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Genetic analysis and QTL mapping of agro-morphological traits in sunflower (*Helianthus annuus* L.) under two contrasting water treatment conditions

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Abstract

The present study was undertaken to investigate the genetic basis and map quantitative trait loci (QTLs), controlling agronomic traits in sunflower under well-watered and water-stressed conditions. Recombinant inbred lines (RILs) coming from the cross between sunflower parental lines PAC2 and RHA266 were evaluated in a rectangular 8×9 lattice design with two replications in each treatment conditions. High genetic variability and transgressive segregation was observed for all studied traits in both water treatment conditions. Significant correlations were observed among studied traits. QTL-mapping was performed using a recently developed SSR sunflower linkage map. One to eleven QTLs were found for studied trait across two water treatment conditions. The percentage of phenotypic variance (R^2) explained by QTLs ranged from 0.23 to 48.89%. Based on overlapping support intervals, the co-location of QTLs for studied traits was determined. QTLs controlling most of the traits were overlapped on different linkage groups, which was in accordance with the phenotypic correlation results among the traits. A comparative analysis of identified QTLs herein with those described in previous studies for drought adaptive traits revealed a number of QTLs in common. These QTLs have potential use in marker-assisted selection.

Keywords: composite interval mapping, drought stresses, drought tolerance, genetic gain, recombinant inbred lines, rectangular lattice design, transgressive segregation.
Abbreviations: AFLP: amplified fragment length polymorphism, BIO: total dry matter, CIM: composite interval mapping, GLM: general linear model, GYP: grain yield per plant, HD: head diameter, HGWL: 100-grain weight, LAD: leaf area duration, LN: leaf number per plant, NA: number of achene, PL: petiole length, PH: plant height, QTL: quantitative trait loci, RILs: recombinant inbred lines, SD: stem diameter, SSD: single seed descent, SSR: simple sequence repeat.

Introduction

Crop responses to drought stresses involve processes modulated by water deficit at morphological, anatomical, cellular and molecular levels. The changes which occur in all plant organs in response to water stress decrease plant photosynthesis resulting in grain yield deduction (de la Vega et al., 2007; Richrds, 2006). It would be very useful to develop effective strategies to reduce drought stress damage to crop plants. A strategy involves producing a high yielding genotype with traits leading toward drought tolerance (Tardieu and Tuberosa, 2010). Since the genetic mechanism of drought tolerance in crop plants is very complex and seed yield is strongly influenced by genotype and environmental conditions (Karamanos and Papatheohari, 1999; Andjelkovic and Thompson, 2006), conventional breeding methods did not result in significant progress in this field. Recently, molecular marker technologies have been successfully used to decipher the nature of crop plants responses to drought stress (Poormohammad Kiani et al., 2007a,b; 2008; 2009; Rauf 2008). Sunflower (*Helianthus annuus* L.) is one of the most important oil crops worldwide. Among abiotic stresses, the major limiting factor for sunflower yield is drought stress. The effect of water stress on sunflower has been studied in several research works (Haddadi et al., 2011a; Nezami et al. 2008; Poormohammad Kiani et al. 2009; Razi and Assad 1998). Nezami et al. (2008) showed that plant height, biological yield, stem diameter, head size, seed number per head and 1000-grain weight decreases under dried and semi-dried conditions. Razi and Assad (1998) found that irrigation led to an increase in days to physiological maturity, head size, stem diameter, number of leaves per plant, plant height, 1000-grain weight, harvest index, and grain yield. Haddadi et al. (2011a) and Poormohammad Kiani et al. (2009) detected genomic regions associated with leaf-related traits and yield components under well-irrigated and partially irrigated conditions. QTL mapping aims at understanding the genetic basis of economically important traits and facilitate plant breeding via marker-assisted selection (Sarrafi and Gentzbittel, 2004). However very few studies have been carried out to detect molecular marker associated with QTLs in sunflower under water-stressed conditions (Haddadi et al., 2011a; Poormohammad Kiani et al., 2009). The objectives of the present study were to investigate the genetic variation of sunflower under well-watered and water-stressed conditions and identifying QTLs, controlling sunflower's agro-morphological traits under above conditions using a population of RILs.

Results

Phenotypic variation

Combined analysis of variance showed that watering condition significantly influenced most of studied traits in

different genotypes of sunflower (Table 1). The mean squares due to interactions between genotype and water treatment were non-significant for plant high (PH) and leaf number per plant (LN). Slicing significant interaction effects revealed that there was significant difference in head diameter (HD), stem diameter (SD), number of achene (NA), grain yield per plant (GYP) and total dry matter (BIO) among studied genotypes in both watering conditions. While petiole length (PL), plant high (PH) and leaf area duration (LAD) was only differed significantly among examined genotypes in well-watered condition (Table 1). The genetic parameters and phenotypic variation observed among RILs and their parents are presented in Table 2. The average value for each studied traits over RILs were higher than those of parents in water treatment regimes except for PL, HD, and leaf number per plant. LN and the average value of RILs were less than those of parents in both water treatment conditions. However, the difference between mean of RILs ($\overline{X}_{\text{RILs}}$) and their parents ($\overline{X}$ p) was significant only in 100-grain weight (HGW) under water-stressed condition (Table 2). The performance of the RHA266 was better than that of PAC2 in all characters except for BIO, PH and PL in both watering conditions. The difference between parents was not significant for all examined traits in both watering conditions except for NA that their difference was significant under water-stressed condition (Table 2). High standard deviation was observed for all studied traits in both watering conditions. Genetic gain calculated as difference between the mean values of 10% selected best RILs ($\overline{X}_{\text{10\%bestRILs}}$) and the mean of parents ($\overline{X}$ p) was significant for all studied traits in both watering conditions (Table 2). The average value of most of traits decreased under water stressed condition in comparison with well-watered condition. In general, transgressive segregation was observed for all studied traits in studied RILs population.

Correlation analysis

The phenotypic correlations between watering conditions and all studied traits were positive and highly significant except for the HGW which did not show any correlation with plant height (PH) and NA under well-watered condition (Table 3). Correlations among HGW, LAD, BIO and GYP were positive and significant under water-stressed condition. A significantly positive correlation was also observed between SD, NA, LN and plant height (PH) under water-stressed (Table 3). Positive correlation was observed between GYP and NA, LAD, and PL, LN and PH as well as among HGW, HD and LN. There was a significant negative correlation between HGW and NA under water-stressed condition. In addition, plant high also negatively correlated with SD, HGW, and HD in water-stressed condition (Table 3). Pair-wise correlations between traits in two water treatment conditions for all studied traits are summarized in Table 3. High significant correlations were observed between water treatments for all studied traits except for PL and LAD.

QTL analysis

The map position and characteristics of QTLs associated with the studied traits under well-watered and water-stressed conditions are presented in Table 4 and 5. QTL names were constructed using the trait abbreviation name suffixed with numbers presenting the linkage group and order of QTL on the linkage group.

The QTLs names were also followed by either W or S presenting well-watered and water-stressed conditions, respectively. For an easier overview of overlapping QTLs between traits and water treatment conditions, an image of all QTL regions was presented in Supplementary data 1. A total of 64 QTLs were identified for studied traits in two watering conditions. The QTLs correspond to various traits in different watering conditions were located throughout the genome except on linkage group 1, 4 and 7 (Supplementary data 1). One to eleven QTLs were found for studied trait across two watering conditions. Individual QTLs explained 0.23 to 48.89% of phenotypic variance. The sign of additive gene effects showed that favourable alleles for studied traits come from both parental lines. 18.75% of the identified QTLs (12 of 64) were co-localized on several linkage groups (Table 4, 5, Supplementary data 1). In well-watered condition, overlapping QTLs were found on linkage group 9, 10 and 17. In water-stressed condition, overlapping QTLs were found on linkage group 13 and 17. Some QTLs identified for some traits in well-watered condition were co-localised with QTLs of the same traits in water-stressed condition (Table 4, 5, Supplementary data 1). For PH, eight and one QTLs were identified under well-watered and water-stressed conditions, respectively. The phenotypic variance varied from 0.7 to 16.95%, and positive alleles come from both parents. The major QTL (PHW.10.1) located on linkage group 10 which was responsible for 16.95% of phenotypic variation under well-watered condition and maternal line 'PAC2' contributed to positive allele. Two QTLs were identified for HD, one on linkage group 14 controlling trait in well-watered condition and other on linkage group 17 controlling trait in water-stressed condition. These QTLs had small effects, responsible for less than 1% of the phenotypic variation. The positive alleles for these QTLs come from PAC2.

Five QTLs were identified for SD of those three QTLs control trait under well-water condition and two in water-stressed condition. The phenotypic variance explained by QTLs ranged from 0.23 to 32.43%. The positive alleles for these QTLs come from both parents. Among five QTLs detected for SD, one QTL located on linkage group 17 (SDW.17.1; SDS.17.1) was common in both water treatment conditions.

Two major QTLs were identified for SD on linkage group 10 (SDS.10.1; SDS.10.2) under water-stressed condition, accounting for 39.1% of the phenotypic variance. Nine QTLs were detected for GYP on linkage groups 12, 13, 14, 16 and 17, of those five QTLs control trait in well-watered condition and four in water-stressed condition, accounting for 0.4 to 9.85% of the phenotypic variation. Among identified QTLs, three QTLs were co-localized on linkage groups 12, 13 and 17. The major QTL (GYP.14.1) for GYP was located on linkage group 14 and explained 9.85% of the phenotypic variance, which was specific to water-stressed condition. The positive allele for this QTL comes from RHA266. Four QTLs were detected for HGW on linkage groups 10 and 17 which those two QTLs control trait in well-watered condition and the other two in water-stressed condition with phenotypic variance accounting for 0.26 to 16.8%. Among identified QTLs for HGW, two QTLs were detected only in water-stressed condition and the other two were common in both water treatment conditions. Positive alleles of three QTLs come from PAC2 and for other QTL come from RHA266. Five QTLs were identified for PL on linkage groups 3, 5, 9 and 16, with the phenotypic variance

Table1. Mean squares of agro-morphological traits in sunflower recombinant inbred lines (RILs) and their two parents under two water treatment conditions

Source of variation	Df	MS									
		PH	HD	SD	GYP	HGW	BIO	NA	PL	LN	LAD
Environment	1	76.08^{ns}	62.59***	2.53***	452.61***	1.45*	1314.85**	165304.71***	30.54***	32.02**	63868.6***
Replication (Environment)	2	58.23^{ns}	98.09***	3.57***	347.21***	4.28*	3506.57***	41270.23**	72.7***	62.28***	30673.17***
Genotype	71	171.09***	12.23***	0.15***	141.19***	4.92***	938.57***	25292.69***	4.09***	12.23***	5816.27***
G ×E	69	91.42^{ns}	4.63**	0.07*	60.34**	1.07*	265.24***	12132.34*	3.01**	2.91^{ns}	3142.12*
Blok (Environment × Replication)	32	117.07^{ns}	5.71***	0.1**	54.92*	1.5*	317.25***	11083.83^{ns}	3.08*	4.93^{ns}	4335.49**
Residual	94	85.58	2.42	0.045	33.75	1.06	124.38	7967.37	1.74	4.14	2145.54
GE effect sliced by E for G											
Water-stressed		85.61^{ns}	7.03***	0.06 *	69.19**	2.78***	391.66***	8408.66*	2.21^{ns}	6.31*	2280.21^{ns}
Well-watered		128.9*	6.53***	0.11***	109.26***	2.87***	651.62***	20716***	2.87*	7.89**	4916.74***
CV%		11.95	14.99	18.1	27.42	21.78	26.43	28.52	19.73	10.93	30.05

PH, plant height; HD, head diameter ; SD, stem diameter; GYP, grain yield per plant; HGW, 100-grain weight; BIO, total dry matter per plant; NA, number of achene; PL, petiole length; LN, leaf number per plant and LAD, leaf area duration. ns, not significant; *, **, *** significant at 0.05, 0.01 and 0.001 probability level.

Table2. Genetic parameters and gain for PH , HD, SD, GYP, HGW, BIO, NA, PL, PL and LAD in sunflower RILs and their two parents under two water treatment conditions.

Conditions	Item	Traits									
		PH	HD	SD	GYP	HGW	BIO	NA	PL	LN	LAD
Well-watered	PAC2 (P1)	69.3	8.4	0.941	8.5125	3.665	32.57	196.3817	7.5	18.6	100.09
	RHA266 (P2)	67.30	10.70	1.11	16.09	4.44	28.4625	463.24	6.80	20.40	106.428
	P1-P2	2	-2.3	-0.17	-7.5725	-0.77	-4.103	-266.857	0.7	-1.8	6.342
	$\overline{X}_{P}$	68.3	9.55	1.026	16.26	4.05	40.74	339.69	7.15	19.5	103.25
	Max	115.80	17.60	2.35	57.45	10.50	117.68	846.17	12.00	27.20	480.51
	Min	52.20	6.60	0.60	6.27	2.68	14.67	118.07	2.40	13.40	38.64
	$\overline{X}_{RIL}$	76.99	10.86	1.27	17.04	4.83	44.69	401.54	7.03	19.04	138.29
	$\overline{X}_{RIL}$- $\overline{X}_{P}$	8.69	1.31	0.24	0.78	0.78	3.95	61.85	-0.12	-0.46	35.04
	$\overline{X}_{10\%bestRIL}$	99.54	15.54	1.93	38.34	7.73	91.22	635.38	10.03	24.20	279.87
	GG10%= $\overline{X}_{10\%bestRIL}$- $\overline{X}_{P}$	31.24	5.99	0.90	26.04	3.68	60.71	305.57	2.88	4.70	176.61
	STDEV	11.62	2.53	0.35	9.58	1.43	22.84	139.82	1.62	2.65	68.39
	LSD (0.05%)	12.19	2.96	0.29	8.34	1.25	15.05	123.45	1.54	3.36	60.22
Water stressed	PAC2 (P1)	83	9.2	0.875	16.02	3.895	39.69	379.2152	5.8	19.5	67.2
	RHA266 (P2)	73.3	10.1	1.259	16.51	4.1625	41.795	423.8768	5.5	20.3	86.408
	P1-P2	9.7	0.9	0.204	-0.49	0.2675	2.105	-44.6616	0.3	-0.8	19.208
	$\overline{X}_{P}$	78.15	9.65	1.057	12.29	4.03	30.51	286.51	5.65	19.9	76.80
	Max	106.20	21.60	2.11	41.00	10.25	92.82	519.00	12.40	25.60	325.58
	Min	48.40	5.60	0.56	4.11	2.41	11.69	112.14	2.60	13.00	32.14
	$\overline{X}_{RIL}$	77.94	9.93	1.08	14.26	4.68	39.95	329.81	6.36	18.33	106.88
	$\overline{X}_{RIL}$- $\overline{X}_{P}$	-0.21	0.28	0.021	1.97	0.65	9.44	43.3	0.71	-1.57	30.08
	$\overline{X}_{10\%bestRIL}$	94.57	15.20	1.71	30.52	7.88	77.67	455.33	10.17	22.66	218.69
	GG10%= $\overline{X}_{10\%bestRIL}$- $\overline{X}_{P}$	16.42	5.55	0.65	14.26	3.85	36.93	53.78	4.52	2.76	141.89
	STDEV	9.51	2.65	0.31	7.82	1.57	19.37	97.53	2.05	2.47	55.84
	LSD (0.05%)	9.79	1.75	0.23	4.78	1.23	10.86	80.41	1.71	11.45	49.73

$\overline{X}_{P}$: Mean of parents. $\overline{X}_{RILs}$: Mean of all RILs. $\overline{X}_{10\%SRILs}$: Mean of the 10% Selected RILs for each measured characters. GG10%: Genetic gain when the mean of 10% selected RILs is compared with the mean of parents. $LSD_{0.05}$: Least significant differences calculated using $t_{0.05}$ and error mean square of each experiment.

ranging from 2.33 to 20.86%. Two QTLs out of fiver were specific to water-stressed condition. For all QTLs except for QTL located on linkage group 9 that high contribution (11.80%) in phenotypic variance, positive alleles come from the maternal line (PAC2). A total of 13 QTLs were detected for NA across water treatment conditions. Identified QTLs accounted for 2.23 to 48.89% of phenotypic variance and the positive alleles come from both parental lines (PAC2 and RHA266). Seven QTLs were detected for the LAD across both watering conditions of those two QTLs controlling trait in both watering conditions were co-localized. The variation explained by QTLs ranged from 1.28 to 25.4%. PAC2 contributed positive alleles at 5 QTLs and RHA266 at 2 QTLs. In the present study eight QTLs for LN across both watering conditions were detected that explained 1.54 to 16.47% of phenotypic variance. One QTL detected on linkage group 15, comprise 16.47% of phenotypic variance and was identified only in water-stressed condition. One QTL were detected for BIO on linkage group 17. This QTL was co-localized with several QTLs controlling different traits across both two water treatment conditions.

Discussion

Phenotypic variation and the effect of water stress

The significant sunflower genotype and water treatment interaction for most of the examined traits revealed that the genotypes respond differently to water treatment regimes. Detection of genotype × environment interaction is important in QTL studies not only to understand how the genes interact with the environment but also to correctly document the relative effect of QTLs (Maloof, 2003). The parents did not show any difference for studied traits in each of water treatment regimes except for NA that they showed significant difference in water stressed condition. No significant difference between the mean of RILs and the mean of parents for the studied traits shows that the RILs used in this study were representative of possible recombination of the cross 'PAC2 × RHA266'. A wide range of variation was observed among the RILs for all studied traits in both watering conditions. Genetic gain presented as the differences between the mean of the selected 10% RILs and the mean of the parents, was significant for all studied traits (Table 2). Transgressive segregation that would be the result of the accumulation of positive alleles from both parental lines was observed for most of the studied traits. The positive and negative signs of additive effect at different loci indicate the contribution of both parental lines and confirm the transgressive segregation observed at the phenotypic level. Transgressive segregation for morphological and agronomical traits as well as for water status traits under well-watered and water-stressed conditions has been also reported by Rachid Al-Chaarani et al. (2004) and Poormohammad Kiani et al. (2007a,b) in sunflower.Yield is a resultant trait that is maximized by cumulative effects of large number of factors, and then it is expected to be varying under various environments. Based on this fact, improvement of seed yield may occur if selection is based on a yield contributing traits showing highest association with yield in specific environment (Richards, 1996). Correlation analysis indicated that HGW, NA, BIO, and LAD directly and positively influenced the grain yield of sunflower under both water treatment conditions. Therefore it was suggested that these agronomic traits are very important for sunflower yield breeding (Table 3). Significant negative correlation was found between NA and HGW under water-stressed condition (Table 3). This was in good agreement with the result of Thomson et al. (2003) that have reported grain per panicle was negatively correlated with grain weight in rice. PH was negatively correlated with HGW and HD under water-stressed condition (Table 3). This suggests that drought stress can be managed by modifying the plant morphology or incorporation of some traits that help plants to cope with drought stress successfully (Yordanov et al., 2000). Highly significant correlations between performances under two water treatments for the traits studied showed that the phenotypic value under well-watered condition explained a large proportion of the variation for performance under water-stressed conditions (Table 2). This result suggests that selection under well-watered conditions could partly be effective to improve grain yield and other agronomical traits under water-stressed a conditions. The same results have been reported in rice (Zou et al. 2005) and sunflower (Poormohammad Kiani et al. 2009) RILs.

Co-localization of QTLs for GYP and other agro-morphological traits

The QTLs identified in the present study revealed that several putative genomic regions were involved in the expression of the studied traits under well-watered and water-stressed conditions. We have identified QTLs that associated with more than one trait. For example, HPW.9.1 and LADW.9.1, co-located on linkage group 9 at 42.01 cM that associated with HP and LAD phenotypes; HPW.10.1 and SDW.10.1 co-located on linkage group 10 at 37.01 cM that associated with HP and SD phenotypes; the co-localized QTLs, HPW.12.1 and GYPW.12.1 on linkage group 12 at 65-69 cM that associated with HP and GYP phenotypes; the co-localized QTLs, SDW.17.1, BIOW.17.1, GYPW.17.1, HGWW.17.1 and LADW.17.1, on linkage group 17 at 10.1-11.1 cM that associated with SD, BIO, GYP, HGW and LAD phenotypes (Table 4, Supplementary data 1). Overlapped QTLs were also identified in water-stressed condition. For example, GYPS.13.1 and LADS.13.1 co-located on linkage group 13 at 54.01 cM that associated with GYP and LAD phenotypes; LADS.16.2 and PLS.16.1 on linkage group 16 at 55-60 cM that associated with LAD and PL phenotypes; and SDS.17.1, HDS.17.1, GYPS.17.1, HGWS.17.1 and LADS.17.1 on linkage group 17 at 10.1-11.1 cM that associated with SD, HD, GYP, HGW and LAD phenotypes (Table 5, Supplementary data 1). These findings were supported by the correlation analysis among traits. The co-locality of QTLs for different traits implies the likely presence of pleiotropic or close linkage between the QTLs that control traits. This signifies the plural selection efficiency by selecting marker(s), closely associated with these traits (Tuberosa et al., 2002a, b; Hittalmani et al., 2003). The differences in type and number of identified QTLs under two water regimes suggests that the regulation and expression of genes was not completely in the same manner under both watering conditions. However, in a number of cases, some QTLs of some traits in water-stressed condition were co-localised with some QTLs of some traits in well-watered condition. For example, in interval 65-69 cM on linkage group 12 overlapped QTLs were detected for HP and GYP (HPW.12.1, GYPW.12.1 and GYPS.12.1). Other overlapped QTLs between two water treatment regimes was observed in interval 53-57 cM on linkage group 14 near markers ORS391 and SSU227 for HP, HD and NA (HPS.14.1, HDW.14.1 and NAW.14.1). The most important stable QTL across two water treatment conditions was detected on linkage group 17 in interval 10.01-11.01 near marker ORS169 for HD, SD,

Table3. Correlation among traits in sunflower RILs under well-watered and water-stressed conditions.

Trait	PL	BIO	GYP	HD	PH	HGW	LAD	LN	NA	SD
well-watered condition										
PL	1									
BIO	0.31**	1								
GYP	0.69***	0.29*	1							
HD	0.8***	0.27*	0.68***	1						
PH	0.42**	0.37**	0.34***	0.52***	1					
HGW	0.68***	0.26*	0.7***	0.58***	0.15 ns	1				
LAD	0.64***	0.5***	0.45***	0.57***	0.34**	0.47***	1			
LN	0.5***	0.35**	0.43***	0.49***	0.47***	0.31**	0.32**	1		
NA	0.37**	0.25*	0.69***	0.5***	0.43***	0.19 ns	0.24**	0.43***	1	
SD	0.55***	0.4**	0.39***	0.63***	0.67***	0.29*	0.54***	0.37**	0.29*	1
water-stressed condition										
PL	1									
BIO	0.2 ns	1								
GYP	0.11 ns	0.77***	1							
HD	0.09ns	0.16 ns	0.14 ns	1						
PH	0.04 ns	0.19 ns	0.18 ns	-0.71***	1					
HGW	0.004 ns	0.23*	0.32**	0.97***	-0.75***	1				
LAD	0.65***	0.36**	0.31**	0.05 ns	0.17 ns	0.29*	1			
LN	0.02 ns	0.03 ns	0.05 ns	0.96***	0.77***	0.99***	0.23 ns	1		
NA	0.06 ns	0.45***	0.59***	0.25*	0.47***	-0.33***	0.15 ns	0.36****	1	
SD	0.08 ns	0.15 ns	0.11 ns	0.98***	-0.73***	0.97***	0.05 ns	0.96***	0.31*	1
Phenotypic correlations between studied traits measured under two different water treatments conditions										
	0.16ns	0.5***	0.35**	0.51***	0.32**	0.64***	0.18ns	0.53***	0.29*	0.44***

PH, plant height; HD, head diameter; SD, stem diameter; GYP, grain yield per plant; HGW, 100-grain weight; NA, number of achene; LAD, leaf area duration; BIO, total dry mater; PL, petiole length and LN, leaf number per plant. ns: non significant; *, **, *** significant at 0.05, 0.01 and 0.001 probability level, respectively.

GYP, BIO, HGW and LAD, with the phenotypic variance accounted for 0.23% to 16.75% of the variation. The positive allele for this QTL comes from PAC2. Identification of co-localized QTLs between the two water treatment conditions could be the reason for high correlation coefficients seen across the two water treatment conditions.

Co-localization of QTLs for agro-morphological traits with QTLs for plant water status and oil quality traits identified in previous studies

Some QTLs identified herein for agronomic traits showed co-locality with QTLs identified for water status traits, chlorophyll fluorescence parameters and yield-related traits in Poormohammad Kiani et al. (2007a; 2008; 2009) studies. For example, we have identified QTL for LP on linkage group 5 (LNS.5.1) that co-localized with QTLs identified for osmotic potential under water-stressed condition (OP.WS.5.2) and day from sowing to flowering under well-watered condition (DSFW.5.2) in Poormohammad Kiani et al. (2007a; 2009) studies. Co-localized QTLs were also identified for GYP under well-watered conditions and number of leaf per plant on linkage group 14 under both water treatment conditions (GYPW.14.1, LNI.14.1 and LNN.14.1) (Poormohammad Kiani et al., 2009), for GYP and osmotic potential under well-watered conditions (GYPS.14.1 and OP.WW.14.1) (Poormohammad Kiani et al., 2007a), for PH, the actual efficiency of PSII electron transport and relative water content under well-watered conditions (HPW.17.1, ΦPW.17.1 and RWC.WW.17.1) (Poormohammad Kiani et al., 2008), for PH, SD, BIO and LAD (PHW.10.1, SDW.10.1, BIOW.10.1 and LADW.10.2) under well-watered condition (Poormohammad Kiani et al., 2009), and for NA under well-watered conditions and osmotic potential at full turgor under water-stressed conditions (NAW.17.1 and OPF.WS.17.1) (Poormohammad Kiani et al., 2007a). Maintaining turgor potential under water-stressed condition is necessary for cell division and expansion, and consequently for plant growth and productivity. It has been reported that various biochemical and physiological responses, such as photosynthesis photochemistry and stomatal conductance under water-stressed condition depend on turgor potential in sunflower (Turner and Jones, 1980; Morgan, 1984; Maury et al., 1996; 2000; Poormohammad Kiani et al., 2007a; 2008; 2009). Therefore, overlapping QTLs for turgor potential and agronomical traits suggest the common genetic basis for turgor maintenance and plant growth and development. Some QTLs detected herein were co-localized with QTLs identified for fatty acids, protein and oil content under well-watered and water-stressed conditions in Haddadi et al. (2010) studies. For example, HPW.8.4 was co-localized with QTL controlling head diameter on linkage group 8. HPW.9.1 was co-localized with QTLs controlling leaf area at flowering and percentage of seed protein on linkage group 9. HPW.10.1 or SDW.10.1 was co-localized with QTLs controlling percentage of seed protein on linkage group 10. NAW.11.1 was co-localized with QTL controlling oleic acid on linkage group 11. HPW.12.1 was co-localized with QTL controlling leaf area at flowering linkage group 12. HPS.13.1 was co-localized with QTL controlling grain yield per plant and GYPS.13.1 was co-localized with QTL controlling plant high on linkage group 13. LNW.14.1 was co-localized with QTL controlling oleic acid content on linkage group 14. NAW.17.1 was co-localized with QTL controlling percentage of seed oil linkage group 17. In conclusion, we have detected several specific and non specific QTLs under well-watered and water-stressed conditions for agro-morphological traits. Detection of QTLs influencing various traits could increase the efficiency of marker-assisted selection and increase genetic progress.

Material and methods

Plant materials and experimental design

A population of RILs was developed through single seed descent from a cross between the public sunflower parental lines PAC2 and RHA266 (Flores Berrios et al., 2000). RHA266 was developed from the across between wild *H. annuus* and peredovik by USDA. PAC2 was an INRA-France inbred line developed from a cross between *H. petiolaris* and HA61 (Gentzbittel et al., 1995). RHA266 was a branched line with higher value in yield and 1000-grain weight in comparison to PAC2 (Rachid Al-Chaarani et al., 2004). Seeds of RILs and their two parents which kindly were provided by INRA (France) were evaluated in both well-watered and water-stressed conditions using a rectangular 8×9 lattice design with two replications in each condition. The experiment was arranged in accordance with the research farm of Urmia University, Iran. The latitude and longitude of region is 37° and 32' north and 45° and 5' east and its height is 1313m above the sea level. Climate of the region is cold and semidry and the average rainfall and the area temperature according to 16 years statistics are 184 mm and 12°C, respectively. Each plot comprised 1 line with 8 m long, and a spacing of 75×25 cm between lines and plants, respectively. The distance between well-watered and water-stressed experiment was considered 5 m. The water deficit treatments were applied by changing in irrigation intervals. Irrigations were carried out when an amount of evaporated water (from Class 'A pan' evaporation) reached to 60 (well-watered), and 180 (water-stressed) mm, respectively (Akbari et al., 2008; Pourtaghi et al., 2011). Amount of irrigation applied identical for all treatments from the beginning of planting time until the complete establishment of sunflower plants (eight-leaf (V8) stage) (Akbari et al., 2008; Pourtaghi et al., 2011). After this stage, the plots were irrigated according to their prescribed treatment.

Trait measurements

Six traits including plant height (PH; cm), stem diameter (SD; cm), head diameter (HD; cm), leaf number per plant (LN), leaf area duration (LAD; cm2 days) and petiole length (PL; cm) were measured on 5 random plants per plot in each well-watered and water-stressed experiment at flowering stage. Grain yield per plant (GYP; gr), number of achene (NA), 100-grain weight (HGW; gr) and total dry matter per plant (BIO; gr) were measured at maturity stage by harvesting five plants per replication.

Statistical analysis

Analysis variance of phenotypic data was performed with PROC GLM using SAS software (SAS Institute Inc.). Phenotypic correlations between traits in each watering conditions and between traits across watering conditions were determined using PROC CORR using SAS software.

Map construction and QTL analysis

The linkage map used in this study was the improved map that recently described by Haddadi et al. (2011b). Briefly, some important tocopherol pathway-related genes, enzymatic antioxidant-related genes, drought-responsive genes and phosphoglyceride transfer - related genes were used to

Table4. Map position and effect of QTLs detected for agro-morphological traits in sunflower RILs in well-watered condition

Trait	QTL	LG	Position (cM)	LOD	Additive effects	[a] R^2	Trait	QTL	LG	Position (cM)	LOD	Additive effects	[a] R^2
PH	PHW.8.1	8	80.51	3.73	0.87	1.29	NA	NAW.2.1	2	49.01	3.97	121.78	48.00
	PHW.9.1	9	42.01	3.58	-1.22	4.27		NAW.6.1	6	16.01	3.65	-26.46	21.46
	PHW.10.1	10	37.01	3.53	-1.82	16.95		NAW.8.1	8	62.61	4.04	-24.15	10.98
	PHW.11.1	11	24.01	3.53	-7.87	3.62		NAW.8.2	8	89.71	3.10	-6.21	2.54
	PHW.12.1	12	69.01	4.01	-1.87	4.97		NAW.11.1	11	14.01	4.32	126.38	48.89
	PHW.13.1	13	59.01	3.81	0.80	1.25		NAW.12.1	12	45.01	4.47	18.79	2.37
	PHW.15.1	15	6.01	3.45	-1.52	4.96		NAW.14.1	14	18.01	4.61	30.44	6.93
	PHW.17.1	17	8.01	3.99	-1.30	3.30		NAW.14.2	14	75.01	4.59	-32.84	11.31
								NAW.15.1	15	29.01	4.00	-28.81	5.16
HD	HDW.14.1	14	57.01	3.77	0.03	0.27		NAW.16.1	16	128.01	4.98	-25.26	24.71
								NAW.17.1	17	32.01	3.43	12.64	2.23
SD	SDW.10.1	10	37.01	3.61	-0.14	32.43							
	SDW.10.2	10	57.01	3.16	-0.03	6.67	LAD	LADW.9.1	9	42.01	4.42	-10.24	1.56
	SDW.17.1	17	10.01	3.78	0.03	1.85		LADW.10.1	10	61.01	4.22	14.64	3.76
								LADW.16.1	16	46.01	3.41	-2.36	2.93
GYP	GYPW.12.1	12	65.01	7.70	-0.28	0.40		LADW.17.1	17	11.01	4.93	5.55	1.28
	GYPW.13.1	13	54.01	7.29	-0.35	2.72							
	GYPW.14.1	14	67.01	6.60	0.29	0.15	LN	LNW.14.1	14	34.01	3.67	2.01	73
	GYPW.16.1	16	2.01	6.44	-1.07	2.24		LNW.16.1	16	49.01	5.8	0.02	1.54
	GYPW.17.1	17	10.01	6.51	-0.02	0.65							
HGW	HGWW.17.1	17	11.01	8.96	0.47	16.75							
PL	PLW.3.1	3	33.01	3.50	0.66	10.38							
	PLW.5.1	5	9.01	2.70	0.11	2.33							
	PLW.8.1	8	6.01	2.77	0.42	3.91	BIO	BIOW.17.1	17	11.01	4.95	1.77	1.31

PH: plant height; HD: head diameter; SD: stem diameter; GYP: grain yield per plant; HGW: 100-grain weight; NA: number of achene; LAD: leaf area duration; BIO: total dry matter; PL: petiole length and LN: leaf number per plant. The positive additive effect shows that PAC2 allele increase the trait and negative value shows that RHA266 allele increases the trait. [a]Percentage of phenotypic variance explained by the individual QTLs.

Table5. Map position and effect of QTLs detected for agro-morphological traits in sunflower RILs in water-stressed condition

Trait	QTL	LG	Position (cM)	LOD	Additive effects	[a] R^2	Trait	QTL	LG	Position (cM)	LOD	Additive effects	[a] R^2
PH	PHS.14.1	14	57.01	10.41	0.55	0.70	NA	NAS.10.1	10	22.01	3.40	-31.17	5.69
								NAS.15.1	15	8.01	2.90	-30.38	6.47
HD	HDS.17.1	17	11.01	3.84	0.01	0.43							
							LAD	LADS.13.1	13	54.01	6.16	23.26	25.40
SD	SDS.14.1	14	5.01	2.90	0.00	0.23		LADS.16.1	16	55.01	3.23	20.28	17.01
	SDS.14.2	17	11.01	5.68	0.03	1.88		LADS.17.1	17	11.01	7.62	11.13	7.97
GYP	GYPS.12.1	12	66.01	4.85	-0.40	2.25	LN	LNS.2.1	2	30.01	2.69	-0.35	2.19
	GYPS.13.1	13	54.01	3.80	2.11	1.70		LNS.3.1	3	35.01	4.10	0.52	2.19
	GYPS.14.1	14	28.01	4.81	-7.85	9.85		LNS.5.1	5	34.01	3.99	-0.51	5.95
	GYPS.17.1	17	11.01	6.80	1.29	1.68		LNS.8.1	8	68.51	3.52	-0.49	7.44
								LNS.12.1	12	50.01	3.09	-0.49	3.35
HGW	HGWS.10.1	10	12.01	3.16	-1.26	16.80		LNS.15.1	15	15.01	3.95	-1.12	16.47
	HGWS.17.1	17	11.01	3.92	0.27	3.74							
	HGWS.17.2	17	26.01	4.08	1.04	0.26	BIO	BIOS.17.1	17	11.01	4.55	5.30	9.72
PL	PLS.9.1	9	68.01	3.11	-0.56	11.80							
	PLS.16.1	16	60.01	3.30	0.68	20.86							

PH: plant height; HD: head diameter; SD: stem diameter; GYP: grain yield per plant; HGW: 100-grain weight; NA: number of achene; LAD: leaf area duration; BIO: total dry matter; PL: petiole length and LN: leaf number per plant. The positive additive effect shows that PAC2 allele increase the trait and negative value shows that RHA266 allele increases the trait. aPercentage of phenotypic variance explained by the individual QTLs.

improve the old version developed by Poormohammad et al. (2007a). Genotyping was done by SNP-based CAPS marker and high resolution melting (HRM) as well as directly on agarose gel. Each linkage group was numbered according to the sunflower reference map (Tang et al., 2002) and presumed to correspond to one of the 17 chromosomes in the haploid sunflower genome (x=17). The chromosomal locations of QTLs were resolved by composite interval mapping (CIM), using Win QTL Cartographer, version 2.5 (Wang et al., 2005) with the mean values of two replications each comprising 5 samples for each RIL in each condition. The genome was scanned at 2-cM intervals; with a window size of 15 cM. Up to 15 background markers were used as cofactors in the CIM analysis with the program module Srmapqtl (model 6). Additive effects of the detected QTLs were estimated with the Zmapqtl program (Basten et al., 2002). The percentage of phenotypic variance (R^2) explained by each QTL was estimated by Win QTL Cartographer.

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Supplementary data 1

Genetic map showing the location of putative QTLs associated with agro-morphological traits in sunflower under two contrasting water treatment conditions is available as supplementary data 1.

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