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Genetic Variability and QTL Mapping for Seed Germination Characters under Water Stress in Sunflower

Asa Ebrahimi[1*] and Ahmad Sarrafi[2]

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[1]Dpartment of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2]Department of Biotechnology and Plant Breeding, Toulose University, France

*Corresponding author: e-mail: dr.asaebrahimi@gmail.com

Abstract

The objective of this research was to study the genetic control of germination variables under normal and water deficit conditions in sunflower. A population of 100 recombinant inbred lines obtained from a cross between PAC2 and RHA266 was used in the present study. The experiment was performed as a randomized complete block design with three replications. Germination variables were time to starting germination (TSG), time to maximum germination (TMG), time to 50% germination (T50%G) and percentage of seed germination (PSG). The putative causes of variation in germination, oil content (OC) and 1000 grain weight (1000GW) were also measured. Positive correlations were obtained between different times to germination for normal and stress conditions. Positive correlations were observed between PSG and OC, as well as between 1000 GW and the different times to germination. Several non-specific QTLs to water deficit were detected for germination parameters. "*HA1837*", "*SSL27*"and "*ORS671_1*" were the SSR markers associated with QTLs detected for germination characters independent of germination environments. The most important specific QTL was associated with "*ORS677*" SSR marker in the water stress condition, explaining 24% of the phenotypic variance for PSG.

Keywords: Drought stress; QTL; SSR marker; Times to germination

Introduction

Seed germination and early seedling growth are the most sensitive stages under environmental stresses (Cook 1979; Jones 1986). Water stress is one of the most severe limitations of crop growth in the semi-arid and arid regions of the world as it impacts a vital effect on plant metabolism at all growth stages. Therefore, depending on plant species, water stress could be critical for germination and seedling development.

Genetic studies have shown that the relative ability to germination in salt or osmotic solution is heritable in tomato (Foolad and Jones, 1991; Saleki *et al*. 1993). On the other hand, Bettey *et al*. (2000) reported lower heritability of mean germination time under normal and stressed conditions (14 and 24%, respectively) in *Brassica oleracea*. Foolad *et al*. (2003) reported significant genetic correlations among seed germination rate under cold, salt and PEG treatments in tomato. According to Ecker (*et al*. 1994), the genes with additive action controlled high germination speed while slow germination speed appeared to be induced by genes with pleiotropic effects in *Eustoma grandiflorum.* Significant genetic correlation was observed between germination rate and grain weight in soybean (Singh *et al*. 1978).

There is a wide variation among species for the minimum water required for plant growth (Evans and Etherington 1990). Since the water potential of dry seed is very low (generally between -350 and -50 MPa) and the gradient for water uptake is large, when water in the

environment is in the physiological range, germination can occur, from 0 to approximately -2 MPa (Roberts and Ellis 1989). According to Heydecker *et al.* (1975) polyethylene glycol (PEG) is commonly used as an osmotic-priming agent, because of its relatively inert and non-toxic nature. PEG was used as drought treatment in sunflower germination with different concentration (Lenzi *et al.* 1995; Turhan and Baser 2004).

Seed germination in oilseed plants involves degradation of oil bodies by sequential and/or collective action of many hydrolytic enzymes such as proteases, phospholipases, lipoxygenase and lipase at different stages of lipolysis (Lin and Huang 1983; Wang and Huang 1987; Feussner *et al.* 1996; May *et al*. 1998; Sadeghipour and Bhatla 2002). Ujjinaiah *et al*. (1989) reported that higher seed oil percentage will lead to higher germination percentage in sunflower. While, Ahmad (2001) observed a negative correlation between oil content and germination percentage in sunflower.

Ebrahimi *et al*. (2012) identified several AFLP markers associated with germination percentage under drought stress in sunflower mutant lines. Three mutant genes were identified in *Arabidopsis thaliana* that decreased water potential for seed germination in saline or osmotic (mannitol) solutions (Saleki *et al*. 1993). Two genes, encoding isocitrate lyase (*IL*) and malate synthase (*MS*), were detected for germination and post-germination characters in the programmes studying gene expression. *IL* and *MS* were important glyoxylate cycle enzymes which involved in stored lipids mobilization of plant seedlings (Terry 1993).

In-vitro screening methods are considered as rapid and easy screening tests for studying the response of genotypes to water stress conditions. Moreover, genetic markers have become an important tool in the selection and screening of plants under environmental stresses (Jahromi 1996). Germination and emergence characteristics are complex traits, likely to be controlled by a number of genes, and therefore require quantitative trait loci (QTL) analysis. This analysis integrates molecular marker linkage maps with data obtained on quantitative traits to give information on the effects and locations of the loci controlling these characters (Kearsey 1998). Al-Chaarani *et al.* (2005) detected four QTLs for germination percentage and two QTLs for time of 50% germination in sunflower in normal condition. The highest phenotypic variance (18%) that was explained by QTL, was observed for time of 50% germination. Davar *et al*. (2015) found 58 QTLs for seed germination, seedling vigour and growth and developmental traits in sunflower. In the water stress condition, one QTL was obtained for mean germination time in *Brassica oleracea* (Betty *et al*. 2000). Some QTLs were identified for salt, cold and drought tolerance during seed germination in tomato (Foolad *et al*. 1998; Foolad 1999; Foolad *et al*. 2003). Barreto Dias *et al*. (2011) determined distinct QTLs for germination under sub- and supra-optimal temperatures on chromosomes 3, 5, 7, 8 in *Medicago truncatula.* An initial set of putative candidate genes was identified in the light of the role of abscissic acid/gibberellin balance in regulating germination at high temperatures (e.g. *ABI4, ABI5*), the molecular cascade in response to cold stress (e.g. *CBF1, ICE1*) in *Medicago truncatula* (Barreto

Dias *et al*. 2011). DeRose-Wilson and Gaut (2011) identified genomic regions involved in germination response under saline condition in *Arabidopsis thaliana*.

As far as our knowledge, the QTL analysis for germination characters of sunflower under water stress conditions has not been reported in the literature. The aim of this study was to identify the chromosomal regions which affect the seed germination characters under normal and PEG treatment conditions.

Materials and Methods

Plant material and experimental conditions

A population of 100 recombinant inbred lines (RILs) was developed through single-seed descent from a cross between PAC2 and RHA266. The RIL population and their two parents were used in order to determine the genetic variability of seed germination characters.

The experiment was performed under normal and polyethylene glycol treatment conditions. Sterile germination medium containing 0.3% w/v phytagel was prepared for both conditions. The germination medium for the water stress treatment included also 0.28% w/v polyethylene glycol (PEG 6000), corresponding to –1.3 MPa of water potential (Lenzi *et al*. 1995; Foolad *et al*. 2003). The seeds were surface-sterilized with 0.5% calcium hypochloride and washed three times in sterile, distilled water and briefly blotted. The seeds were then transferred to petri dishes (95 mm diameter) containing the special medium. Each Petri dish contained 20 seeds (per replication). Petri dishes were arranged as factorial design in incubators maintained in dark where the temperature was 25°C for day (16 h) and 20°C for night (8h). The experiment was carried out as randomized complete block design with three replications.

Trait measurements

The number of germinated seeds was determined for each experimental unit, two times a day, until the time when the percentage of germination was stable at five successive observations. The time to starting germination (TSG) was determined when the protrusion of the radicle was observed for at least one seed in the Petri dish. The time to maximum germination (TMG) was determined when for the first time the percentage of seed germination (PSG) got stable. The time to 50% germination (T50%G) corresponded to 50% of total seed germination in the Petri dish. Oil content (OC) was determined by Nuclear Magnetic Resonance and 1000 grain weight (1000GW) was measured in every experimental unit.

Statistical analyses and QTL mapping

The data was analysed using the SAS PROC GLM (SAS Institute Inc, NC, USA, 1996). A mixed model with water treatment as the fixed factor, and genotype (RILs and parents) as random factor was used for analysis of the data under both conditions. Sunflower reference map recently constructed by Poormohammad Kiani *et al*. (2007), was used for detection of QTLs. This map contained 304 AFLP and 191 SSRs markers with mean density of 3.7 cM per locus. For mapping of QTLs and estimation of their effects, composite interval mapping model 6 on WinQTL Cart (Version 2.5) was used (Wang *et al*. 2005). The control marker number and the window size were 15 and 15 cM, respectively. The experiment-wise threshold level to declare linkage was

calculated from 1000 permutations of each genotype marker against the phenotype in the population. Linkage was reported as significant if the two statistics for a marker were greater than the critical value at P=0.05 as described by Kassem *et al*. (2007).

Results

Phenotypic variation and correlations

Analysis of variance of the RILs and their parents ("PAC2" and "RHA266") showed highly significant variation among genotypes and water regimes for all of the germination variables studied (Table 1). Water regime × RIL was also significant indicating a difference among RILs for the germination response to water regimes. Phenotypic performance of RILs and their parents for all traits are summarized in Table 2. Difference between the parents was significant for PSG and TMG under water stress condition. Genetic gain, calculated as the difference between the mean of the top 10% selected RILs ($\overline{X}$ 10%RIL) and the mean of the parents ($\overline{X}_P$), was significant for PSG under stress environment.

Table 1. Mean squares of some germination characters in the sunflower recombinant inbred line

Source of variation	df	TSG	T50%G	TMG	PSG
Drought	1	564696.0**	1270960.1**	2940700.0**	237208.1**
Block	2	338.4	2793.1	6180.2	880.2
Drought× block	2	4259.2***	5088.8***	8058.2***	419.7**
RIL	99	2801.1***	2193.2***	1907.4***	1429.1***
Drought ×RIL	99	2149.6***	1607.6***	1796.8***	922.3***
Error	396	525.8	548.9	685.5	83.0
Total	599				

TSG, time to starting germination (h); T50%G, time to 50% germination (h); TMG, time to maximum germination (h); PSG, percentage of seed germination.
*. **. *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 2. Genetic variability and genetic gain for germination characters in the normal and PEG treatment conditions

	TSG		T50%G		TMG		PSG	
	Normal	PEG	Normal	PEG	Normal	PEG	Normal	PEG
PAC2 (P1)	26.0+	58.0	29.0	98.0	45.0	186.0	96.7	81.7
RHA266 (P2)	29.0	77.0	29.0	89.0	53.0	143.0	93.3	66.7
P1-P2	-3.0 ^{ns}	-19.0 ^{ns}	0.0 ^{ns}	9.0 ^{ns}	-8.0 ^{ns}	43.0 *	3.4 ^{ns}	15.0 *
$\overline{X}_P$ = (P1 + P2)/2	27.5	67.5	29.0	93.0	49.0	164.5	95.0	74.2
MAX RIL	47.0	266.0	64.0	263.0	106.0	282.0	100.0	93.3
MIN RIL	18.0	40.0	20.0	66.0	34.0	114.0	51.7	3.3
$\overline{X}_{RIL}$	24.2	90.0	33.2	125.8	58.2	198.7	94.3	54.2
10% SRIL	18.3	52.6	23.0	81.0	38.3	148.2	100.0	90.0
GG10%=10%RIL- $\overline{X}$ P	-9.2^{ns}	-14.9^{ns}	-6.0^{ns}	-12.0^{ns}	-10.7^{ns}	-16.3^{ns}	5.0^{ns}	15.8*

10%SRIL: the mean of the top 10% selected RILs; GG10%: genetic gain when the mean of the top 10% selected RILs is compared with the mean of the parents. TSG, time to starting germination (h); T50%G, time to 50% germination (h); TMG, time to maximum germination (h); PSG, percentage of seed germination; OC, oil content (%); 1000GW, 1000-grains weight; *Significant at 0.05 probability level; ^{ns} non-significant.
$^{+}$Mean of three replication

Table 3. Correlations among some germination characters under normal (1) and water stress (2) conditions

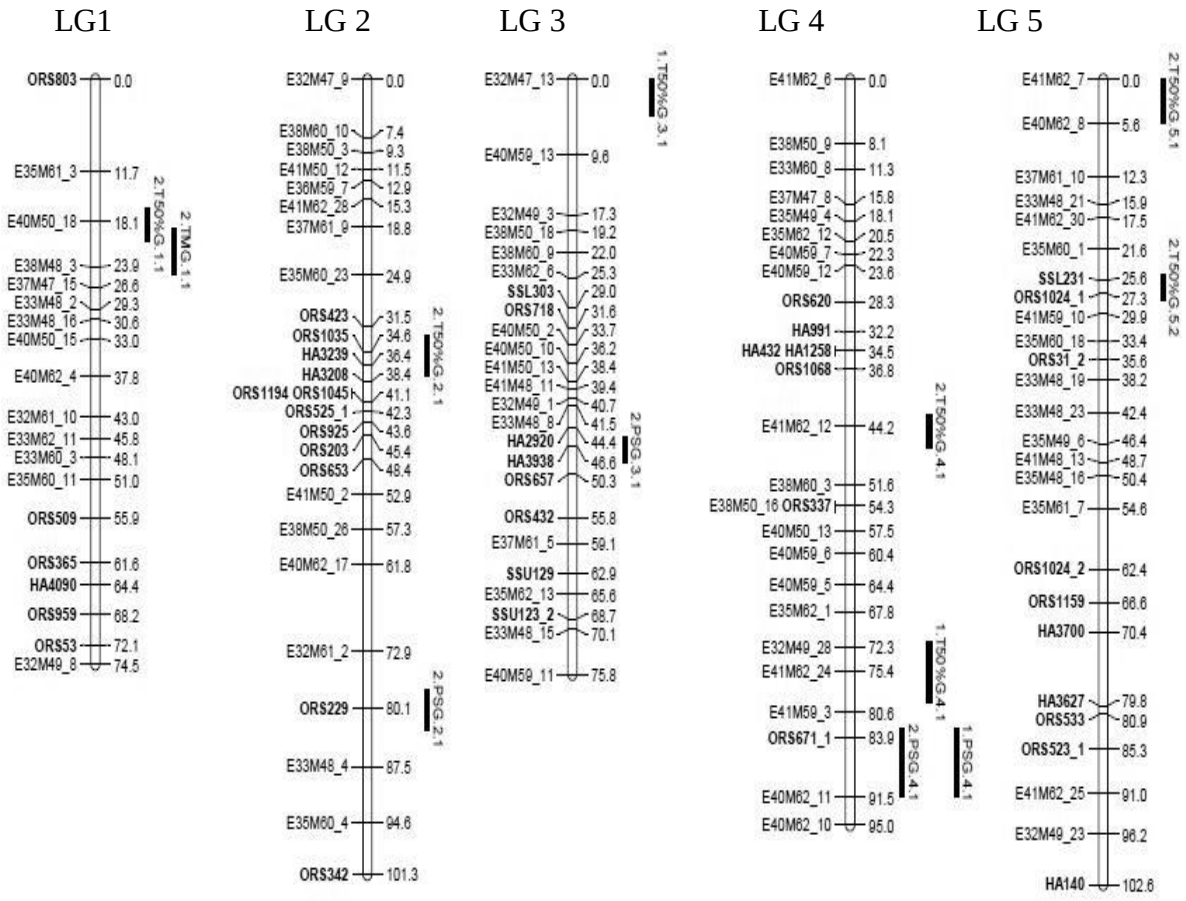
		TSG	T50%G	TMG	PSG	OC
T50%G	1	0.83***				
	2	0.64***				
TMG	1	0.35***	0.60***			
	2	0.40***	0.71***			
PSG	1	-0.62***	-0.50***	-0.06		
	2	-0.36***	-0.65***	-0.53***		
OC	1	-0.39***	-0.37***	-0.13*	0.40***	
	2	-0.34***	-0.47***	-0.36***	0.44***	
1000GW	1	0.83***	0.042	-0.07	-0.12*	-0.32***
	2	0.28***	0.49***	0.42***	-0.49***	-0.30***

TSG, time to starting germination (h); T50%G, time to 50% germination (h); TMG, time to maximum germination (h); PSG, percentage of seed germination.
*' *** Significant at 0.05 and 0.001 probability levels, res

There were positive correlations among the times of starting, 50% and maximum germination (TSG, T50%G and TMG) under both conditions (water stress and normal) (Table 3). Furthermore, positive correlations were observed between PSG and OC in both conditions, as well as between 1000 GW and the times of starting, 50% and maximum germination. Negative correlations of OC and PSG with time of starting, 50% and maximum germination were detected in both conditions.

QTL mapping

The map position and characteristics of QTL associated with the studied traits are summarized in Table 4. QTLs were designated by 1 and 2, for normal and water stress conditions, followed by the abbreviation of the traits (TSG, T50%G, TMG, PGS), the corresponding linkage group and the number of QTLs in the group. For an easier overview of overlapping QTLs between traits and culture media (normal and PEG treatment conditions), all QTL regions were illustrated in Figure 1. The QTLs explained from 5% to 29% of the phenotypic variance (R^2) of the traits. The phenotypic variances explained by the QTLs for the time to starting germination (TSG) ranged from 5% to 20%. The positive alleles for the most important QTL of TSG (*2.TSG.11.1*) under water stress condition (R^2= 20%) came from RHA266, C62, C89, C101, C115 and LR 16a which were the best genotypes for germination under normal environment. The most important QTLs for T50%G were 1.T50%G.11.1, 1.T50%G.10.1, 2.T50%G.5.2 and 2.T50%G.1.1 which explained 29, 24, 17 and 13% of the phenotypic variance of this trait, respectively under normal and stress media. Positive alleles for these QTLs came from RHA266 except for "1.T50%G.10.1". For the time to maximum germination (TMG), the most important QTL (1.TMG.11.2) was on LG11; the favourable alleles which decreased TMG came from RHA266. The most important QTL for PSG (2. PSG.17.3), was located on LG 17 (R2= 24%) in the water stress medium and positive alleles for this QTL came from PAC2.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LG 4
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E33M62_11 45.8
E33M60_3 48.1
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ORS365 61.6
HA4090 64.4
ORS959 68.2
ORS53 72.1
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2.TMG.1.1
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E36M59_7 12.9
E41M62_28 15.3
E37M61_9 18.8
E35M60_23 24.9
ORS423 31.5
ORS1035 34.6
HA3239 36.4
HA3208 38.4
ORS1194 ORS1045 41.1
ORS525_1 42.3
ORS925 43.6
ORS203 45.4
ORS653 48.4
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E38M50_26 57.3
E40M62_17 61.8
E32M61_2 72.9
ORS229 80.1
E33M48_4 87.5
E35M60_4 94.6
ORS342 101.3
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2.PSG.2.1
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E38M60_9 22.0
E33M62_6 25.3
SSL303 29.0
ORS718 31.6
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E40M50_10 36.2
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E32M49_1 40.7
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HA3938 46.6
ORS657 50.3
ORS432 55.8
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E33M60_8 11.3
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E40M59_7 22.3
E40M59_12 23.6
ORS620 28.3
HA991 32.2
HA432 HA1258 34.5
ORS1068 36.8
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E38M50_16 ORS337 54.3
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E41M59_3 80.6
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ORS31_2 35.6
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E35M49_6 46.4
E41M48_13 48.7
E35M48_16 50.4
E35M61_7 54.6
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ORS1159 66.6
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HA3627 79.8
ORS533 80.9
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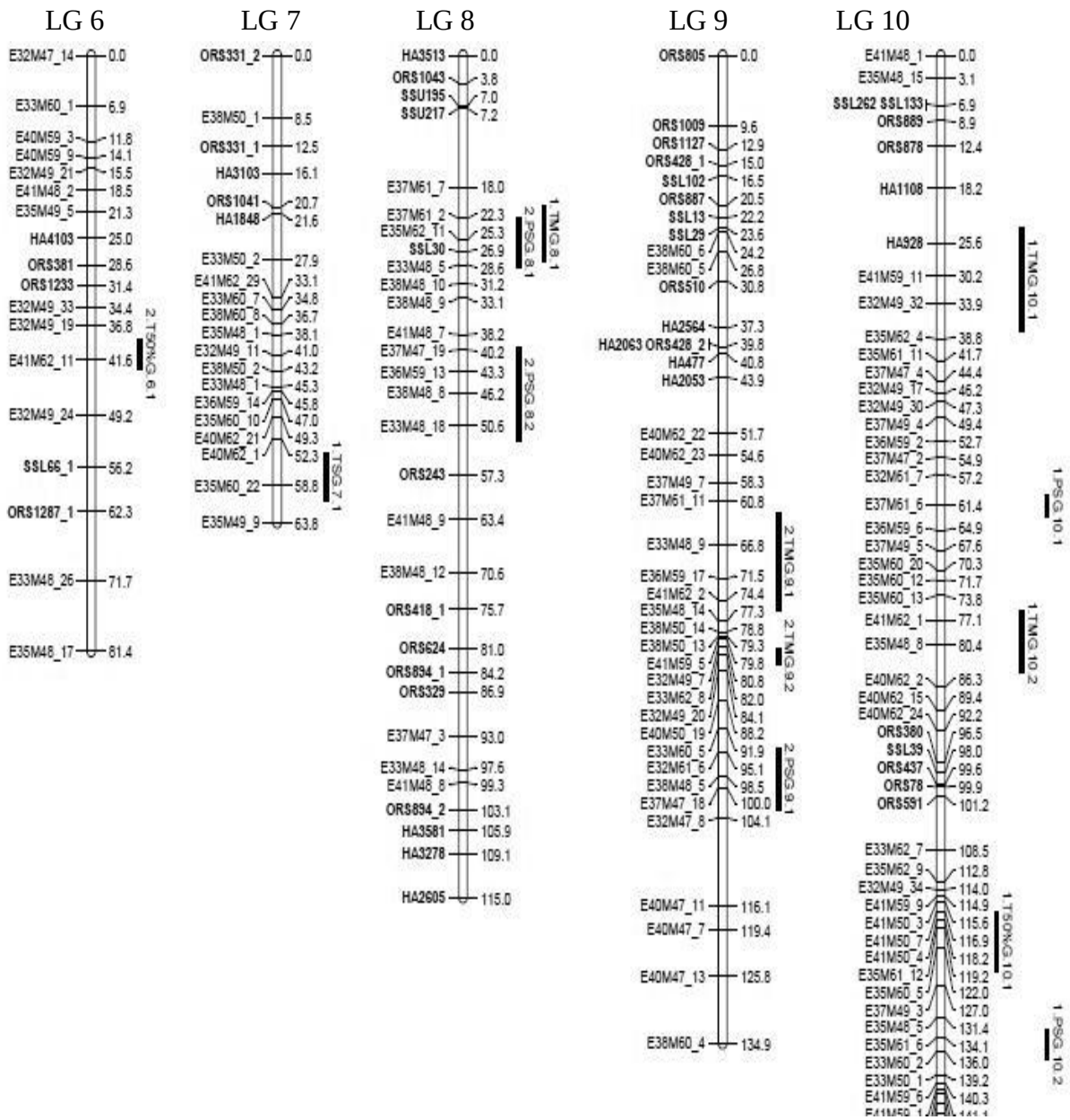
2.T50%G.5.2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HA4103 25.0
ORS381 28.6
ORS1233 31.4
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E32M49_19 36.8
E41M62_11 41.6
E32M49_24 49.2
SSL66_1 56.2
ORS1287_1 62.3
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E35M48_17 81.4
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ORS329 86.9
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E41M48_8 99.3
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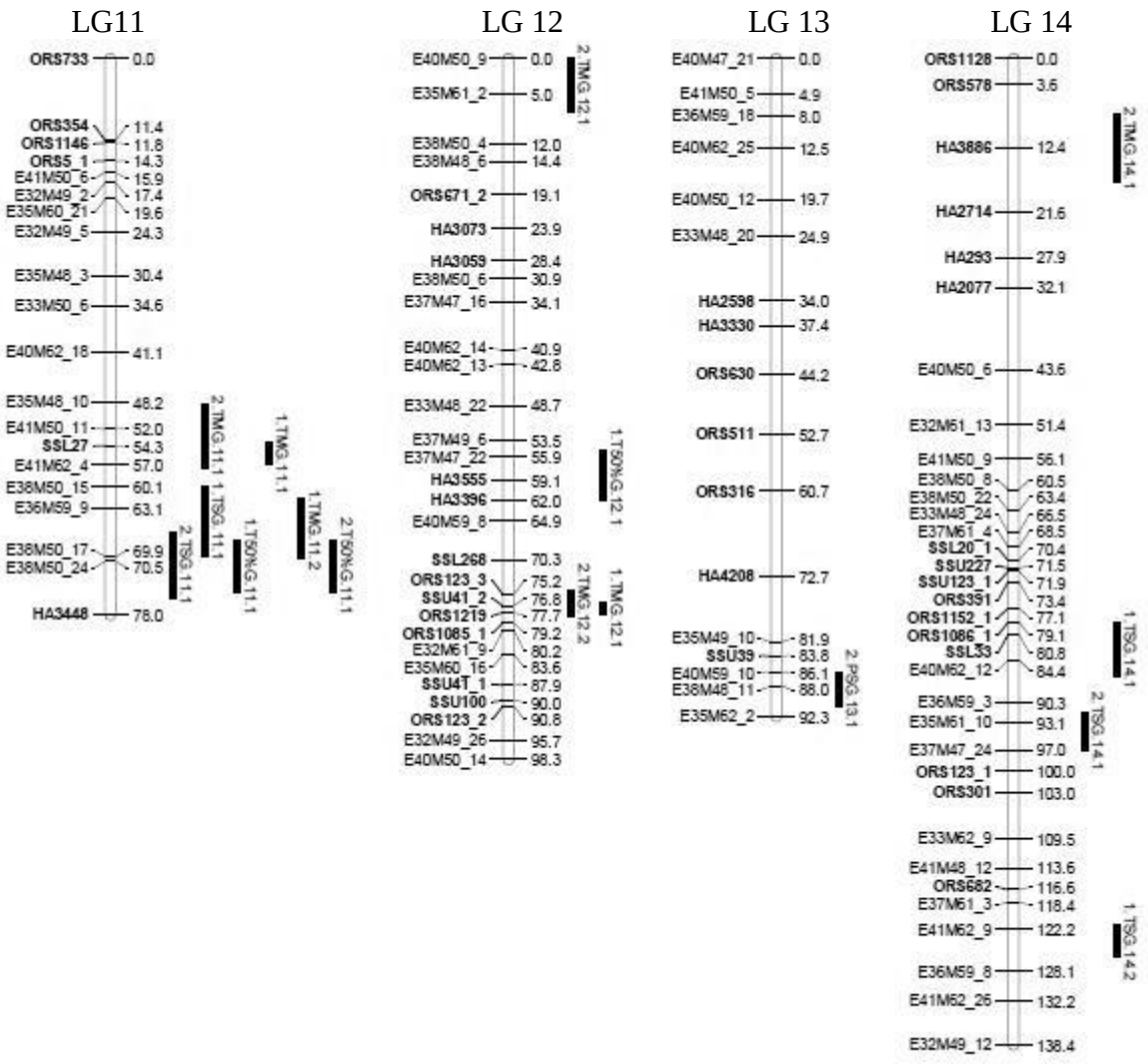
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2 TMG 12.2
LG 13
E40M47_21 0.0
E41M50_5 4.9
E36M59_18 8.0
E40M62_25 12.5
E40M50_12 19.7
E33M48_20 24.9
HA2598 34.0
HA3330 37.4
ORS630 44.2
ORS511 52.7
ORS316 60.7
HA4208 72.7
E35M49_10 81.9
SSU39 83.8
E40M59_10 86.1
E38M48_11 88.0
E35M62_2 92.3
2 PSG 13.1
LG 14
ORS1128 0.0
ORS578 3.6
HA3886 12.4
HA2714 21.6
HA293 27.9
HA2077 32.1
E40M50_6 43.6
E32M61_13 51.4
E41M50_9 56.1
E38M50_8 60.5
E38M50_22 63.4
E33M48_24 66.5
E37M61_4 68.5
SSL20_1 70.4
SSU227 71.5
SSU123_1 71.9
ORS391 73.4
ORS1152_1 77.1
ORS1086_1 79.1
SSL33 80.8
E40M62_12 84.4
E36M59_3 90.3
E35M61_10 93.1
E37M47_24 97.0
ORS123_1 100.0
ORS301 103.0
E33M62_9 109.5
E41M48_12 113.6
ORS682 116.6
E37M61_3 118.4
E41M62_9 122.2
E36M59_8 128.1
E41M62_26 132.2
E32M49_12 138.4
2 TMG 14.1
1 TSG 14.1
2 TSG 14.1
1 TSG 14.2

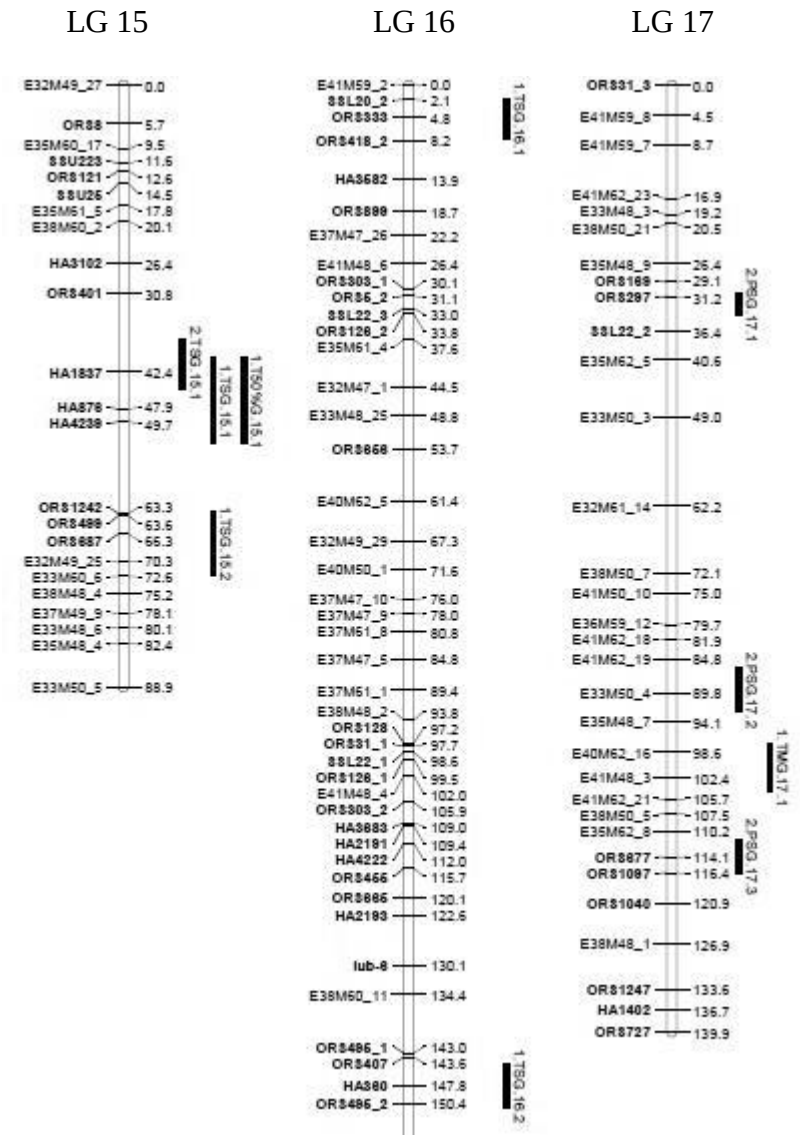

Figure 1. Improved molecular genetic linkage map of sunflower based on 304 AFLP and 191 SSR markers using 100 'PAC2 × RHA266' recombinant inbred lines (RILs). The position of QTLs were presented on the right side of linkage groups. Bars represent intervals associated with the QTLs. The numbers before QTLs name: 1. Normal; 2. PEG treatment. TSG; Time to starting germination (h), T50%G; Time to 50% germination (h), TMG; Time to maximum germination (h), PSG; Percentage of seed germination LG; Linkage group. (A)

Discussion

Phenotypic variation among genotypes

Water stress affected all of the germination characters significantly. In addition, there were differences among the RILs in terms of their response to water deficit. Turhan and Baser (2004) and Lenzi *et al.* (1995) showed significant differences for response to water deficit among their sunflower genotypes. Furthermore, Ebrahimi and Sarrafi (2012) presented similar findings in a population of sunflower mutant lines obtained by gamma radiation. Water treatment×RIL interaction was significant, suggesting that response by a genotype in relation to other genotypes varies between the two germination media.

Correlations were positive among all times to starting, 50% and maximum germination (TSG, T50%G and TMG) under normal and water stress conditions. Foolad *et al.* (1998) also detected positive correlation between impotent times to germination in tomato under salt stress. The strong or moderate correlations among the times to germination indicated that some similar or identical genes and physiological mechanisms control the different germination characters (TSG, T50%G and TMG) in sunflower under normal and water stress conditions. Davar *et al*. (2015) reported a significant correlation between days to seedling emergence and V4 stage in sunflower. Negative correlations between PSG and times to starting, 50% and maximum germination (TSG, T50%G and TMG) were obtained under both conditions, which corresponds with the results of Al-Chaarani *et al*. (2005) under the normal condition in sunflower. These results indicated that rapid germination is associated with a high percentage of germination. Negative correlations between seed oil content and time to starting, 50% and maximum germination suggested that genotypes with a high percentage of seed oil content have a rapid seed germination. Negative correlation was observed between 1000 GW and PSG.This was also reported by Singh *et al.* (1978) in soybean and by Torres *et al*. (1991) in sunflower. Ebrahimi and Sarrafi (2012) also showed that some sunflower mutant lines had high germination percentage under water stress condition.

Significant differences were observed between the parents under water stress condition for PSG and TMG (Table 2). This result suggests that parental lines carry different genes for adaptation to water stress. Genetic gain, considered as the difference between the mean of the 10% selected RILs and the mean of the parents ($\overline{X}$ P), was significant for PSG under the water stress treatment, showing transgressive segregation for this trait. Davar *et al*. (2015) reported significant genetic gain for speed of germination and days to seedling emergence in sunflower. Some studies indicated that selection for rapid germination under stress condition results in progeny with improved germination under normal condition in tomato (Foolad *et al*. 1999; Foolad *et al*. 2003). These studies supported a previous suggestion that similar physiological mechanisms may control the rate of seed germination under different environmental conditions (Bradford 1995). However, for

practical purposes, it is important to determine whether the same or different genes control the rate of tomato seed germination under water stress and normal conditions.

QTL analysis

QTLs identified in the present study showed that several putative genomic regions were involved in the expression of the germination traits under two experimental conditions (Table 4). Two types of QTLs for germination characters were identified: QTLs which affected germination characters in the normal and water stress media (non-specific QTL) and QTLs which affected germination traits only in one environment, either normal or water stress condition (specific QTL). The identification of non-specific QTLs indicated the presence of relationships between germination characters in the water stress and normal conditions. However, whether such genetic relationships were due to pleiotropic effects of the same genes, physical linkage of different genes, or combination of the two, it could not be determined in this study. Isolation and characterization of functional genes affecting germination rate under different conditions may resolve this issue. The detection of non-specific QTLs however is consistent with the significant correlations observed among germination characters under normal and water stress conditions (Table 3). The results are also in agreement with positive correlation obtained between rate of tomato seed germination of non-stress and stress (cold and salt) conditions (Foolad and Lin 1999; Foolad *et al.* 1999).

Linkage group 11 was most important in relation to times to starting, 50% and maximum germination in sunflower with seven QTLs for TSG, T50%G and TMG under both conditions (non-specific). All of the favorable alleles for the times to starting, 50% and maximum germination on linkage group 11 came from RHA266 except 2.TMG.11.1 QTL \TMG under both conditions (non-specific). All of the favorable alleles for the times to starting, 50% and maximum of germination on linkage group 11 came from RHA266 except 2.TMG.11.1 QTL. Five of these QTLs (1.TSG.11.1, 2.TSG.11.1, 1.T50%G.11.1, 2.T50%G.11.1, 1.TMG.11.2) were overlapped; this region probably has pleiotropic effect for the times to starting, 50% and maximum germination in sunflower. Two other QTLs (1.TMG.11.1,2.TMG.11.1) were also overlapped in this linkage group (11). Thus this linkage group is important for times to starting, 50% and maximum germination in the normal and water stressed conditions. The high number of overlapping QTLs for germination characters was confirmed also by positive correlations observed among all time to starting, 50% and maximum germination (Table 3). Bettey *et al*. (2000) also detected the overlapping for times to germination in *Brassica oleracea*. Barreto Diase *et al*. (2011) also reported an overlapped QTL for times to 20% and 50% germination rate in the linkage group 5 in *Medicago truncatula*. We detected two SSR markers: "*HA1837*" under the normal and water stress media and "*ORS499*" under the normal condition which were linked to TSG on linkage group 15. Another SSR marker (*SSL33*) was linked to TSG (*1.TSG.14.1*) which has been observed by Al-Chaarani *et al*. (2005) for seedling shoot length under non-stressed condition. Davar *et al*. (2015) also presented "ORS499" as an

associated marker linked to dry shoot weight in sunflower. We detected also marker "*E37M61_6*" linked to PSG which was reported for percentage of normal seedling by Al-Chaarani *et al*. (2005). The QTL for time to maximum germination (*1.TMG.8.1*) was overlapped with the QTL of PSG (*2.PSG.8.1*) on the linkage group 8.This region has probably pleiotropic effects for these traits. In the linkage group 4, non-specific QTLs (*1.PSG.4.1* and *2.PSG.4.1*) were detected which determined 9% and 17% of the phenotypic variation (R^2), respectively; The favorable alleles came from PAC2. This region could be important in breeding programs as it is linked to SSR marker "*ORS671-1*" controlling PSG under normal and water stress conditions in sunflower. This finding indicates the presence of germination-related common QTL/genes in sunflower which affect germination rate under different conditions. Foolad *et al*. (2007) also presented two non-specific QTLs for germination under cold, salt and drought stress on the linkage group 1 in tomato.

There were important overlapping QTLs for TSG (*1.TSG.15.1*, *2.TSG.15.1*) under both water treatments. These QTLs were linked to SSR marker "*HA1837*" and the favorable alleles for both QTLs under both water treatments came from RHA266. The presence of common QTL suggests presence of genetic relationships between the ability to germinate rapidly under different conditions and the expectation that selection and improvement of seed germination under one condition would lead improved germination under another condition.

The SSR marker "*SSL27"* was linked to TMG under both water treatments in the linkage group 11. The favorable alleles for the QTLs (*1.TMG.11.2*, *1.TMG.11.2*) came from different parents under each water treatment condition which show the existence of different alleles for drought tolerance in different parents for the identified QTLs. We found also two QTLs for times to starting, 50% and maximum germination which were reported for seed oil content by Ebrahimi *et al*. (2008). These common QTLs in both studies confirm again the effect of seed oil content for reducing times to starting, 50% and maximum germination.

Linkage group 17 was interesting because of controlling tolerance to water stress treatment with three QTLs for PSG. The most important (*2.PSG.17.3)* was linked to the SSR marker "*ORS677*" explaining 24% of the phenotypic variance (R^2). Several specific QTLs for PSG were identified for water stress tolerance which were linked to SSR markers in the linkage groups 2, 3, 4, 8 and 17.

As far as we know, there is only one QTL study on germination of sunflower in which four QTLs for PSG was detected (Al-Chaarani *et al*. 2005) with only one linked SSR (*ORS5*3) marker. Among 10 detected QTLs of PSG, six of them were associated to the SSR markers *ORS229*, *HA3938*, *ORS671-1*, *SSL30*, *ORS297* and *0RS677*. Two QTLs (*SSL30*, *ORS297*) were linked to palmitic and linoleic acid content, respectively (Ebrahimi *et al*. 2008).

In conclusion, 52 QTLs were identified for germination characters under normal and water stress conditions in the present study. A low rate of seed germination under water stress condition was mainly due to the reduced water potential of

the germination medium (Bradford 1995). Therefore, it is expected that seeds with rapid germination under water stress environment would also germinate rapidly under normal condition. This is in agreement with our findings, which indicated the presence of a significant correlation for the rate of germination under normal and water stress conditions. These results support the suggestion that the same genes or physiological mechanisms may control the rate of seed germination under both normal and water stress conditions. Isolation, characterization and comparison of functional genes, which facilitate rapid seed germination in normal and water stress conditions, would be necessary to verify this suggestion.

The identification of specific and non-specific QTLs for germination-related traits, indicate that marker assisted selection may result in the development of germplasm with improved germination under both normal and water stress conditions.

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