQTL®6.0 (Van Ooijen, 2009), to identify QTL positions on the genome of tomato for measured traits. Interval mapping is used for estimating the position of a QTL within markers. A permutation test (P<0.05) per germination trait was performed to determine the significance of LOD threshold per chromosome. A LOD score of > 2.0 was used as a threshold level to declare the significance of QTLs.

3. RESULTS

Mean of RILs and Comparison with Parents

In total, 101 tomato RILs along with two parents were studied for seed germination potential under salt stress, high temperature and control. Strong significant difference ($p<0.001$) observed among RILs for all germination traits. For example, high genetic variation observed among RILs for speed of germination under salt stress (Fig. 1) and high temperature at 35^{0}c

(Supplementary Fig. 1 S1) that support our hypothesis. This might be due to the RILs population was exposed to stress condition. However, some inbred lines were germinated between 50 and 100 hrs whereas other took more than 200 hours for germanium, indicated that a longer time for rate of germination. On other hand, few of them were not germinated under both stress conditions.

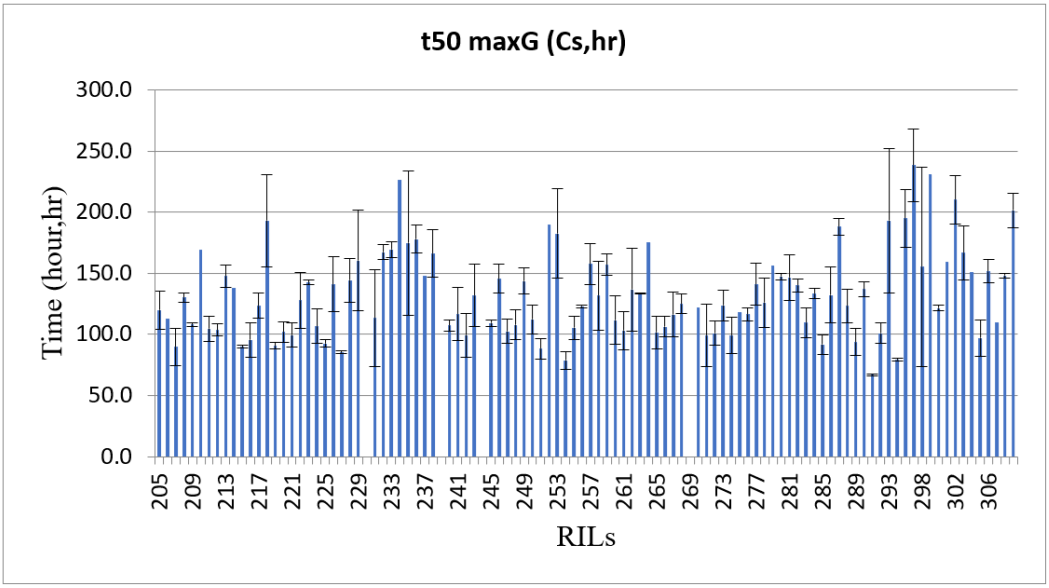

Figure 1: Average and standard error (± SE) of recombinant inbred lines and parents for t50 under salt stress condition

The mean values of RILs for different germination parameters were mostly found between two parents under different growth conditions (Table1). In most cases, RILs of seeds germinated were faster than money maker but slower than pimp, suggesting that the faster germinated seed of progenies inherited from pimp parent.

Table 1: Mean of germination traits for two parents and RILs under high temperature, salt and control conditions

Tomato genotypes	Treatments	Germination parameters				
		t50 ± SE	t10 ± SE	U8416 ± SE	maxG ± SE	AUC ± SE
Money	15 ml H_2O/control	63.88 ± 0.6	52.26 ± 2.9	19.65 ± 6.4	97.49 ± 0	33.50 ± 1
Pimp	15 ml H_2O/control	30.50 ± 0.9	26.12 ± 1.6	7.23 ± 2.25	99.43 ± 0.5	68.90 ± 0.5
RILs	15 ml H_2O/control	45.72 ± 2.57	36.51 ± 1.95	15.84 ± 2.85	94.99 ± 2.0	50.68 ± 2.91
Money	-0.5MPa NaCl	201.62 ± 14.03	137.79 ± 9.35	117.42 ± 8.7	72 ± 14.0	0.21 ± 0.1
Pimp	-0.5MPa NaCl	95.12 ± 8.43	73.31 ± 7.31	37.74 ± 4.5	97 ± 8.0	10.46 ± 4.7
RILs	-0.5MPa NaCl	132.97 ± 15.04	98.31 ± 11.26	62.54 ± 14.05	62 ± 15.0	4.21 ± 1.97
Money	High temp (35 ^{0}C)	115.7 ± 22.7	65.4 ± 5.5	104.1 ± 41.3	8 ± 2.0	0.8 ± 0.1
Pimp	High temp (35 ^{0}C)	39.5 ± 3.1	31.1 ± 1.5	14.75 ± 2.8	99.5± 0.5	59 ± 3.7
RILs	High temp (35 ^{0}C)	65.7 ± 5.6	48.4 ± 5.0	31.3 ± 5.7	70.± 6.0	29.1 ± 4.5

Tomato genotypes; Money, Pimp, RILs = recombinant inbred lines

Germination parameters; t t10 (hr) = time to germinate 10 % of seeds, t50 (hr) = time to germinate 50% of seeds, U8416 (hr) = time interval between 16 and 84 % seeds germinated, AUC = area under the germination curve between 0 and 100 hr, maxG = maximum germination (%) and SE = standard error.

The study tried to compare money maker, Pimp and the derived RILs for germination parameters. The seeds of Pimp germinated earlier (the time required in hours to reach 10 %) (Fig. 2) than money maker under salt stress. However, 13 RILs population showed early set germination under salt than their parents suggesting that transgressive segregation in the RILs population (Fig. 2). Similarly, Pimp germinated faster rate (lower t50) than seeds of money maker under salt stress, high temperature (35^{0}C) and non-stress conditions (Fig. 3). At -0.5 MPa of NaCl, salt stress delayed onset (t10), germination rate (t50) and maximum germination than high temperature as well as non-stress conditions. Among all, RIL of 291 showed early set, high speed, uniform and reached maximum germination curve (Fig. 4). Also, Line 291 reached 60% that beyond of theoretical hour (at 50 hour) than pimp and money maker under salt condition, suggesting that this derived line is a great opportunity for tomato seed growers under optimum and stress conditions.

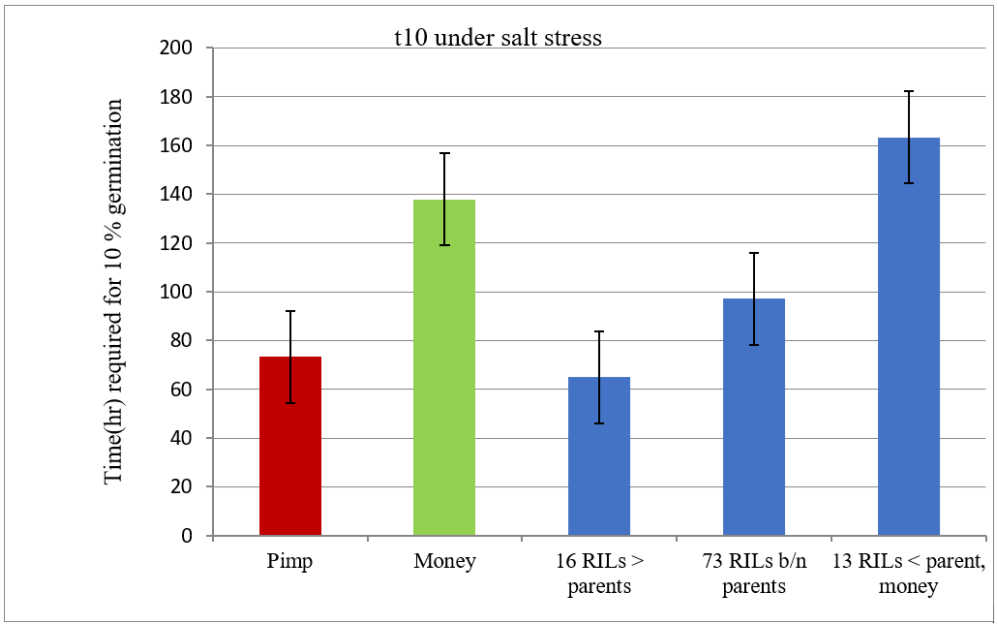

Figure 2: Comparison within RILs and parents for early set germination under salt stress

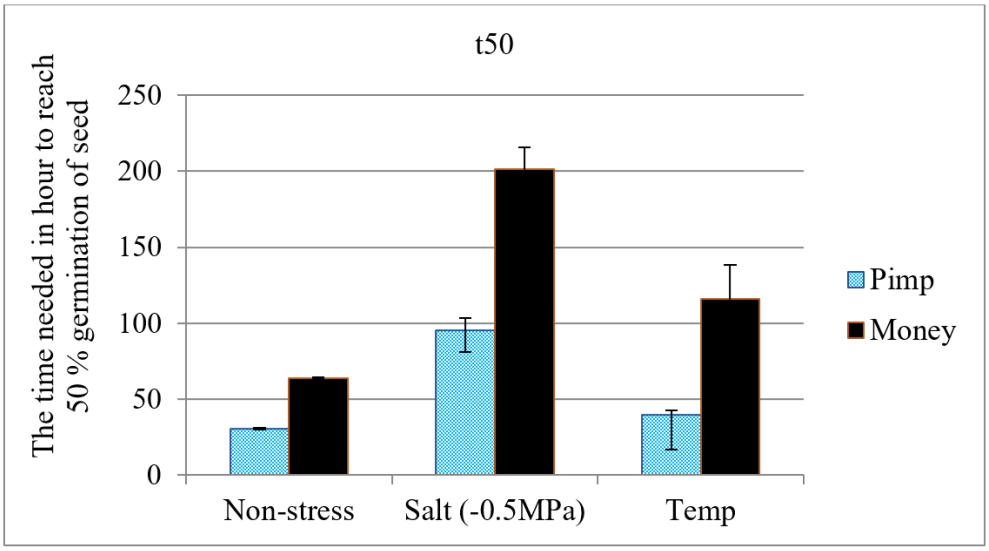

Figure 3: Average and standard error (± SE) of two parents, Pimp (*Solanum pimpinellifolium)* and money *(Solanum lycopersicum*) for t50 under control, salt (-0.5MPa) and high temperature (35 ^{0}c)

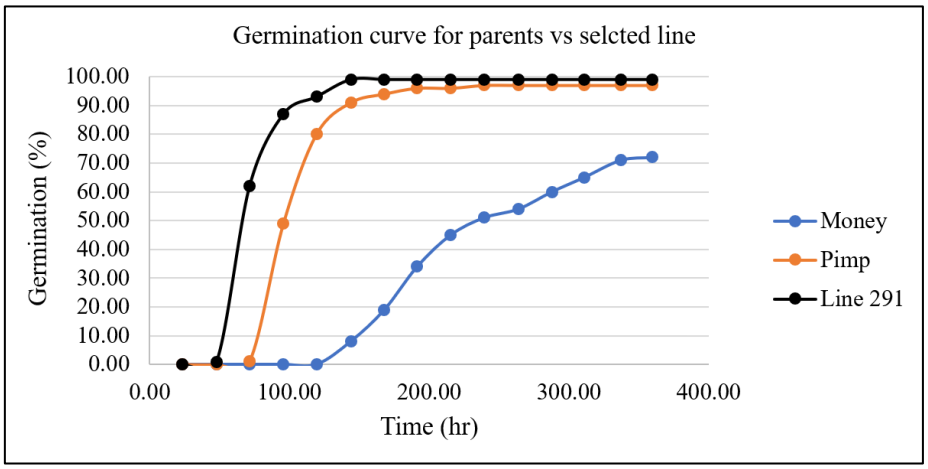

Figure 4: Germination curve for selected line 291 vs parents (Pimp and money maker*)* at -0.5 MPa, salt stress

Detection of QTLs under Salt Stress and High Temperature Growth Conditions

QTL analysis revealed that 29 significant QTL detected and 1 to 4 significant QTLs per traits were identified with LOD scores between 2.01 to 3.69 and explained variance of each QTL ranged from 8.8 % to 15.2 % per trait. It was highlighted that single QTLs was identified on chromosome for different germination traits under three growth conditions (Supplementary Tabel 2 S1). For example, 14 significant QTLs were located on chromosomes 1, 6, 9 and 11 for different seed germination traits at high temperature whereas very few QTLs detected under salt stress, suggesting that salt stress might be affect germination than high temperature.

Moreover, significant co-located QTLs were detected on different chromosomes for different germination traits across three growth conditions. For instance, 4 significant co-located QTLs were found on chromosomes 9, two of each controlling for t10 and rate of germination (t50), each ranging from 9.9 % to 13.2 % per traits under salt stress and high temperature (Figure 5). Also, 12 significant QTLs clustered on chromosomes 1 and 11 for t10, t50 and AUC traits under non-stress and high temperature conditions, ranging from 49.7 to 65.4% explaining variance (Supplementary Tabel 2 S1).

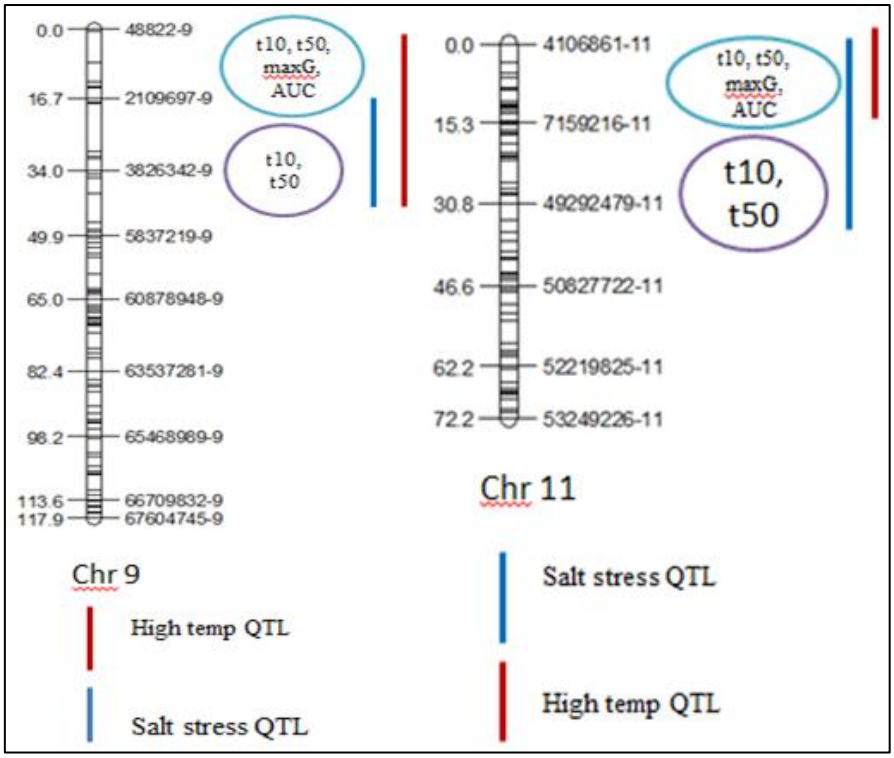

Figure 5: Co-located QTLs locations on chromosomes 9 and 11 for t10; onset and t50; rate of germination under salt and temperature growth conditions. The blue andred colours vertical lines at the right of the chromosomes indicate the approximate locations of QTL for germination traits under high temperature and salt conditions

4. DISCUSSION

In the present study, we harnessed the genetic variation and QTL detection for seed germination to explore the effects of salt and high temperature in RILs tomato. The result demonstrated that high genotypic variation observed among RILs for seed germination under stress than non-stress growth conditions that supported our hypothesis This might be due to RILs exposed to stress condition resulted in variability. The other explanation might be due to money maker cultivar crossed with wild relative resulted in high transgressive segregation in the RILs population. Comparison to two parents (money maker vs pimp), Pimp had the lower t50 value suggested that the faster time to reach a 50 % germinating seeds. This could be due to the fact that the Pimp is a wild relative that might be adapted to fluctuated environmental conditions. Among all RILs, line of 291 exhibited rapidly germinated seed under salt, high temperature and non-stress conditions. Thus, line of 291 might be the most economical useful to grow under saline soil and high temperature condition. On other hand, some RILs population were not germinated under salt and high temperature.

The study showed that salt stress at -0.5 MPa NaCl solution delayed all germination parameters; early germination, germination rate, uniformity and germination percentage as compared to high temperature and control. This statement agreed with (Cuartero and Fernandez-Munoz, 1998) who reported that salinity reduced germination percentages and delays in the germination rate. The fact that salinity stress affected seed germination might be decreasing the rate of water uptake and increase the accumulation of ions and may

change certain enzymatic activities during seed germination process (Delachiave and Pinho.2003). Similarly, high temperature effect on initial seed germination and slow down other germination parameters. Our result agreed with (Heidari *et al*., 2014) who reported extreme temperature significant effect on speed, uniform and maximum germination.

Several significant single and co-located QTLs were detected for the different germination traits across growth conditions. For instance, co-located QTLs were found on chromosomes 9 and 11 for onset and germination rate under salt and high temperature conditions, suggesting that the same QTLs were controlling the physiological traits of different germination traits across the two conditions. This is in line with (Foolad *et al.,* 2007) who reported that the common base QTLs control different physiological germination traits under different conditions. For possible explanation, co-located QTLs controlling for the onset and germination rate traits under salt stress might be contributed to early and speed germination traits under high temperature. It is likely that common genes tolerance to salt stress during onset and speed germination are also these genes encoding heat shock proteins associated with tolerance to high temperature.

Genotype by salt stress expressed very few significant QTLs under salt condition that not supported our hypothesis. This implies that QTLs might highly sensitive to salt stress conditions (Zhu 2002) reported that salt stress might be damage the DNA activities and can induce the production of reactive oxygen species. In addition, the ionic effect of salt can inhabit the enzyme activities and water uptake during seed germination process (Ashraf *et al.,* 2002). These might be the reason why a few of RILs seeds failed to germinate under salt stress in the present study.

5. CONCLUSION

Our results highlighted that high genetic variation observed among RILs of tomato for all germination traits under three growth conditions. Salt at -0.5 MPa showed a major influence on seed germination than high temperature and control. RILs of 291 showed rapidly germinated seed and performed under three growth conditions.

Co-located QTLs were detected on same chromosome for the same traits and / or different traits under salt, high temperature and non-stress. This suggest that common genes controlling different physiological processes which contribute to different germination traits expression that controls multiple traits. Further investigation is required on co-located QTLs through fine mapping, validation and will provide valuable insights into the genetics base of seed tomato for germination under salt stress and high temperature conditions.

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