The influence of salt stress and high temperature on seed germination of recombinant inbred lines in tomato

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Abbreviations

^{0}C = degree Celsius

cM = centiMorgan

LOD = Logarithm of the odds; for significance of QTLs

ml = milliliter

MPa = Mega Pascal

NaCl = Sodium chloride

QTL = Quantitative trait loci

RILs= Recombinant inbred lines

SE = Standard error

Acknowledgment

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Abstract

Seed quality is composed of genetic, physical and physiological characteristics that enable germination and to produce healthy seedlings under field conditions. However, this quality is influenced by genotype, and genotype x environment interactions. The experiment was conducted at seed laboratory of plant physiology at Wageningen University and Research Center during 2014/2015. A total of 101 RILs along with two parents were used in a randomized complete design (RCD) with two replications under non-stress, salt and high temperature conditions. The objective of this experiment was to evaluate the seed germination of tomato RILs under salt stress, high temperature and non-stress conditions as well as identification of QTLs affecting different germination traits under these conditions. Significant (t-test, $P<0.05$) differences were observed among the RILs for all germination parameters under salt, high temperature and non-stress conditions. Bigger genetic variation was observed under stress than non-stress condition due to exposure to stress. At -0.5 MPa of NaCl, salt stress was delaying seed germination parameters than high temperature, 35 ^{0}c and non-stress conditions. However, the magnitude of their response to stress varied among RILs. In most cases, average of the germination traits of the RILs were intermediately found between the values of the two parents for different germination parameters across the three conditions. However, transgressive segregation was also observed in the population due to combination of alleles from both parents for the expression of the studied phenotypes. By using RILs, several QTLs were identified for the seed germination traits under non-stress, high temperature and salt stress conditions. At least 1 to 4 significant QTLs per trait were identified and each of these QTLs had an explained variance ranging from 8.8 % to 15.2 %. Overall, 29 significant QTLs were detected for five germination traits under the three different environmental conditions. Among these, 16 QTLs were detected for onset and germination rate under the three conditions which implies that these QTLs affect initial and rapid seed germination under the three conditions. Several co-located QTLs were found on the different chromosomes for different traits under different conditions. This might be an indication of the same gene responsible for controlling such different traits or due to pleiotropic effects that control multiple traits. On the other hand, other QTLs were also found on separated chromosomes suggesting that different genes influence different physiological processes which contribute to different seed quality traits under the different conditions.

Key-words: Seed germination, salt stress, high temperature, Quantitative trait loci (QTL), tomato

1. Introduction

1.1. Background and justification

Seed is a ripened ovule consisting of an embryonic plant, stored food and protective coat. It is a living propagules that is used for planting purposes. Among agricultural inputs; seed is the vital input to enhance crop production. According to FAO (http://faostat.fao.org) about 162 million tonnes of tomato was produced worldwide in 2012. Such production depends on various factors. Among these, seed quality is one of the most important, since low seed quality was found to influence the emergence and number of seedling that can be established in the field (Finch-Savage, 1995).

Seed quality is composed of genetic, physical and physiological characteristics that enable germination and production of healthy seedlings under wide environmental conditions. This quality is judged by different end users such as farmers, processors and industries. Farmers expect to obtain high quality seeds that are able to germinate and produce normal seedlings under field conditions (Ligterink *et al*., 2012), because poor germination directly affects the crop yield. On the other hand, food processors may require seeds having high nutrient content like starch and/or oil (Nesi *et al*., 2008). After post-harvest, seed processors can improve the germination of seed via seed processing. However, seed quality and its traits are determined by both genetic background of seed and environmental conditions. This means, seed and seedling of the plants are not only influenced by genes inherited but also by maternal effects.

Maternal effect can be genetic and non-genetic effects. The genetic maternal effect may happen due to genetic transmission in different organelles such as mitochondria and chloroplast of DNA (Platenkamp and Shaw, 1993). On the other hand, non-genetic maternal effect occurred in environmental conditions that may influence the performance of seedlings and the ability of the mother plant to provide seed. For instance, the deficiency of nutrients or toxicity of minerals in soil, and temperature stresses can affect seed germination, seedling growth, seed development and maturation stage (Probert, 2000; Galloway, 2001). Similarly, the effect of the maternal environment on seed quality including seed germination and size was previously reported (Hilhorst and Toorp, 1997; Gutterman, 2000; Luzuriaga *et al*., 2006). Thus, seed quality is influenced by many factors and its traits are controlled by genomic and environment interactions (Koornneef *et al*., 2002). These interactions can also influence seed germination and subsequently post-germination characteristics such as uniformity and health of the seedling in the field.

Germination is defined as the process in which seeds begin to uptake water, followed by elongation of the embryo and penetration of the radicle through the endosperm and seed coat (Bewley and Black, 1994). Accordingly, a visible sign of germination in tomato appears when the embryonic axis/radicle

penetrates its surrounding structures. According to Bradford (1995) seed germination in general involves three distinct phases: phase I, phase II (lag phase) and phase III. In the last phase, the embryo elongation is initiated and germination is considerably completed

Unfavourable environmental conditions such as extreme temperature and high salt level can affect tomato growth and hence its yield. 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 of the crop (Cuartero and Fernandez-Munoz, 1998; Ashraf and Foolad, 2005). Similarly, extreme temperature can delay and eventually inhibit seed germination (Hampson and Simpson, 1990). However, these environmental effects depend on the intensity and duration of stress as well as its interaction with the genetic background of the crop.

1.2 Problem statement

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. For instance, breeders mostly gave attention to develop tomato varieties for abiotic tolerance such as salt and drought tolerance (Munns and Tester, 2008) and stimulated to screen germplasm based on phenotypic expressions that cope with these stresses. Earlier reports revealed that most commercial tomato cultivars are sensitive to salt stress (Foolad and Lin, 1997) and limited genetic variation exists. But, the variability exists within cultivated tomato and more pronounced in related wild species for abiotic stress tolerance (Kazmi *et al*., 2012). Thus, this variability is the novel choice to study seed germination under stressful environmental conditions. However, studies that have been conducted on tomato seed germination and it's traits under stressful environmental conditions using an integrated quantitative trait loci (QTL) approach are relatively limited. Besides, less attention has been given to environmental stress conditions (salt condition and/or high temperature) that may highly affect the germination and quality of seedlings of tomato. Taken together, this suggests that further investigation is still required to investigate the effect of these factors in tomato species.

1.3 Research interest

Genetic variation exists within tomato species including land races, cultivated tomato and related wild species. The wild relative tomato species *Solanum pimpinellifolium* showed genetic variation within them and are a good source of genetic material for salt tolerance compared to cultivated tomato (Foolad and Lin, 1997). Also, the presence of variation within an interspecific population for seed and seedling quality has been reported (Khan *et al*., 2012). The existence of this genetic variability provides a good source of experimental material to investigate seed and seedling quality of tomato under stress conditions.

Moreover, seed and seedling quality traits are complex, since they are governed by the genome of the crop and environmental interactions. In such traits, several genes are involved and thus, are suitable for QTL analysis .These QTLs represent the genome regions that are explaining the phenotypic variation. The association of QTLs with germination traits have been studied in different crops under different stress conditions (Foolad, 1999). By using a recombinant inbred line (RIL) population of tomato, some QTLs were identified for germination traits under different stress conditions (Foolad *et al*., 2007; Kazmi *et al*., 2012).

Among wild related species, Pimp, *Solanum lycopersicum* is the most closely related species to cultivated tomato and crossing between them is feasible and can produce fertile seed. Therefore, it is useful to generate a RIL population between them and will be also important to study their variation in germination potential and growth traits under different environmental conditions. In this minor thesis report, the RIL population of tomato derived from *Solanum lycopersicum* (CV. money maker) crossed with *Solanum pimpinellifolium* (Pimp) (Kazmi *et al*., 2012) was used as source of genetic material for germination studies and identification of QTL that influence the germination traits under different environmental conditions.

1.4. Objective

The aim of this study was to assess the influence of salt stress and high temperature on seed germination of tomato and to observe the variation among RILs in germination potential. Besides, QTLs that determine the germination traits under non-stress and stress conditions were identified.

1.5. Hypothesis

High genetic variation within RILs would be expected under stress compared to non-stressful environmental conditions for different germination parameters. The occurrence of more genetic variation would be expected to result in detection of more QTLs for different traits.

1.6. Research questions

- Do the RILs differ from each other under stress and non-stress environmental conditions for different germination parameters?
- What is the influence of high salt and high temperature during tomato seed germination?
- Will QTLs be detected for germination traits?
- Will co-located QTLs be detected for germination traits under different conditions?

2. Materials and methods

2.1. Source of plant materials

The tomato RIL population derived from a cross between two parents: cv. moneymaker (*Solanum lycopersicum)* and pimp (*Solamun pimpinellifolium*) was used. In total 101 RILs along with two parents were used for seed germination experiments.

2.2. Experimental setup

The experiment was conducted at Wageningen University and Research Center at the Wageningen Seed Lab of the Plant Physiology laboratory chair group during 2014/2015. The lab is located at $51^0$59’11’’N, $5^0$39’52’’E, Wageningen, 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 conducted independently using non-stress (control), salt and high temperature as experimental treatments. 15 ml of water was applied on each tray for non-stress condition. For the salt stress condition, 15 ml (-0.5 MPa) salt solution was added to each germination tray. For high temperature stress condition (35 ^{0}C), 15 ml of water was added to each tray. Each tray (21 cm x 15 cm) contained three different sets of RILs and approximately 40 seeds of each line were sown under aseptic conditions for three conditions of treatment independently. Maximum of 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.

Figure 1. Germination trays covered in plastic bag.

2.3 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.

2.4 Data Collection

2.4.1 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 8 consecutive days during the germination. After 8 days, the remaining seeds were scored as non-germinated seeds.

2.4.2 Imaging and seed size measurement

Each germination tray was placed under a digital camera (Nikon D80 camera with a 60 mm objective) fixed to a stand and connected to a computer in order to take an image of the seeds. Each picture was automatically saved with date, time and image of samples. According to Joosen *et al*., (2010) the seeds pictures were analysed using the open source software Image-j to measure the seed size based on colour thresholds.

2.5 Statistical analysis

2.5.1 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 this script summarized the results of averages for the different parameters including graphs, standard error and student t-tests ($p<0.05$).

The germination curves were used to calculate five parameters;

a) t10 (the time required in hours to reach 10 % germinated seed. This is the initiation of germination or onset of germination.

b) t50 (the time needed in hours to reach 50 % germination of seeds). This is the rate of germination

c) Gmax (%) the maximum germination capacity of the seed batches

d) U8416 (%), uniformity of germination: this is the time between 16 % and 84 % germination.

e) AUC, the area under the germination curve: this is the integration of the fitted curve between t= 0 and a user defined endpoint (x). In this study x was 100 hrs.

2.5.2 QTL analysis

Complete linkage map consisted of 13 individual groups that correspond to the 12 chromosomes of tomato based on a previous constructed map. The RIL population of F_8 stage developed from two parents, *Solanum lycopersicum* (cv.moneymaker) and *Solanum pimpinellifolium* (pimp). These RILs were genotyped by 716 Single Nucleotide Polymorphisms (SNP) markers and the average distance between markers was about 10 cM.

QTL analysis was performed by using mapping software; MapQTL®6.0, 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. Each significant QTL explained the corresponding phenotypic variance (%).The marker close to the highest LOD peak was taken for QTL significant.

3. Results

In total 101 tomato RILs along with two parents were studied for seed germination potential under non-stress, salt and high temperature conditions. This population is derived from a cross between cv. money maker *(Solanum lycopersicum*) and pimp (*Solanum pimpinellifolium)*. The germinator curve script calculated five parameter; t10maxG (t10), t50maxG (t50), maxG (%), U8416 and AUC. Statistical output summarized the result of average, standard error and student t-test. The results are presented in the following paragraphs.

3.1 Variation, mean and distribution of germination parameters for RILs

3.1.1 Variability among RILs population for germination parameters

The germinator package analyses showed significant (t-test, $P<0.05$) differences among the RILs for all germination parameters under stress and non-stress conditions (Fig 10, 11, and 12 in Appendix). Genetic variations exist among RILs population for t10, t50, Gmax, U8416 and AUC. Higher genetic variation was observed under stress condition, salt and high temperature than non-stress condition (Fig 10, 11, and 12, Appendix). This might be due to the RILs population was exposed to stress condition. Seed germination of RILs population showed different time to germinate under salt and high temperature conditions. But, the magnitude of their response to these stresses was varying among RILs. For instance, among RILs, genotype 291 showed the earlier to reach t10, the faster to achieve t50, the shorter for U8416 and achieved maximum germination under stress conditions. However, some RILs of seeds were not germinated under stress conditions.

Except for Gmax parameter under non-stress, seeds of pimp showed significant ($p < 0.05$) difference when compared to seeds of money maker for germination parameters under all conditions. Comparing the two parents, seeds of pimp germinated more rapidly than seeds of money maker under salt, high temperature (35^0C) and non-stress conditions (Table 1, Fig 2 and 3). For instance, the time required to reach t10maxG and t50maxG for the two parents varied under the three conditions. In most case, money maker showed the longer germination time under three conditions that associated with a greater likelihood non-stress tolerance. For all RILs, at -0.5 MPa of NaCl, salt stress delayed onset (t10maxG), germination rate (t50maxG) and other germination parameters compared to high temperature and non-stress conditions (Fig 2, Fig 3 and Fig 10-12 in appendix). This implies that salt at -0.5 MPa and high temperature, 35 ^{0}C have different effect on tomato seeds germination.

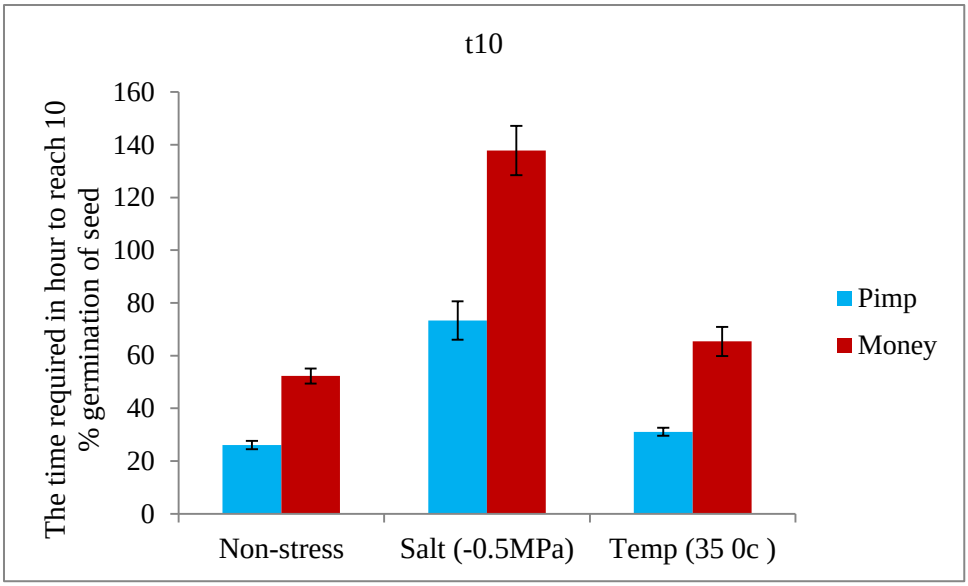

Figure 2. Average and standard error (± SE) of two parents, Pimp (*Solanum pimpinellifolium)* and money *(Solanum lycopersicum*) for t10 under control, salt (-0.5MPa) and high temperature (35 ^{0}c).

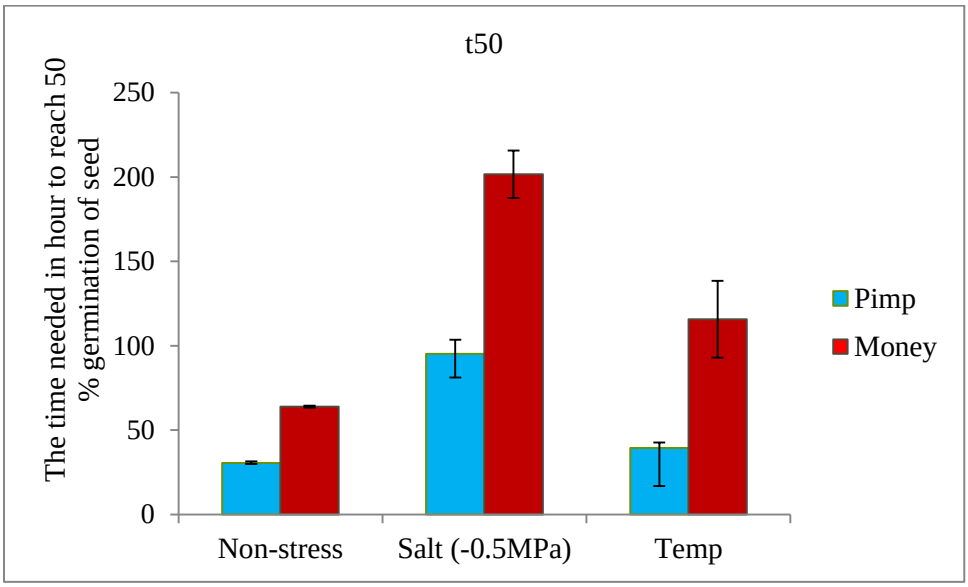

Figure 3. Average and standard error (± SE) of two parents, Pimp (*Solanum pimpinellifolium)* and money *(Solanum lycopersicum*) for t50 under control, salt (-0.5MPa) and high temperature (35 ^{0}c)

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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). However, a low germination percentage was observed for money maker at 35 ^{0}C (Fig 6). This implies that the money maker showed low seed germinated from cumulative germination data under high temperature. Similarly, money maker showed the longer to achieve maximum germination percentage and had low value than pimp under salt conditions (Fig 5). From the germination curve, salt and high temperature were showed low maximum germination of seeds compared to non-stress condition. This shows that the clear effect of salt stress, -0.5 MPa and high temperature at 35 ^{0}C on seed germination characteristics

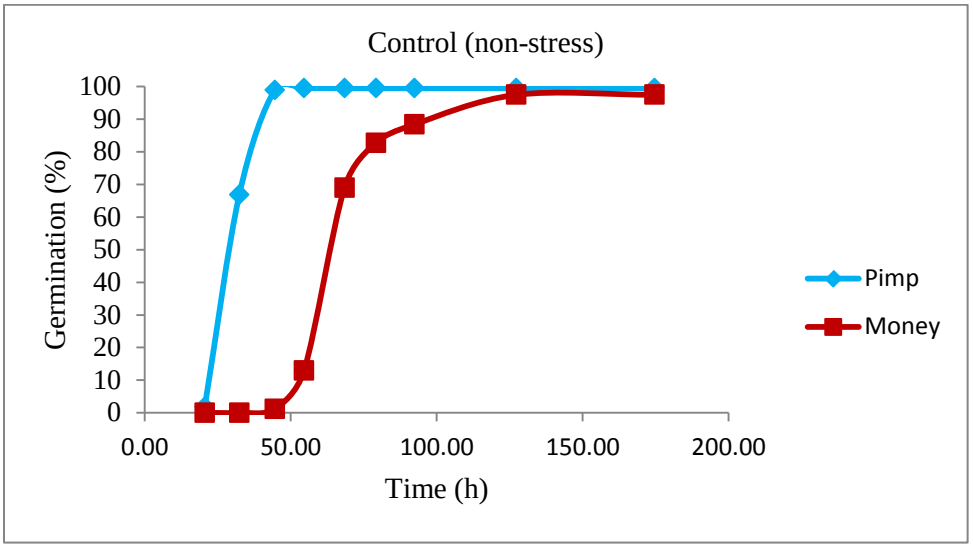

Figure 4. Germination curve of Pimp (*Solanum pimpinellifolium)* and money *(Solanum lycopersicum*) under control condition

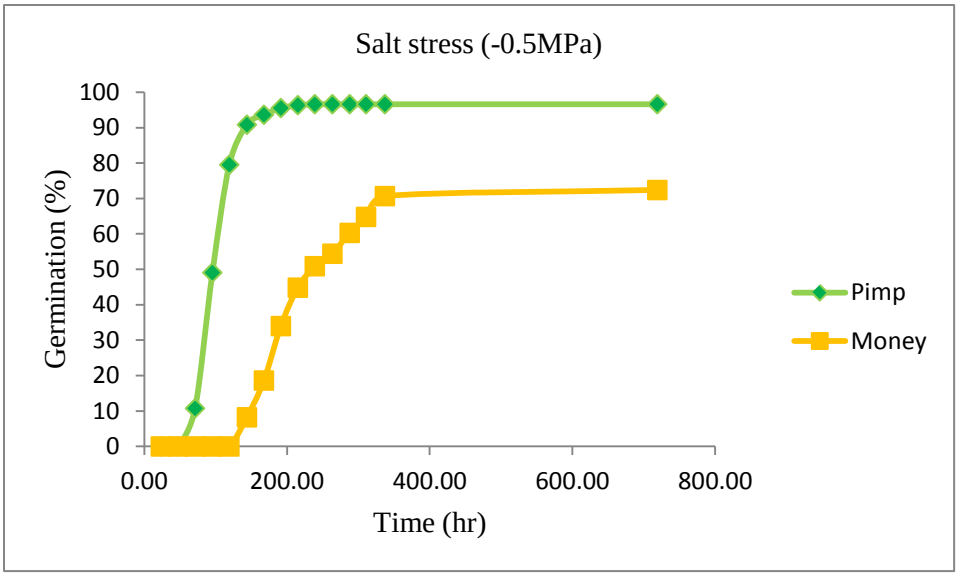

Figure 5. Germination curve of Pimp (*Solanum pimpinellifolium)* and money *(Solanum lycopersicum*) under salt stress

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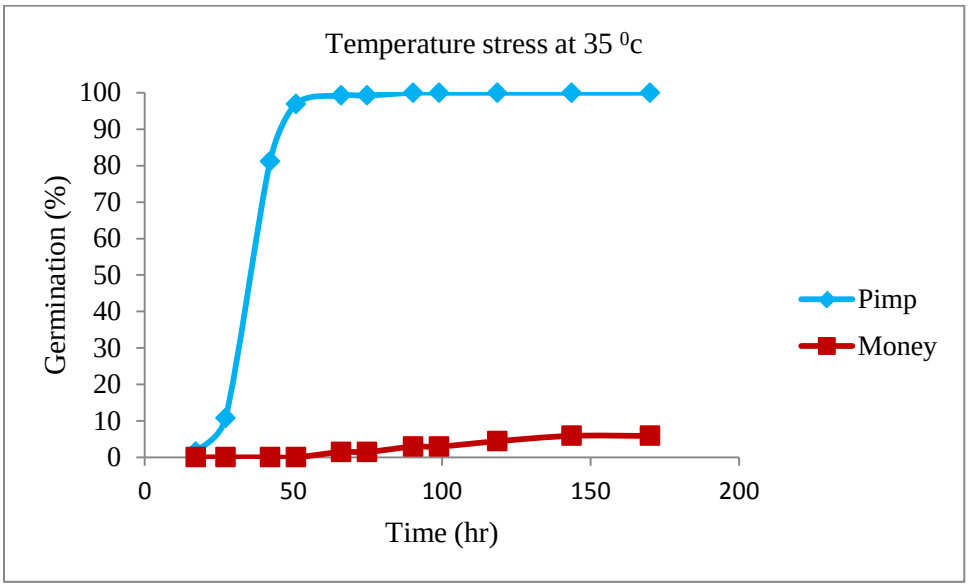

Figure 6. Germination curve of Pimp (*Solanum pimpinellifolium)* and money *(Solanum lycopersicum*) at high temperature (35 ^{0}c).

3.1.2 Mean and distribution of RILs population

The average values of all RILs for the different germination parameters were mostly found between two parents under different conditions (Table1). Except for maxG, in most cases, seeds germinated from the RILs populations were faster than money maker but slower than pimp. It is more likely that the faster germinated seed of progenies inherited from pimp parent.

Table 1. Mean and standard errors (± SE) of germination traits for parents and average of RILs under non-stress, salt and high temperature

		Germination parameters[2]				
Genotypes[1]	Treatments	t10 ± SE	t50 ± SE	U8416 ± SE	AUC ± SE	maxG ± SE
Money	15 ml H_2O	52.26 ± 2.9	63.88 ± 0.6	19.65 ± 6.4	33.50 ± 1	97.49 ± 0
Pimp	15 ml H_2O	26.12 ± 1.6	30.50 ± 0.9	7.23 ± 2.25	68.90 ± 0.5	99.43 ± 0.5
RILs	15 ml H_2O	36.51 ± 1.95	45.72 ± 2.57	15.84 ± 2.85	50.68 ± 2.91	94.99 ± 2.0
Money	-0.5MPa NaCl	137.79 ± 9.35	201.62 ± 14.03	117.42 ± 8.7	0.21 ± 0.1	72 ± 14.0
Pimp	-0.5MPa NaCl	73.31 ± 7.31	95.12 ± 8.43	37.74 ± 4.5	10.46 ± 4.7	97 ± 8.0
RILs	-0.5MPa NaCl	98.31 ± 11.26	132.97 ± 15.04	62.54 ± 14.05	4.21 ± 1.97	62 ± 15.0
Money	High temp (35 ^{0}C)	65.4 ± 5.5	115.7 ± 22.7	104.1 ± 41.3	0.8 ± 0.1	8 ± 2.0
Pimp	High temp (35 ^{0}C)	31.1 ± 1.5	39.5 ± 3.1	14.75 ± 2.8	59 ± 3.7	99.5± 0.5
RILs	High temp (35 ^{0}C)	48.4 ± 5.0	65.7 ± 5.6	31.3 ± 5.7	29.1 ± 4.5	70.± 6.0

[1]Money = *Solanum lycopersicum*; Pimp = *Solanum pimpinellifolium*; RILs = recombinant inbred lines; [2]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.

Histograms showed that the distribution of RILs population for each germination parameter varied under different conditions (Fig 7-9). In these histograms, high numbers of RILs had values for the germination parameters intermediately between two parents under three conditions for different germination parameters (Fig 7-9). However, transgression was observed in opposite direction for certain parameters. For instance, high transgression was observed for U8416 and maxG (%) parameters under control conditions (Fig 7). Similarly, several numbers of transgressive RILs were observed for AUC and maxG (%) parameters under salt condition (Fig 9).

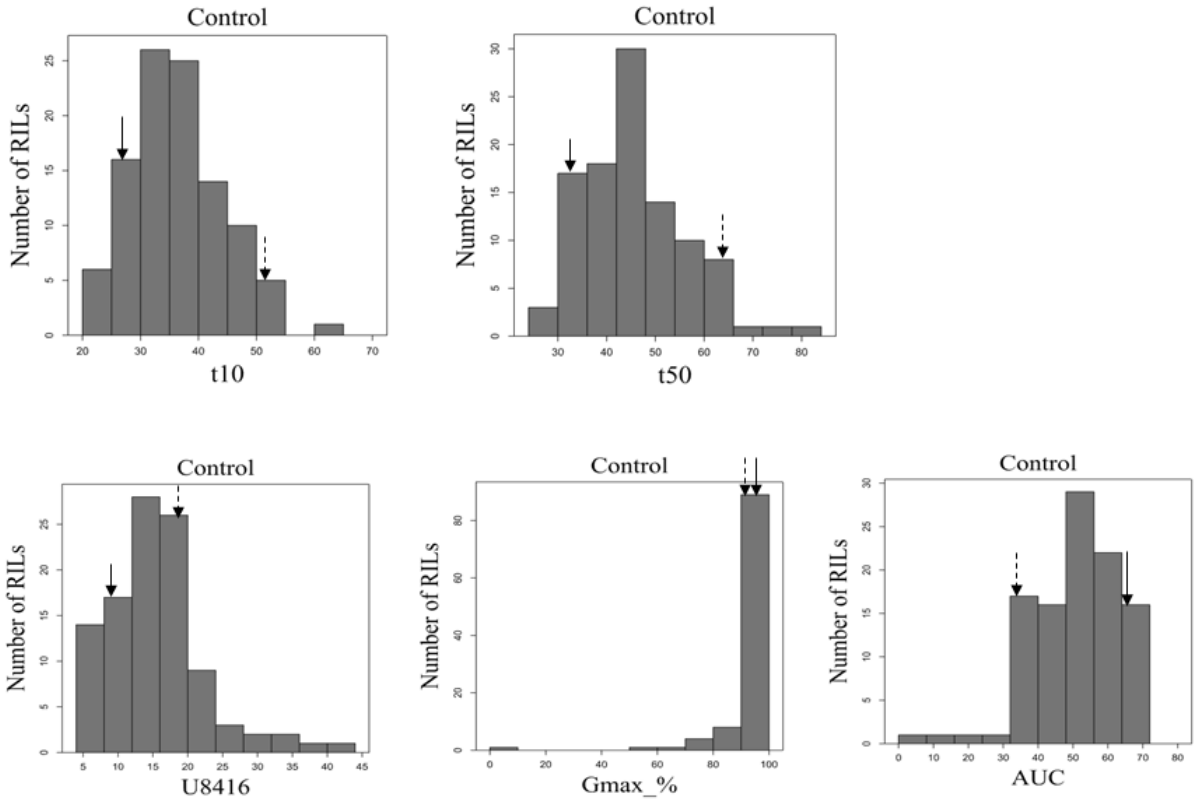

Figure 7. Frequency distribution of number of RILs for different germination parameters under control condition for the *Solanum pimpinellifolium* and *Solanum lycopersicum* recombinant inbred lines. The average parental line value is shown with a solid arrow for Pimp (*Solanum pimpinellifolium*) and a dashed arrow for money-maker (*Solanum lycopersicum)*. t10maxG or t_{10} (hr) : the time needed to achieve 10 % germinated seed; t50maxG or t_{50} (hr) : the time required to reach 50% germination seeds; U8416 (hr) : = time interval between 16 and 84% seeds germinated; Gmax (%) : maximum germination; AUC : area under the germination curve between 0 and 100 hours.

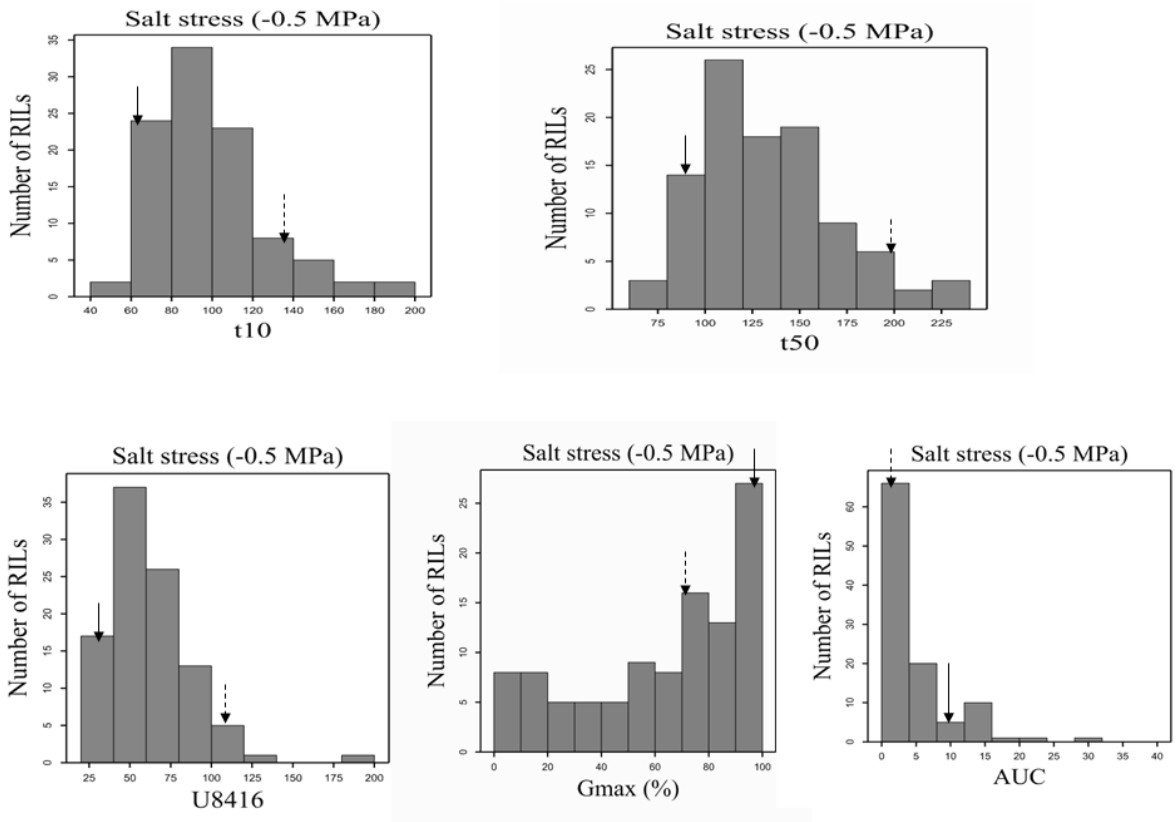

Figure 8. Frequency distribution of number of RILs for different germination parameters under salt stress (-0.5MPa) condition for the *Solanum pimpinellifolium* and *Solanum lycopersicum* recombinant inbred lines.

The average parental value is showed with a solid arrow for the Pimp (*Solanum pimpinellifolium*) and a dashed arrow for the money-maker (*Solanum lycopersicum)*. t10maxG or t_{10} (hr) = the time needed to achieve 10 % germinated seed; t50maxG or t_{50} (hr) = the time required to reach 50% germination seeds; U8416 (hr) = time interval between 16 and 84% seeds germinated; Gmax (%) = maximum germination; AUC = area under the germination curve.

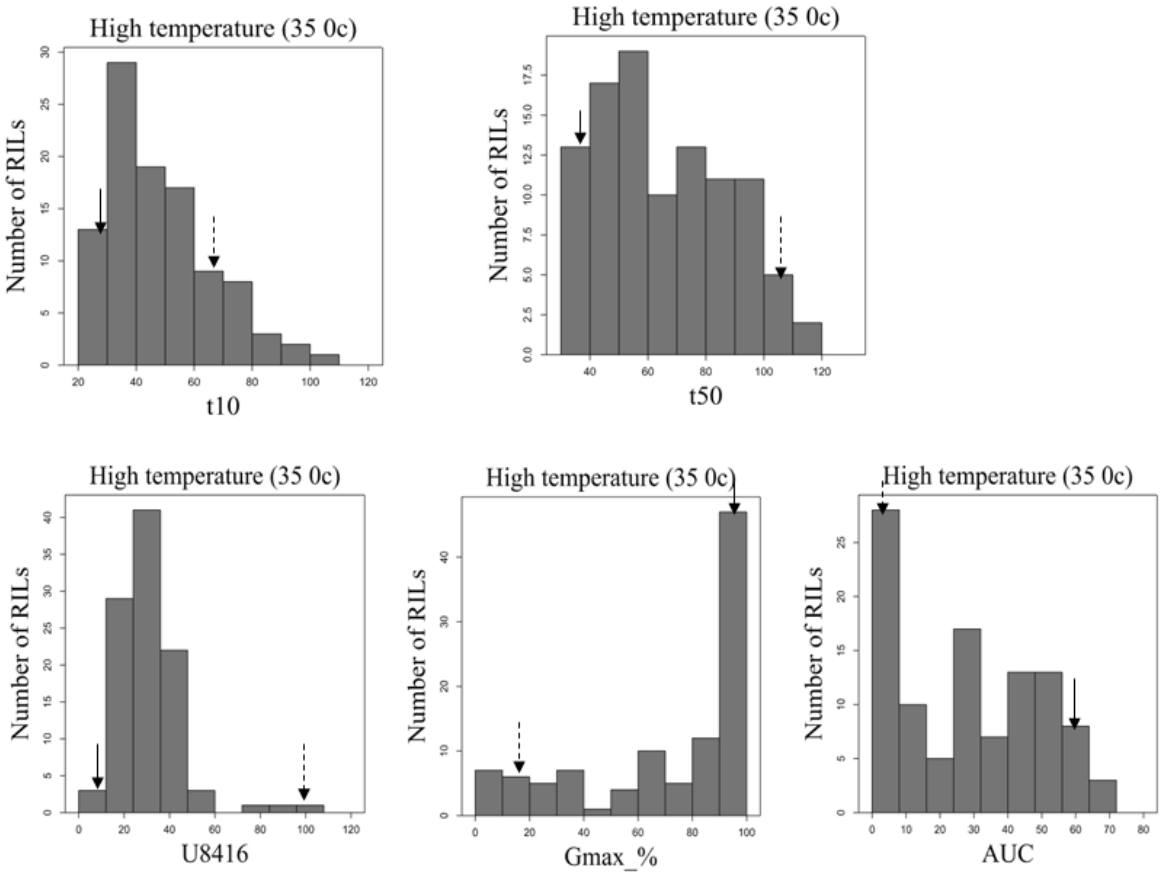

Figure 9. Frequency distribution of number of RILs for different germination parameters at high temperature (35 ^{0}C) condition for the *Solanum pimpinellifolium* and *Solanum lycopersicum* recombinant inbred lines.

The average parental line value is showed with a solid arrow for the Pimp (*Solanum pimpinellifolium*) and a dashed arrow for the money-maker (*Solanum lycopersicum)*. t10maxG or t_{10} (hr) the time needed to achieve 10 % germinated seed; t50maxG or t_{50} (hr) = the time required to reach 50% germination seeds; U8416 (hr) =time interval between 16 and 84% seeds germinated; Gmax (%) =maximum germination; AUC =area under the germination curve.

3.2. Identification of QTLs for seed germination traits under different environmental conditions

The germinator calculated different germination parameters (t10maxG, t50maxG, maxG%, U8416 and AUC) under different environmental conditions. The calculated outputs were used as input data to identify the QTL position on chromosomes for five germination traits under non-stress, salt and high temperature conditions. The QTL analysis revealed that 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.

3.2.1. Identification of QTLs for seed germination traits under control condition

In total 12 significant QTLs on chromosomes 1, 2, 4, 6, 7, 8 and 11were identified for seed germination traits under non-stresses conditions (Table 2). Among these, four QTLs were detected for t10maxG on chromosomes 1, 4, 8 and 11 with a total explained variance of 47.7%. Also, six QTLs detected for t50maxG and AUC traits, two each on chromosomes 4, 11 and one on chromosomes 1and 6, respectively. Two QTL identified for U8416 and maxG (%) traits on chromosomes 2 and 7, respectively. Among all, QTLs that were found on chromosomes 1, 4 and 11 were having the larger effect for early seed germination under control condition. Due to a bigger explained variance for onset as well as germination rate.

3.2.2. Identification of QTL for seed germination traits under salt stress condition

Compared to other conditions, less number of QTLs were found that associated with seed germination traits under salt stress. For t10maxG and t50maxG, two QTLs were identified; one each on chromosomes 9 with the total explained variance of 21.5% (Table 2). These QTLs might have a larger effect for early seed germination under salt stress. One QTL was detected on chromosomes 2 for maxG (%) under salt conditions. However, non-significant QTLs were detected for the U8416 and AUC traits under salt stress.

3.2.3. Identification of QTL for seed germination traits under high temperature condition

Many significant QTLs were found for seed germination traits under high temperature conditions. A total of 14 significant QTLs on chromosomes 1, 6, 9 and 11were identified for seed germination traits at high temperature (35 ^{0}C) stress (Table 2). Among these, 8 QTLs were detected for t10maxG and maxG (%) traits, two each on chromosomes 1, 6, 9 and 11 with a total explaining variance of 87.7%. For t50maxG and AUC traits, 6 QTLs were identified two each on chromosomes 9 and 11, one on chromosome 1 and 6 with a total explained variance of 68.8% (Table 2). Under high temperature condition, most of the QTLs were identified on common chromosomes affecting germination traits.

3.2.4. Co-located QTLs for different traits as well as different conditions.

Some QTLs were found across common chromosomes for different stress conditions. For example, 4 significant co-located QTLs were found on chromosomes 9, each ranging from 9.9 % to 13.2 % per traits overlap under under salt and high temperature (Table 2). Also, 12 significant QTLs cluster on chromosomes 1 and 11 under non-stress and high temperature conditions, ranging from 49.7 to 65.4% overlapping. In addition, two significant co-located QTLs were found on chromosomes 2 under salt and non-stress conditions, ranging from 9.8 to 10.1% overlap.

Several co-located QTLs were found on the same chromosomes for different traits. For instance, co-located significant QTLs were identified for t10maxG, t50maxG and AUC under non-stress condition (Table 2). Similarly, several co-located QTLs were found for different seed germination traits at high temperature. These co-located QTLs were found on chromosome 1, 6, 9 and 11 for almost all traits.

3.2.5. Comparison of QTLs affecting germination traits across environmental conditions

In total 29 significant QTLs were identified 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 contribute for uniform germination across three environmental conditions (Table 2). Therefore, the highest numbers of QTLs were identified for onset and germination rate across three environmental conditions. In comparison between 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.

Table 2. Chromosomal location and significant QTL that was associated to seed germination traits of the tomato RILs population under control, salt and high temperature conditions

Treatment	Trait	Chr.No	Associated marker	position interval (cM)	LOD	Variance explained (%)	effects
Control	t10maxG	1	83444565-1	0-19.12	3.48	14.9	3.05
		4	3154286-4	32.42-32.87	2.18	9.6	-2.49
		8	55776545-8	66.58-69.49	2.29	10.1	2.52
		11	7857508-11	9.88-33.75	3.02	13.1	2.92
	t50maxG	1	83444565-1	0-5.48	3.08	13.3	3.76
		4	31544286-4	32.43-32.87	2.30	10.1	-3.33
		11	48586795-11	13.38-30.75	2.77	12.1	3.61
	U8416	7	55492731-7	39.46-39.84	2.22	9.80	2.12
	Gmax	2	32325113-2	14.26-19.39	2.3	10.1	-0.04
	AUC	4	3154286-4	31.42-32.87	2.35	10.3	4.00
		6	34101367-6	29.47-40.47	2.27	9.9	3.92
		11	5279605-11	11.02	2.01	8.8	-3.74
Salt	t10max	9	3440493-9	21.77-30.73	2.57	11.6	10.62
	t50max	9	3440493-9	23.78-29.39	2.18	9.9	12.14
	Gmax	2	49452377-2	93.76-94.83	2.24	9.8	-0.097
High tem	t10maxG	1	86636577-1	14.39-14.46	2.04	9.3	5.49
		6	43580814-6	92.84-104.33	2.79	12.4	-6.35
		9	2247148-9	17.52-29.39	2.59	11.6	6.90
		11	5174517-11	8.83-12.33	2.08	9.4	5.59
	t50max	6	43580814-6	94.95-104.33	2.66	11.9	-7.51
		9	2247148-9	16.25-29.39	2.99	13.2	8.72
		11	47009022-9	8.57-12.85	2.36	10.6	7.05
	Gmax	1	83852566-1	0-1.73	2.71	11.7	-0.108
		6	35282947-6	46.65-54-45	2.63	11.4	0.108
		9	2247148-9	13.67-24.77	2.36	10.3	-0.109
		11	5279605-11	6.39-11.02	2.62	11.4	-0.109
	AUC	1	83852566-1	0-1.73	2.38	10.4	-6.56
		9	2247148-9	3.52-30.39	3.69	15.16	-8.84
		11	5279605-11	8.83-12.33	2.16	9.4	-6.36

Key: control, non-stress; salt stress, -05MPa (15ml salt solution); high tem, high temperature at 35 ^{0}C; t10maxG (t_{10}), the time required in hrs to achieve 10 % germinated seed; t50maxG (t_{50}), the time

needed in hrs to reach 50 % germination of seed; U8416, time interval between 16 and 84% seeds germinated; Gmax (%), maximum germination; AUC, area under the germination curve; Cr.No, chromosomes number on which significant QTLs were detected; associated marker, nearest marker to the location of the identified QTLs; position interval (cM), confidence interval based on LOD-2 score ; LOD, LOD score of significant QTL peak; explained variance, phenotypic variance that is explained by significant QTL; QTL, Quantitative trait Locus; Effect, difference between mA and mB, where mA carrying an allele of money maker and mB carrying an allele of Pimp at the QTL position. mA and mB were estimated during Map QTL analysis

4. Discussion

This study was carried out to assess the seed germination of recombinant inbred lines (RILs) of tomato under stress conditions (-0.5MPa salt, high temperature; 35 ^{0}C) and non-stress conditions. The discussion is organized into two main parts. The first part discusses about the influence of salt and high temperature on seed germination. Besides, the occurrence of variation among RILs in response to these physical environmental stress conditions for different germination parameters and their implication for seed quality traits will also be discussed. The second part focuses on the identification of quantitative trait loci (QTLs) on chromosomes under different conditions and their interpretation for seed germination traits.

4.1 Effect of salt stress and high temperature on seed germination

In the present study, considerable genetic variation was observed for all physiological seed germination parameters (t10, t50, Gmax, U8416 and AUC) under three environmental conditions. This could be due to existence of natural variation among RILs. Bigger genetic variation was observed under stress conditions than control due to subjected to stress environment. When different genotypes exposed to stress environment, it is expected to different phenotypic expression and high values of genetic variance than non-stress condition. However, the extent of their response to these stresses was variable between RILs.

The finding showed that salt stress delayed all germination parameters as compared to other conditions (Table, 1, Figure 2-3, Fig 5 and Fig 8). This shows that salt stress at -0.5 MPa delayed the onset of germination, decreases the germination rate, decreases germination uniformity, and decreases maximum germination and inhibited germination compared to control. This statement agreed with Cuartero and Fernandez-Munoz (1998); Ashraf and Foolad (2005) who reported that salinity may delay the initiation of germination, reduce the rate, and elongate the time needed to complete germination. Thus directly influence the agronomic seed quality traits. For instance, the shorter time interval was observed for quantifying the uniformity of germination under control, which four times earlier than salt stress conditions. Similarly, most of RILs showed the longer germination time to achieve maximum germination at -0.5 MPa of NaCl when compared to high temperature, 35 ^{0}c and control conditions. The effect of salt stress on the uniformity and maximum germination of seed as observed in the current study may not be desired by plant growers, since farmers need high quality of seed that enable them to germinate high percentage and uniformity of seedlings under required conditions.

Seed germination rate is one of the most important seed germination quality parameters in seed science. Under salt stress, for Pimp, the time required to reach 50 % germinated seed was twice faster

than Money maker. This could be due to the fact that the pimp is a wild relative species that might already be adapted to climatic change as well as buffering against unpredictable environmental conditions. The lower t50 value indicates the faster in time to reach a 50 % germinating seeds. An example, among RILs, genotype 291 showed rapidly germinated seed under salt and high temperature conditions. This genotype also efficiently performed for different germination parameter under these conditions. Such genotype might be the most economical useful to grow in saline soil.

However, understanding the physiological and underlying molecular mechanism how salinity delayed seed germination is quite important. During seed germination, salinity may disorder physiological processes like decreasing the water uptake by seeds. In agreement with this, Foolad *et al*., (2007) observed the delayed onset and low rate of seed germination under salt conditions due to low water potential and ionic (Na^+ and Cl^-) effects. Also, salt stress decreases some enzyme activities, break down starch and causes nuclear deformation during seed germination (Cuartero and Fernandez-Munoz, 1998; 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.

The results from the current study indicate that the seeds of RILs germinated faster in response to high temperature than under salt stress but slower as compared to the control for different germination parameters investigated. This indicates that salt at -0.5 MPa and high temperature, 35^{o}C have different effect on tomato seeds germination. However, the magnitude of their response to high temperature was varied among RILs.

Optimum temperature is speeding up seed germination and other metabolic processes. However, high temperature (beyond the optimum) may disorder the germination process. For instance, comparing high temperature vs control, for money maker, the time to achieve 50 % a germinated seed is twice as high. This implies that high temperature reduced seed germination rate. Similarly, high temperature slowed down for quantifying the germination uniformity and to achieve maximum germination percentage when compared to control conditions (Fig 4 vs 6, 10 vs 12). This is in line with Hampson and Simpson (1990) who stated that extreme temperature can delay, slow down and eventually inhibit seed germination.

The mean of germinated seeds of all RILs were found intermediate between two parents (Table 1). In addition, in most cases, the germinated seed of RILs were frequently distributed between two parents (Fig 7-9). Suggesting that transgressive segregation occurs in the population for germination characters, due to combination of favourable alleles from both parents expected to expresses the phenotypic variant of traits. However, observing the individual values of RILs for the seed germination parameters were not in between two parents. For instance, transgression also observed in opposite direction for some parameters, U8416 and maxG (Fig 7) and AUC and maxG (%) (Fig 9).

This is might be due to over dominance of one parent for phenotypic expression. This phenomena was tends to agree with (Devicente and Tanksley, 1993) who reported that transgressive segregation also observed in opposite direction for same traits due to epistasis interaction or / complementary gene action from two parents. These RILs showed high genetic variation and their phenotypic expression is the result of the cross between two extreme parents.

4.2. Identification of QTLs affecting 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. These detected QTLs explain some of the differences found for the studied seed germination traits under these conditions. Many QTLs were found on different chromosomes for different traits indicating that different QTLs were controlling the same germination traits. This is in agreement with Kazmi *et al*. (2012) who reported that several QTLs also were found on separated chromosomes that controlling the same physiological process that can contribute to different seed germination traits under different conditions.

The findings in the current study show that several significant overlapped QTLs were found for the different traits as well as under different conditions. For instance, for t10, t50 and AUC traits, six significant co-located 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. 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 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 are controlling different physiological germination traits under different conditions. Further explanation could be that the common QTLs that are controlling for the onset as well as germination rate traits under salt stress might be contributing to early germination traits under high temperature. Thus, these QTLs seem to contribute to rapid seed germination under the two stress conditions tested in the present study.

Great genetic variation was more observed under salt stress than two conditions. However, the interaction of genotypes with environment was not expressed more significantly for 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 be sensitive to high stress environmental conditions. Further explanation, at -0.5 MPa NaCl, salt stress might be damaged the nuclear division of DNA due to more ionic accumulation in the media during seed germination process. Besides, study reported that salt stress can induce the production of reactive oxygen species, ROS in plants (Zhu, 2002). Thus, the more generated ROS resulted in more cell member or / DNA damaged and inhibition of enzyme activities during seed germination process, consequently few significant QTL detection.

5. Conclusion

Differences in seed germination among RILs were observed for each germination parameter under non-stress, salt and high temperature conditions. At -0.5 MPa of NaCl, salt stress has a major influence on seed germination than high temperature, 35 ^{0}c and control conditions.

Transgressive segregation also observed in the population for germination characters, due to combination of favourable alleles from both parents and their phenotypic expression is the result of the cross between two extreme parents.

In this study, significant QTLs were identified for different germination traits under control, high temperature and salt stress conditions. Besides, co-located QTLs were found for different germination traits under both stress conditions or / stress and non-stress conditions. Thus, it can be concluded that the common basis QTLs affecting different germination traits under different stress and control conditions. These the common genetic direction of the QTLs with explained high variance percentage for different germination traits under different conditions could be an advantage for breeding purpose. Also, these different traits are found on the same gene and convenient for breeding.

In general it can be concluded from the present results that the tomato seed germination was affected by genetic, the interaction of genotypes with environment, salt stress and high temperature conditions. Under these conditions, tomato seed phenotypes interact across three environments and high source of genetic variation observed that used for study on seed and seedling quality traits.

6. Recommendation

This recommendation is based on results and further research to be conducted.

Co-located QTLs were detected for onset and germination rate under stress conditions. To confirm this result, correlation analysis with seed weight, seed size, the speed of initial down root germination and related parameters should be conducted. If these traits are positive correlated as well as co-located QTLs identified for some traits, it is expected to contribute for germination rate.

In line with co-located QTLs, identification of candidate genes and expression in the QTL regions should be investigated for targeting traits under different conditions. Such candidate genes are more likely involved in seed quality traits.

Further investigation is required on seeds of RILs for germination under high temperature by salt interactions. Such research will most likely result in new findings on the genetic by environment interactions that influence seed and seedling quality.

7. List of Tables

8. List of Figures

9. Reference

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10. Appendix

10.1 Appendix-I: Average output for five germination parameters under control conditions

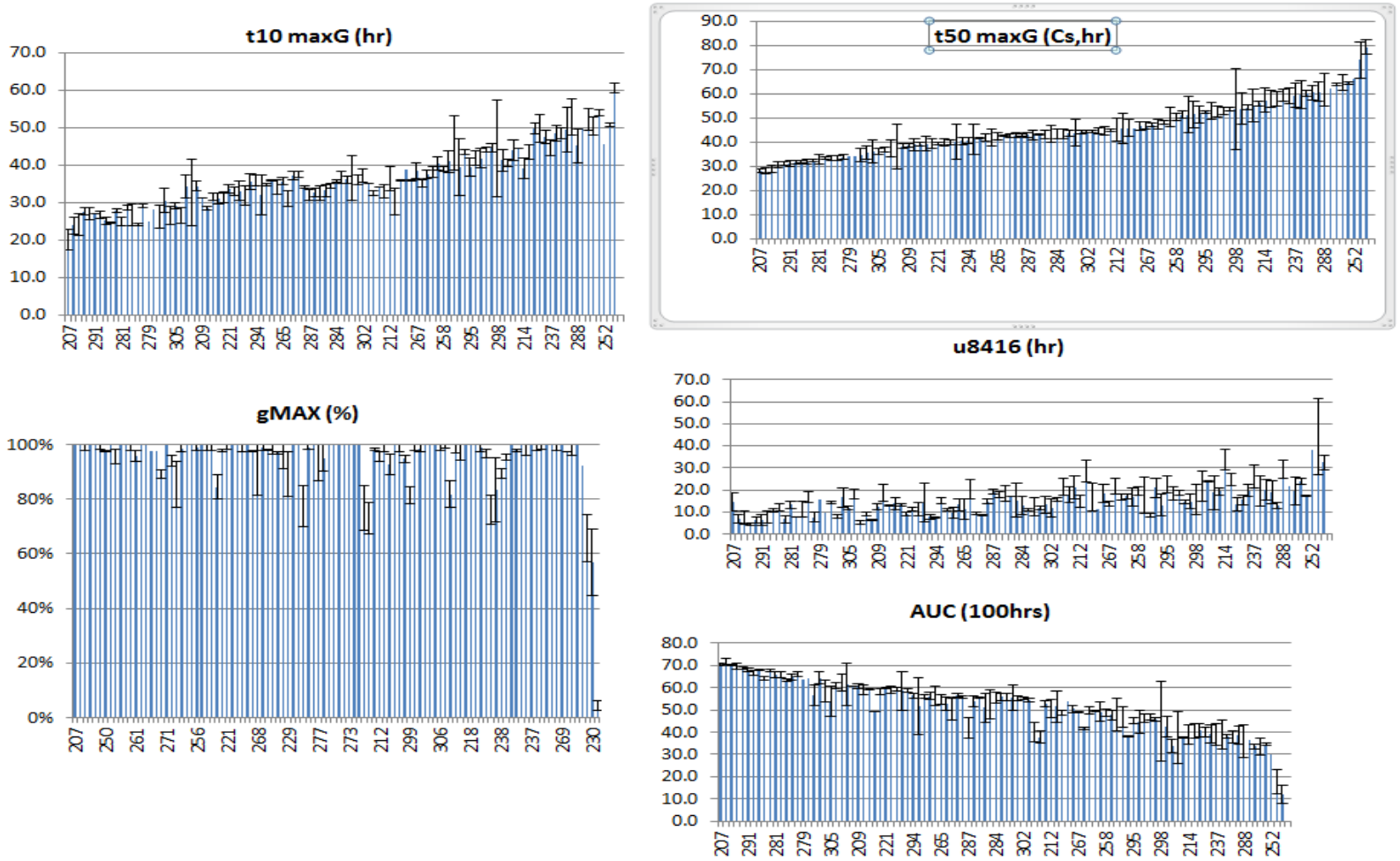

Figure 10 . Average and standard error (± SE) of recombinant inbred lines and parents for t10, t50, MaxG, U8416 and AUC under control conditions

10.2. Appendix-II: Average output for five germiantion parametres under salt stress

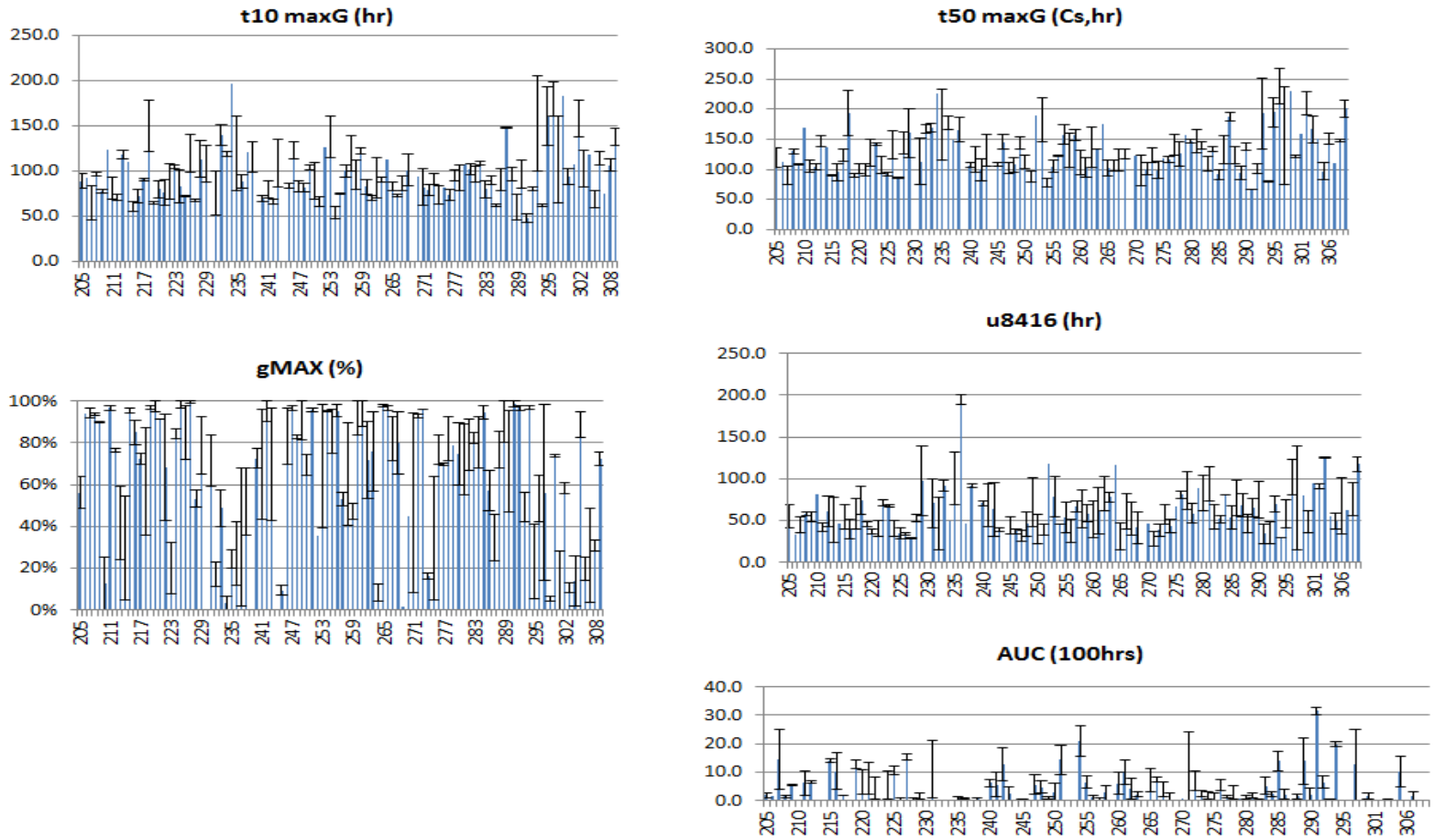

Figure 11. Average and standard error (± SE) of recombinant inbred lines and parents for t10, t50, MaxG, U8416 and AUC under salt stress conditions

10.3. Appendix-III. Average output for high temperature condition

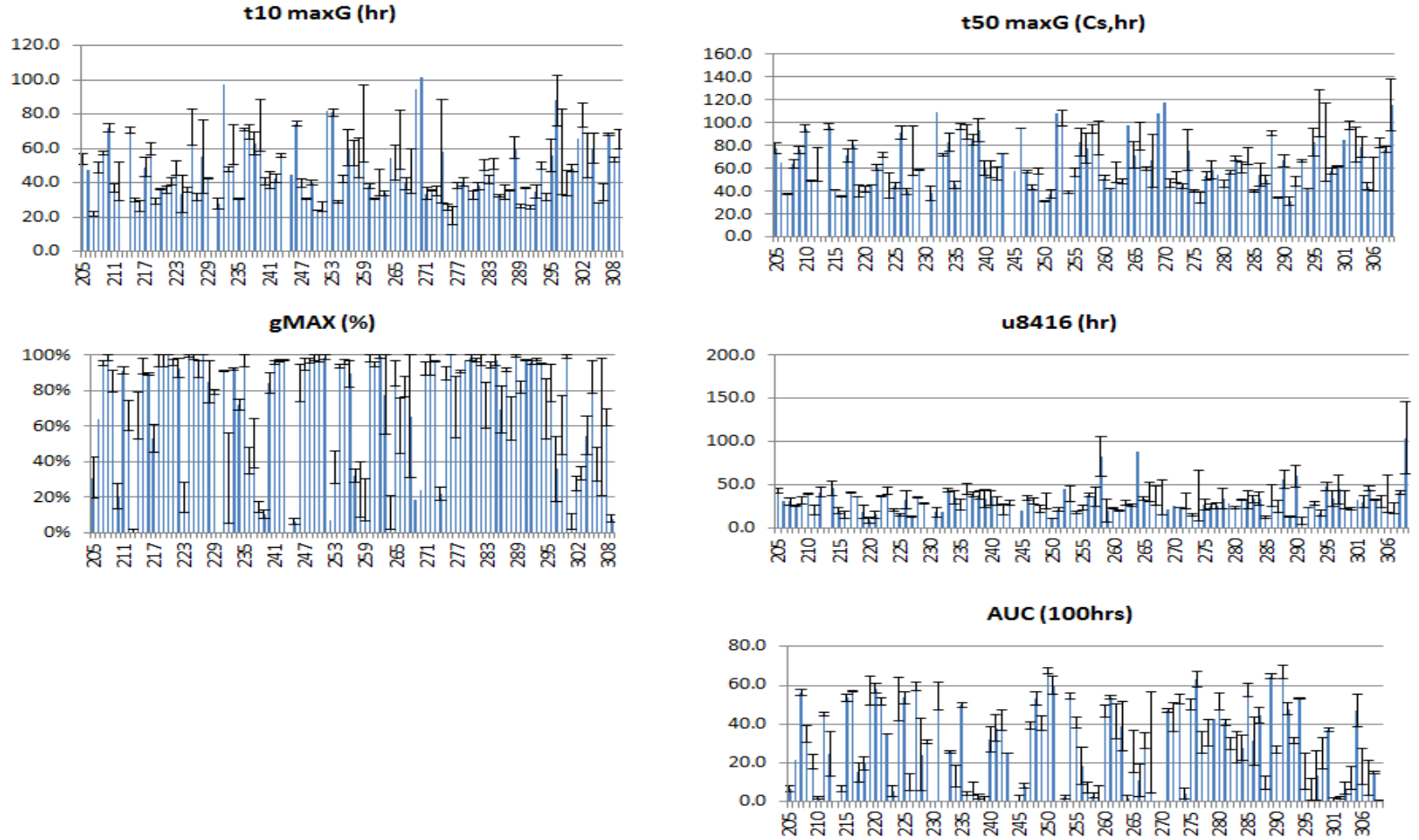

Figure 12. Average and standard error (± SE) of recombinant inbred lines and parents for $t_{10,}$ t50, MaxG, U8416 and AUC under high temperature (35 ^{0}c) condition

Working plan for the month of November 2014- February 2015

S.No	Activities	November	December	January	February
1	Literature study				
2	Introduction to lab. work and pollination				
3	Experimental set up				
4	Data collection				
5	Writing on introduction				
6	Data analysis				
7	Draft report				
8	Presentation				
9	Final report				

Pollination activities in greenhouse