Detection of Quantitative Trait Loci for Seed Germination Traits in Tomato under Salt and High Temperature Conditions

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Abstract

The objective of the experiment was first to evaluate the Recombinant Inbred Lines (RILs) of tomato for seed germination under salt stress, high temperature and non-stress growth conditions and then to identify quantitative trait loci (QTLs) controlling different germination traits under these conditions. A total of 101 RILs along with their two parents were tested under three growths. Significant ($P<0.05$) differences were observed among the RILs for all germination traits under all growth conditions. Germination was more delayed under salt stress at -0.5 MPa of NaCl than two growth conditions. Out of all RILs, genotype 291 showed rapidly germinated seed under three growth conditions. The QTL analysis detected a total of 29 significant QTLs for five germination traits under the three different growth conditions. At least 1 to 4 significant QTLs per trait identified explaining variances ranging from 8.8 to 15.2%. Four significant co-located QTLs were found on chromosomes 9, two of each controlling for onset and rate of germination traits, each ranging from 9.9 % to 13.2 % per traits under salt and high temperature. Similarly, 12 significant QTLs clustered on chromosomes 1 and 11 for onset (t10), speed (t50) and the area under the germination curve *(AUC) traits under non-stress and high temperature conditions, ranging from 49.7 to 65.4% .This might be an indication that the common genes control different traits or a locus has pleiotropic effects that controls multiple traits. Further study is required on Co-located QTL through fine mapping that can identify the candidate genes tolerance to salt and high temperature conditions which is very useful to improve seed germination and the performance of seed production.*

Keywords: common QTLs, rate of germination, salt stress, high temperature

Introduction

Seed quality is composed of genetic, physical and physiological characteristics that enable germination and produce normal seedlings under wide environmental conditions. 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; include germination rate, uniformity and percentage of normal seedling in the field.

Adverse environmental conditions such as salt stress and extreme temperature can affect tomato growth

and hence its yield. Seed germination and early seedling stages are more susceptible to salinity stress than other growth stage (Ashraf and Foolad, 2005). Salt stress delays the seed germination, decreases the rate and increases the dispersion of the germination process and afterwards could lead to poor performance of seedlings in the field resulted in low yield (Cuartero and Fernandez-Munoz, 1998; Ashraf and Foolad, 2005). Similarly, extreme temperature eventually inhibits seed germination and can decrease seedling emergence as well impact on the final yield (Hampson and Simpson, 1990). However, these environmental effects depend on the intensity, duration of stress and its interaction with the genetic background of the crop.

In the last decades, most studies tended to focus on the improvement of the economical outcome and nutritional quality of tomato by considering either the genetic or environmental factors rather than emphasising on the genetic and environmental interactions that governs seed and seedling quality traits. Thus, seed quality traits are complex, since they are governed by the genome of the crop and environmental interactions which primarily expressed germination traits. In such traits, several genes are involved and thus, are suitable for quantitative trait loci (QTL) analysis. In many crops including tomato studies were conducted to identify QTLs for seed germination, QTLs for germination rate and seedling vigor traits that have tolerance to abiotic stress and under optimum conditions (Foolad *et al*., 2007; Kazmi *et al*., 2012). For example, QTLs detected for seed germination traits under cold stress, salt stress and non-stress condition in tomato and rice (Foolad *et al*., 1996; Wang *et al*., 2011). These studies indicated that seed germination and vigor traits are controlled by many genes and are strongly affected by environmental conditions (Bettey *et al*., 2000; Koornneef *et al*., 2002; Finch-Savage *et al*., 2010).

Earlier reports revealed that most commercial tomato cultivars are sensitive to salt stress (Foolad and Lin, 1997) and limited genetic variation exists within cultivated tomato. But, genetic variation exists within tomato species including landraces, cultivated tomato and related wild species. Thus, this variability is a novel choice to study seed germination under adverse environment conditions. Subsequent study is required by crossing cultivated tomato with related wild species for QTL identification that regulate germination traits under fluctuation of environments. Therefore, the objective of this study was to assess the influence of salt stress and high temperature on seed germination of tomato and identify common QTLs that regulate germination traits under the aforementioned abiotic stress conditions.

Materials and Methods

Source of plant materials

The tomato Recombinant Inbred Lines (RILs) population derived from a cross between two parents: cv. Money maker (*Solanum lycopersicum)* and Pimp (*Solanum pimpinellifolium*) was used. In total 101 RILs along with two parents were used for seed germination experiments.

Experimental setup

The experiment was conducted at Wageningen University and Research Center in Seed Lab of the Plant Physiology laboratory during 2015 in the Netherlands.

Germination trays were arranged at random with two replications and a maximum of 35 trays were used for each repetition and one layer of white filter paper was laid on each tray. A total of 101 RILs along with two parents were used in a randomized complete design (RCD) with two replications for each environmental condition in separate experiments.

Three sets of experiments were independently conducted using non-stress (control), salt and high temperature experiments. Fifteen ml of water was applied on each tray for non-stress condition. For the salt stress condition, 15 ml of NaCl salt solution was added to each germination tray and for high temperature stress condition (35 ^{0}C) (Kazmi *et al*., 2012), 15 ml of water was added to each tray. Each tray (21 cm x 15 cm) contained three different sets of RILs and approximately 50 seeds of each line were sown under aseptic conditions for three conditions of treatment independently. Trays were piled up and each stack had one empty tray on the top and bottom, layers of white filter paper laid on each with 15 ml water applied to prevent unequal evaporation.

Growth conditions

The germination trays were mounted according to their order, tightly fitted with lids and the whole stack was wrapped in a closed transparent plastic bag and incubated at 4 ^{0}C for 3 days in the dark for stratification. Later, the plastic bags were put in incubator at 25 ^{0}C under continuous light for salt and non-stress conditions. For temperature stress, the bags were incubated at 35 ^{0}C (Kazmi *et al*., 2012)

Linkage analysis

We use the genetic linkage map by Kazmi *et al*. (2012), in which they used 5,529 SNPs to genotype the RILs tomato population. SNP markers with identical values 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.

Data collection

Germination assessment

Seed germination was counted and scored when radical protrusion was observed. Germination was followed twice per day for non-stressed while daily for stressed conditions for 14 consecutive days during the germination. After 14 days, the remaining seeds were scored as non-germinated seeds.

Statistical analysis

Germination parameters

Statistical analysis was conducted by using the curve-fitting script of the Microsoft excel Germinator package. Cumulative germination data was analyzed by using this script to determine germination parameters (Joosen *et al*., 2010). Statistical output of germination curves were used to calculate five germination traits; 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:

QTL detection

The mean values per recombinant inbred lines of the germination traits were used for QTL detection. 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 two 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. Additive effects of each detected QTL together with phenotypic variation explained by each QTL (explained variance, %) was determined.

Results

Mean of RILs population

The result showed significant difference among RILs for the different germination traits. It was not convenient to show all mean traits output on this paper but average and standard error values for germination rate under salt stress illustrated below (Figure 1).Tremendous genetic variation observed among for speed germination. For example: some of 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.

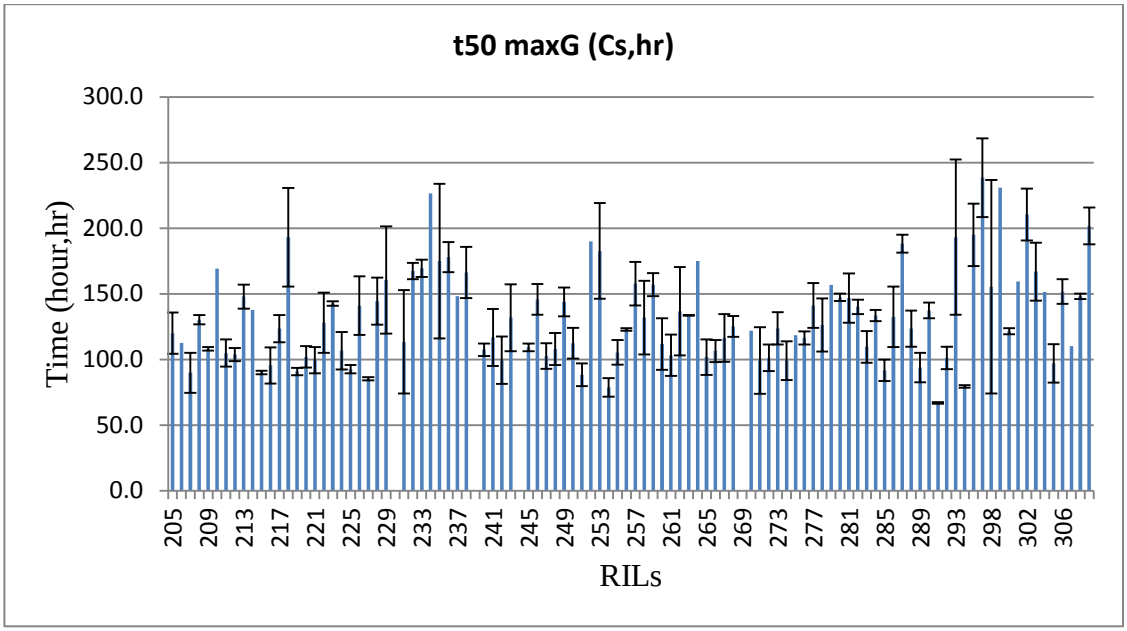

Figure 1. Average and standard error(± SE) of recombinant inbred lines and parents for t50 under salt stress condition

Comparing the two parents, seeds of Pimp germinated earlier (lower t10) and faster rate (lower t50) than seeds of money maker under salt stress, high temperature (35^0C) and non-stress conditions (figure 2 and 3). At -0.5 MPa of NaCl, salt stress delayed onset (t10), germination rate (t50) and other germination parameters compared to high temperature and non-stress conditions.

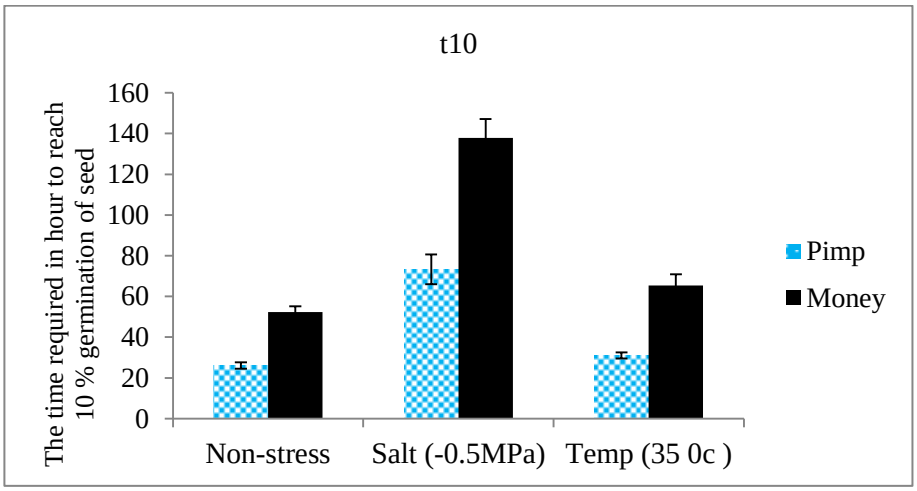

Figure 2. Average and standard error (± SE) of two parents, Pimp (*Solanum pimpinellifolium)* and Money maker (*Solanu mlycopersicum*) for t10 under control, salt (-0.5MPa) and high temperature (35 ^{0}c).

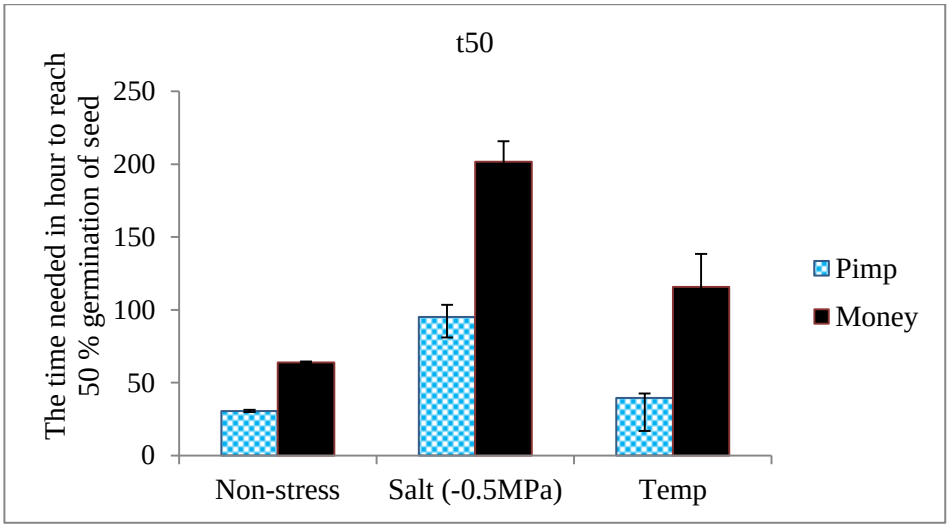

Figure 3. Average and standard error(± SE) of two parents, Pimp (Solanumpimpinellifolium) and Money maker (Solanumlycopersicum) for t50 under control, salt (-0.5MPa) and high temperature (35 0c)

Comparing the two parents, the seeds of pimp quickly achieved maximum germination percentage than money maker under non-stress conditions. For instance, the time to reach 98 % germination for pimp and money maker was 44 hrs and 127 hrs, respectively (Fig 4) under control. Also, pimp increasing sharply and tends to linear while money maker not (Fig 4).Similarly, money maker took longer time to achieve maximum germination percentage and had lower value than pimp under salt conditions (Fig 5). From germination curve, salt showed low germination of seeds compared to non-stress condition. This showed that the clear effect of salt stress on seed germination characteristics.

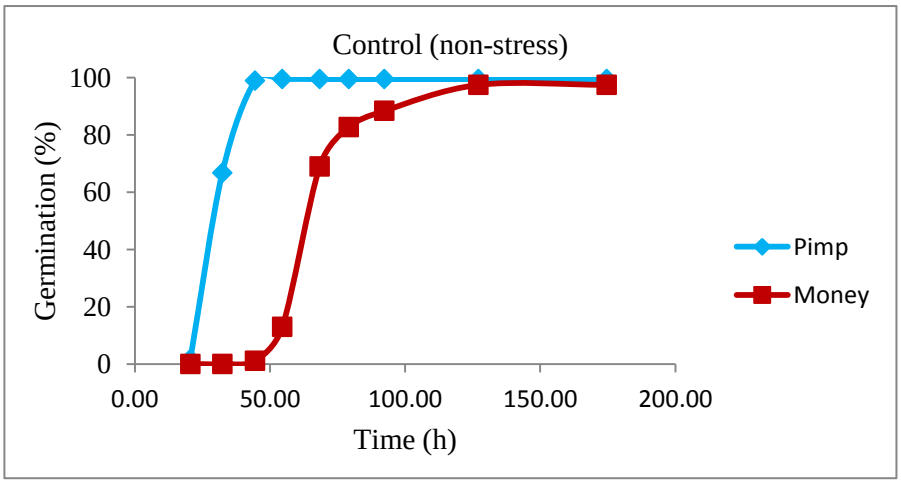

Figure 4.Germination curve of Pimp (Solanumpimpinellifolium) and money (Solanumlycopersicum) under control condition

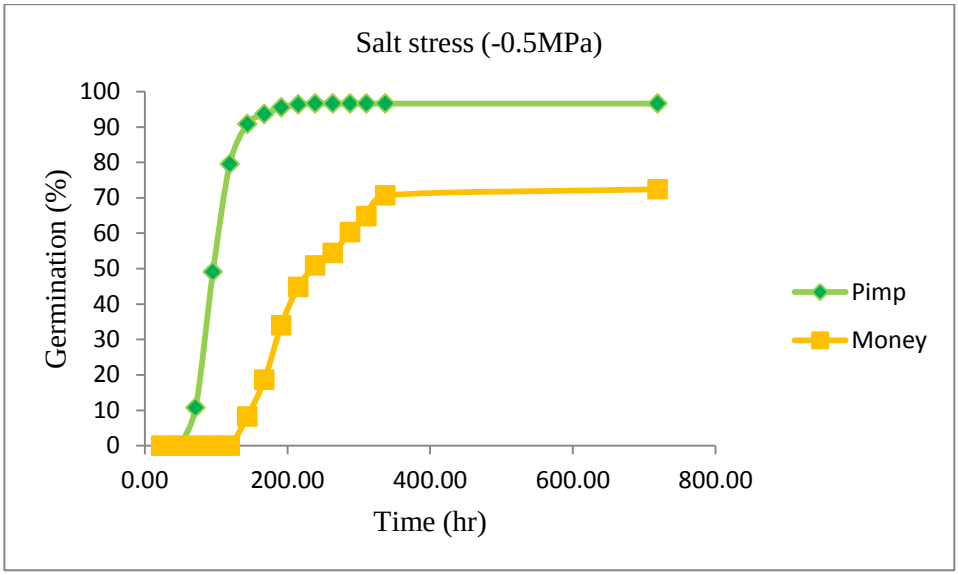

Figure 5. Germination curve of Pimp (Solanumpimpinellifolium) and money (Solanumlycopersicum) under salt stress

Detection of co-located QTLs for onset and germination rate under stress conditions

Several QTLs were found across common chromosomes for different traits under different growth conditions. For example, 4 significant co-located QTLs were found on chromosomes 9, two of each controlling for onset and rate of germination traits, each ranging from 9.9 % to 13.2 % per traits overlap under salt and high temperature (Figure 6). Also, 12 significant QTLs cluster 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 (Figure 7). In addition, two significant co-located QTLs were found on chromosomes 2 for Gmax trait under salt and non-stress conditions, ranging from 9.8 to 10.1% overlap.

Comparison of QTLs affecting germination traits across environmental conditions

In total 29 significant QTLs were detected across three environmental conditions. Among these, 9 QTLs affecting onset of germination, 7 QTLs affecting germination rate, 10 QTLs; each five of QTLs affecting maximum germination and AUC, and one QTL contributes to uniformity of germination across three environmental conditions (data not shown). Therefore, the highest numbers of QTLs were detected for onset and germination rate across three environmental conditions. In comparison among environments, several QTLs (14 QTLs) were detected under high temperature, whereas, 3 QTLs were identified under salt stress conditions. This implies that QTLs might be sensitive to salt stress like at -0.5 MPa of NaCl.

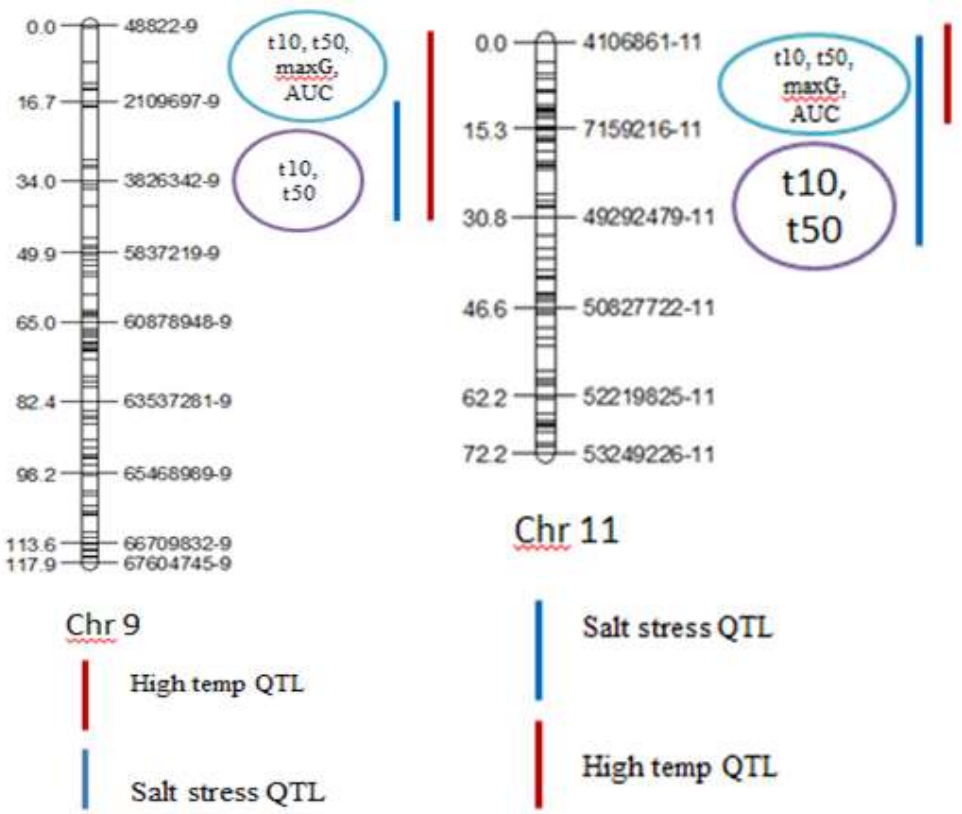

Figure 6. Co-located QTLs locations on chromosomes 9 and 11 for t10; onset and t50; rate of germination under salt and temperature growth conditions. The blue andred colours vertical lines at the right of the chromosomes indicate the approximate locations of QTL for germination traits under high temperature and salt conditions

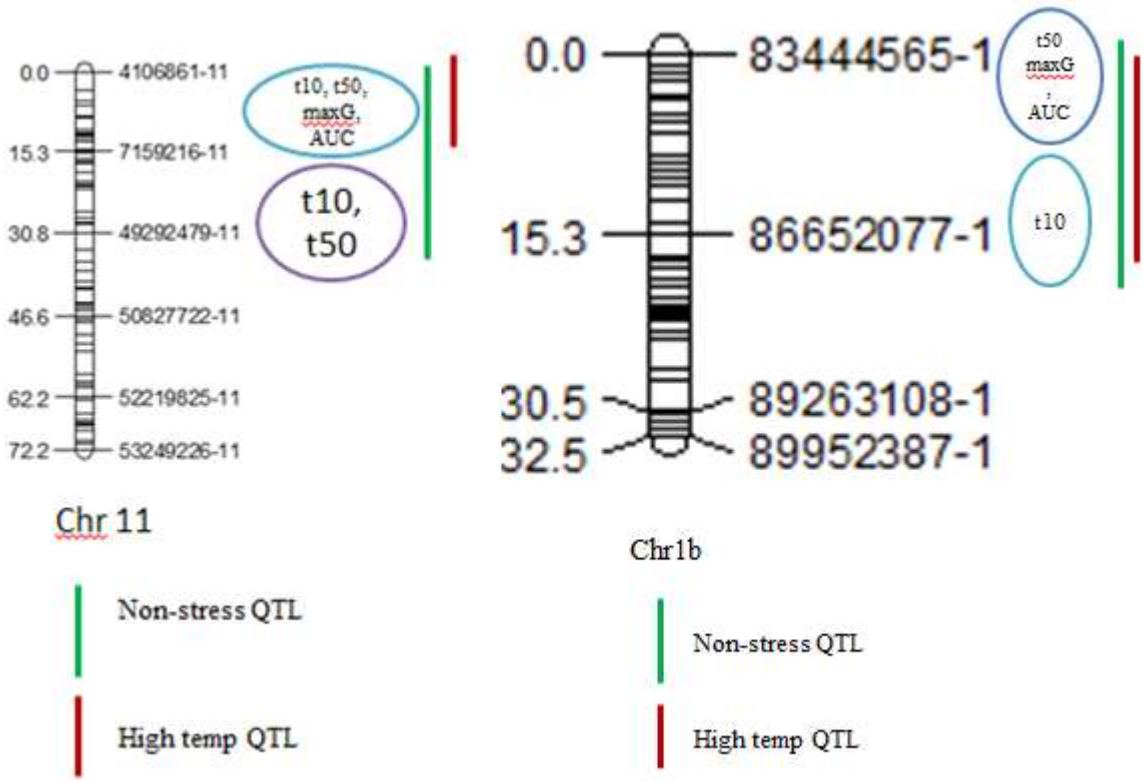

Figure 7. Co-located QTLs locations on chromosomes 11 and 1 for onset, germination rate, maxG and AUC traits under non-stress and high temperature growth conditions. The greenand redcolours vertical lines at the right of the chromosomes indicate the approximate locations of QTL for germination traits under non stress and high temperature growth conditions

Discussion

Effect of salt stress and high temperature on seed germination

Understanding the physiological mechanism how salinity and high temperature affect seed germination and seed quality is quite important. During seed germination, salinity may disorder physiological processes like decreasing the water uptake by seeds. There was evidence in this study under salt stress condition, some RILs population showed slower initial germination and longer time interval for germination traits. Foolad *et al*., (2007) observed that the delayed onset and slow rate of seed germination under salt conditions. Similarly, high temperature may disorder the germination process; like slow down and eventually inhibit seed germination (Hampson and Simpson 1990). This longer and or slower germination times affect seed quality in terms of uniformity of germination, rate of germination, seedling vigour and these attributes to impact on the whole value chain in tomato production.

This study was carried out to assess the effect of salt stress and high temperature on seed germination and identification of QTLs controlling seed germination traits under three growth conditions. Considerable genetic variation was observed for all physiological seed germination parameters (t10, t50, Gmax, U8416 and AUC) under three environmental conditions. Higher genetic variation was observed under stress conditions than in non-stress might be due to genotypes were exposed to stress environment,

The finding showed that salt stress at -0.5 MPa NaCl delayed all germination parameters such as onset germination, germination speed as well germination uniformity and decreased maximum germination as compared to high temperature and control. This statement agreed with Cuartero and Fernandez-Munoz (1998); Ashraf and Foolad (2005) who reported that salinity may delay the initiation of germination and elongate the time needed to complete germination. Thus salt stress directly influences the agronomic seed quality traits. But variation observed among RILs tolerance to salt stress. For example, genotype 291 showed rapidly germinated seed under salt and high temperature conditions. This genotype also efficiently performed for different germination traits under three growth conditions. Thus, this genotype might be the most economical useful to grow under saline soil and high temperature condition, i.e. This genotype is tolerance to salt stress and high temperature during seed germination, due to a mechanisms to regulate salt tolerance and revealing candidate genes or common QTLs that function in response to salt stress as well high temperature (Gong *et al*., 1999). On other hand, parent of Pimp had the lower t50 value indicates the faster in time to reach a 50 % germinating seeds. This could be due to the fact that the Pimp is a wild relative that might already be adapted to climatic change as well as buffering against changeable environmental conditions.

Identification of Co-located QTLs controlling seed germination traits

In this study, in total 29 significant QTLs were detected for different germination traits across three environmental conditions. Among these, 12 QTLs were detected under control, 14 QTLs detected under high temperature and the remaining 3 QTLs were found under salt stress.

The findings in the current study showed that several significant co-located QTLs were found for the different traits as well as under different growth conditions. For instance, for t10, t50 and AUC traits, six significant overlapped QTLs were found, two of each found on chromosomes 4 and 11 under control conditions. It is interesting that the common existence of genes also controlling different traits under control condition. Also, not all but most of the significant co-located QTLs were clustered for most

germination traits under high temperature condition. This implies that most of different germination traits were controlled by common generic QTLs under high temperature condition. This statement tends to agree with other studies (Clerkx *et al*., 2004; Khan *et al*., 2012) suggesting that due to common basis QTLs controlling such multiples traits. Similarly, some clustered QTLs were located on chromosomes 9 and 11 for onset and germination rate traits under salt and high temperature conditions. It is likely that the same QTLs were controlling the physiological characteristics of different germination traits across the two conditions. This is in line with Foolad*et al*., (2007) who suggested that the common QTLs control different physiological germination traits under different conditions. For possible explanation, common 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 for the tolerance to salt stress during onset and speed germination are also these genes encoding heat shock proteins associated with the tolerance to high temperature. Plants have different mechanism for salt tolerance. Zhang *et al*., (2012) reported that FAD2 genes regulate salt tolerance during seed germination and early seedling growth in some crops. For instance, Na^+ extrudes out from the cell and compartmentalizes it into vacuoles membrane using Na^+/H^+ anti-portal. However, we didn't detect such experiment in the present study.

High genetic variation was more observed under salt stress than two conditions. However, genotype by environment interaction was not expressed more number of significant QTLs under salt condition. This result does not support the hypotheses that the occurrence of more genetic variation would be expected to result in detection of more QTLs. No significant QTLs were detected for U8416 and AUC traits under salt conditions implying that some QTLs might highly sensitive to stress environmental conditions. Other study reported that salt stress might be damage the DNA activities and can induce the production of reactive oxygen species, ROS in plants (Zhu, 2002). Also, the ionic effect of salt can inhabit the enzyme activities during seed germination process (Ashraf *et al*., 2002). These might be the reason why seeds of a few RILs fail to germinate under salt stress in the present study. Furthermore, Many QTLs related to the salt tolerance have been reported in vegetable crops includes cucumber, watermelon and tomato (Li *et al*., 2011; Zhang *et al*., 2003). However, we indentified very few significant QTLs detected under salt stress in RILs tomato.

Conclusion

Differences among RILs of tomato and genotype by environment interaction observed for all traits under

salt stress, high temperature and controlled conditions. at -0.5 MPa of NaCl, showed a major influence on seed germination than high temperature, 35 ^{0}c and control conditions. It was interesting that Line 291 showed rapidly germinated seed under salt and high temperature conditions as well efficiently performed for different germination traits under three growth conditions. In this study, significant co-located QTLs found on the same chromosome for the same traits and / or different traits under salt and high temperature stress. This might be an indication of the same genes that controlling different physiological processes which contribute to different germination traits expression or locus has pleiotropic effect that controls multiple traits. Further investigation is required on co-located QTLs through epistasis interaction, fine mapping and candidate genes detection in the QTL regions for target traits.

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