

QTL analysis of agronomic traits in recombinant inbred lines of sunflower under partial irrigation

P. Haddadi · B. Yazdi-samadi · M. R. Naghavi ·
A. Kalantari · P. Maury · A. Sarrafi

Received: 17 May 2010 / Accepted: 21 December 2010 / Published online: 21 January 2011
© Korean Society for Plant Biotechnology and Springer 2011

Abstract The objective of the present research was to map QTLs associated with agronomic traits such as days from sowing to flowering, plant height, yield and leaf-related traits in a population of recombinant inbred lines (RILs) of sunflower (*Helianthus annuus*). Two field experiments were conducted with well-irrigated and partially irrigated conditions in randomized complete block design with three replications. A map with 304 AFLP and 191 SSR markers with a mean density of 1 marker per 3.7 cM was used to identify QTLs related to the studied traits. The difference among RILs was significant for all studied traits in both conditions. Three to seven QTLs were found for each studied trait in both conditions. The percentage of phenotypic variance (R^2) explained by QTLs ranged from 4 to 49%. Three to six QTLs were found for each yield-related trait in both conditions. The most important QTL for grain yield per plant on linkage group 13 (*GYP-P-13-1*) under partial-irrigated condition controls 49% of phenotypic variance (R^2). The most important QTL for 1,000-grain weight (*TGW-P-11-1*) was identified on linkage group 11. Favorable alleles for this QTL come from RHA266. The major QTL for days from sowing to

flowering (*DSF-P-14-1*) were observed on linkage group 14 and explained 38% of the phenotypic variance. The positive alleles for this QTL come from RHA266. The major QTL for HD (*HD-P-13-1*) was also identified on linkage group 13 and explained 37% of the phenotypic variance. Both parents (PAC2 and RHA266) contributed to QTLs controlling leaf-related traits in both conditions. Common QTL for leaf area at flowering (*LAF-P-12-1*, *LAF-W-12-1*) was detected in linkage group 12. The results emphasise the importance of the role of linkage groups 2, 10 and 13 for studied traits. Genomic regions on the linkage groups 9 and 12 are specific for QTLs of leaf-related traits in sunflower.

Keywords Sunflower · Recombinant inbred lines · QTL · Partial-irrigation · Agronomic traits

Abbreviations

QTL Quantitative trait locus
CIM Composite interval mapping

P. Haddadi · A. Sarrafi (✉)
Laboratoire de Symbiose et Pathologie des Plantes (SP2),
IFR 40, INP-ENSAT, 18 Chemin de Borde Rouge,
BP 32607, 31326 Castanet Tolosan, France
e-mail: sarrafi@ensat.fr

P. Haddadi · B. Yazdi-samadi · M. R. Naghavi · A. Kalantari
Agronomy and Plant Breeding Department,
Faculty of Agriculture, University of Tehran, Karaj, Iran

P. Maury
Agrosystèmes et Développement Territorial,
UMR 1248, INRA, INP-ENSAT,
31326 Castanet Tolosan, France

Introduction

Sunflower (*Helianthus annuus* L., $2n = 34$) has been considered for construction of molecular genetic map. A saturated and reference genetic map based on 304 AFLP and 191 SSR markers with a mean density of 1 marker per 3.7 cM was recently constructed (Poormohammad Kiani et al. 2007). Drought stress can have major impacts on plant growth and development. Drought can result in lower yield and possible crop failure. Upon partial water stress, photosynthetic activity decreases mainly due to stomatal closure,

which constitutes the stomatal limitation of photosynthesis (Hugo et al. 2004). Low heritability of yield and large genotype \times environment interaction are the main obstacles in achieving progress in enhancing yield through direct selection (Tuberosa et al. 2002). Many agriculturally important traits such as yield, quality and disease resistance are controlled by several genes and are known as quantitative traits. The regions within genomes that contain genes associated with particular quantitative trait are recognized as quantitative trait loci (QTLs). QTLs controlling important traits such as in vitro regeneration parameters (Flores Berrios et al. 2000a, b, c), resistance to *Sclerotinia sclerotiorum* and *Phoma macdonaldii* (Bert et al. 2004; Micic et al. 2005), physiological parameters (Hervé et al. 2001), resistance to downy mildew and black stem (Rachid Al-Chaarani et al. 2002), agronomic traits (Rachid Al-Chaarani et al. 2004), germination and seedling development (Rachid Al-Chaarani et al. 2005), yield components and percentage of oil in grain (Mestries et al. 1998; Mokrani et al. 2002; Leon et al. 2003), water status traits and osmotic adjustment under well-watered and water-stressed conditions (Poormohammad Kiani et al. 2007) and seed-quality traits (Ebrahimi et al. 2008) have been identified in sunflower.

Gimenez and Fereres (1986) and Prieto Iosada (1992) showed that duration of leaf area is related to rainfed sunflower yield. The yield differences were also associated with variation in total biomass (Alza and Fernandez-Martinez 1997).

According to Flagella et al. (2002), flower bud formation and flowering are critical growth stages for water stress in sunflower. Some researchers have imposed water stress at flower bud formation stage in order to evaluate the effect of water stress on physiological traits such as plant water status and photosynthesis in sunflower (Pankovic et al. 1999; Maury et al. 2000; Tezara et al. 2002). Gimenez and Fereres (1986), and Prieto Iosada (1992) showed that leaf area duration is related to rainfed sunflower yield. The yield differences were also associated with variation in total biomass (Alza and Fernandez-Martinez 1997). The goals of this research are to identify QTLs in recombinant inbred lines (RILs) of sunflower for leaf area at flowering (LAF), leaf area duration (LAD), head diameter (HD), 1,000-grain weight (TGW), grain yield per plant (GYP), days from sowing to flowering (DSF) and plant height (PH) by using a saturated SSR and AFLP linkage map (Poormohammad Kiani et al. 2007) in

Table 1 Analysis of variance (mean squares) for days from sowing to flowering (DSF), plant height (PH), head diameter (HD), 1,000-grain weight (TGW), grain yield per plant (GYP), leaf area at flowering

	df	Conditions	DSF	PH	HD	TGW	GYP	LAF	LAD
RILs	86	WI	34.56**	1,212.70**	54.41**	1,377.1**	1,917.25**	13.59**	65.79**
		PI	28.81**	885.48**	43.45**	1,067.2**	1,340.72**	8.30**	40.19**
Blocks	2	WI	30.27**	640.60 ^{NS}	1.70 ^{NS}	428**	704.02*	2.50 ^{NS}	26.07 ^{NS}
		PI	20.25 ^{NS}	226.59 ^{NS}	9.94 ^{NS}	194 ^{NS}	1,142.34**	5.30 ^{NS}	12.22 ^{NS}
Error	172	WI	6.32	218.01	3.76	77	159.92	4.40	21.58
		PI	8.60	145.10	5.50	86	188.01	3.70	17.97

^{NS} Non-significant

*, ** Significant at 0.05 and 0.01 probability levels, respectively

(LAF) and leaf area duration (LAD) in a population of sunflower (*Helianthus annuus*) recombinant inbred lines (RILs) grown under well-irrigated (WI) and partially irrigated (PI) conditions

Table 2 Simple correlation coefficients (Pearson) days from sowing to flowering (DSF), plant height (PH), head diameter (HD), 1,000-grain weight (TGW), grain yield per plant (GYP), leaf area at

	DSF		PH		HD	TGW		GYP		LAF	
	WI	PI	WI	PI		WI	PI	WI	PI	WI	PI
PH	0.33**	0.29**									
HD	-0.09 ^{NS}	-0.21**	0.33**	0.21**							
TGW	-0.25**	-0.24**	0.25**	0.18**	0.78**	0.80**					
GYP	-0.14*	-0.16**	0.31**	0.29**	0.83**	0.83**	0.79**	0.77**			
LAF	0.15*	0.12 ^{NS}	0.45**	0.36**	0.19**	0.25**	0.19**	0.24**	0.23**	0.24**	
LAD	0.10 ^{NS}	0.12 ^{NS}	0.46**	0.40**	0.22**	0.30**	0.24**	0.30**	0.30**	0.34**	0.65**

^{NS} Non-significant

*, ** Significant at 0.05 and 0.01 probability levels, respectively

flowering (LAF) and leaf area duration (LAD) in a population of sunflower recombinant inbred lines (RILs) grown under well-irrigated (WI) and partially irrigated (PI) conditions

field conditions with well-irrigated and partially irrigated treatments.

Materials and methods

Plant materials and experimental conditions

The recombinant inbred lines (RILs) used in this research were developed through single seed descent from F2 plants, derived from a cross between PAC2 and RHA266 (Flores Berrios et al. 2000a). Both parental lines are sunflower public inbred lines. RHA266 has been obtained from a cross between wild *H. annuus* and Peredovik by USDA, and PAC2 is an INRA-France inbred line from a cross between *H. petiolaris* and ‘HA61’ (Gentzbittel et al.

1995). RHA266 is more resistant to downy mildew with higher values for yield, 1,000-grain weight and oil percentage compared with PAC2 (Gentzbittel et al. 1995; Rachid Al-Chaarani et al. 2004). Two independent experiments were undertaken at Teheran University campus of Karaj. Experimental design was randomized complete block with three replications. Seeds of 87 RILs and their two parents were sown in the field under well-irrigated and partially irrigated conditions. Each genotype per replication consisted of one row, 4 m long, 50 cm between rows and 25 cm between plants in rows. The distance between replications of well-irrigated and partially irrigated treatments was 7 m. The so-called ‘well-irrigated’ condition plots were irrigated once every week, whereas for the second condition (partially irrigated), irrigation was controlled and adjusted by the observation of the wilting

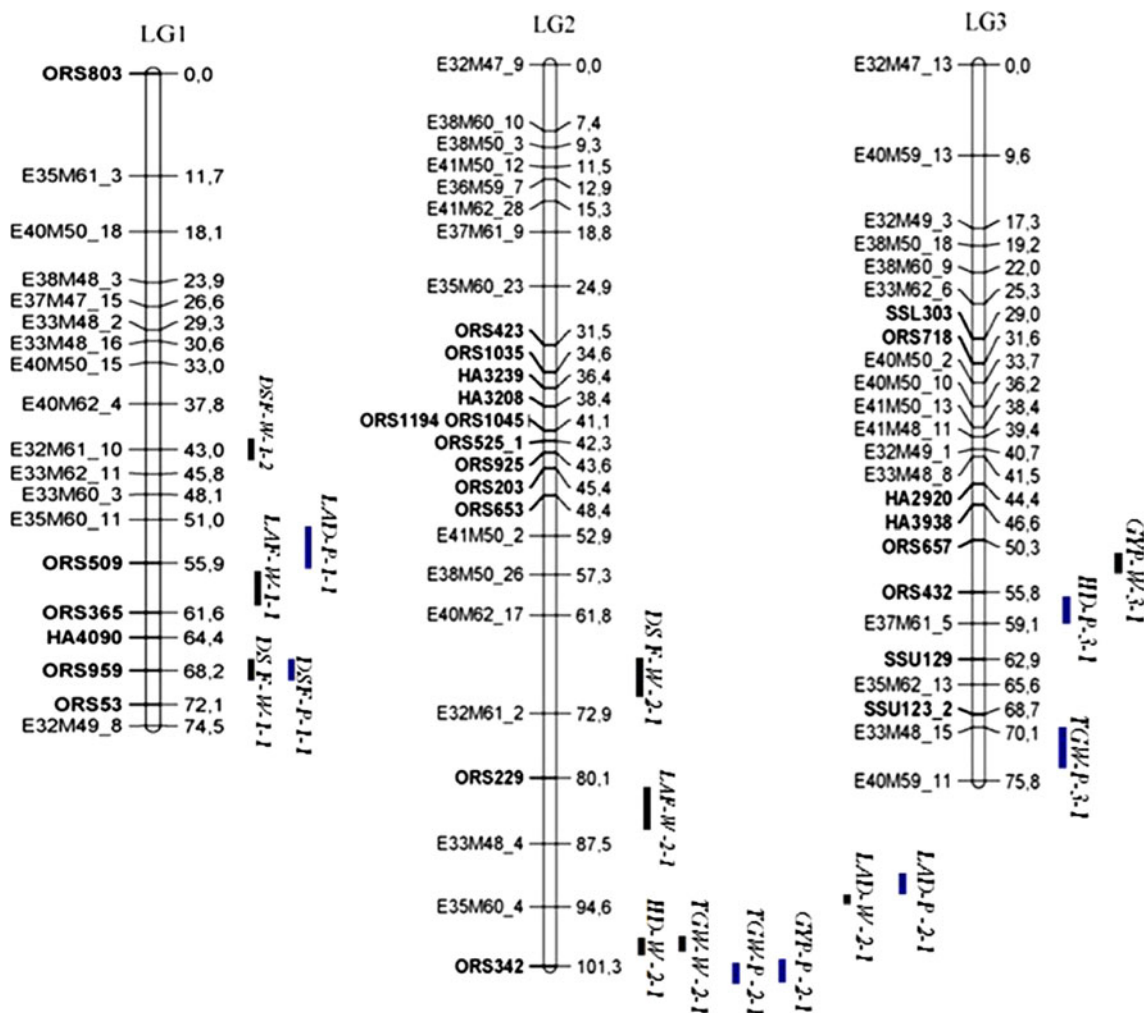


Fig. 1 Molecular linkage groups of the sunflower (*Helianthus annuus*) map presenting QTLs for days from sowing to flowering (DSF), plant height (PH), head diameter (HD), 1,000-grain weight (TGW), grain yield per plant (GYP), leaf area at flowering (LAF) and

leaf area duration (LAD). The positions of QTLs are shown on the right side of the linkage groups (LG1–LG17). Bars represent intervals associated with the QTLs

threshold of the leaves. Partial water deficit was started 45 days after sowing at the stage near flower bud formation and continued up to maturity.

Investigation of agronomic traits

Various traits such as days from sowing to flowering (DSF) and plant height (PH) were measured for RILs and their parental lines at the plot scale (on each line, when 50% of the plants were at flowering stage). Moreover, leaf length (L) and width (W) of all green leaves were measured at flowering stage, and total leaf area at flowering (LAF) was calculated with the formula: $LAF = 0.7 L \times W$ (Alza and Fernandez-Martinez 1997). Green leaf area of the plants was determined weekly from flowering to harvest in order

to evaluate green leaf area with respect to time. An integral of weekly leaf area was considered as being an estimate of leaf area duration (LAD, m² days). At harvest, yield components such as head diameter (HD), 1,000-grain weight (TGW) and grain yield per plant (GYP) were measured. Three plants per genotype per condition per replication were randomly selected for evaluation of the mentioned traits. All traits were measured for RILs and their parental lines in each replication for both experiments.

QTL and statistical analysis

Various traits data were analyzed using SAS PROC GLM (SAS Institute 1996) and SPSS. Statistical analysis was

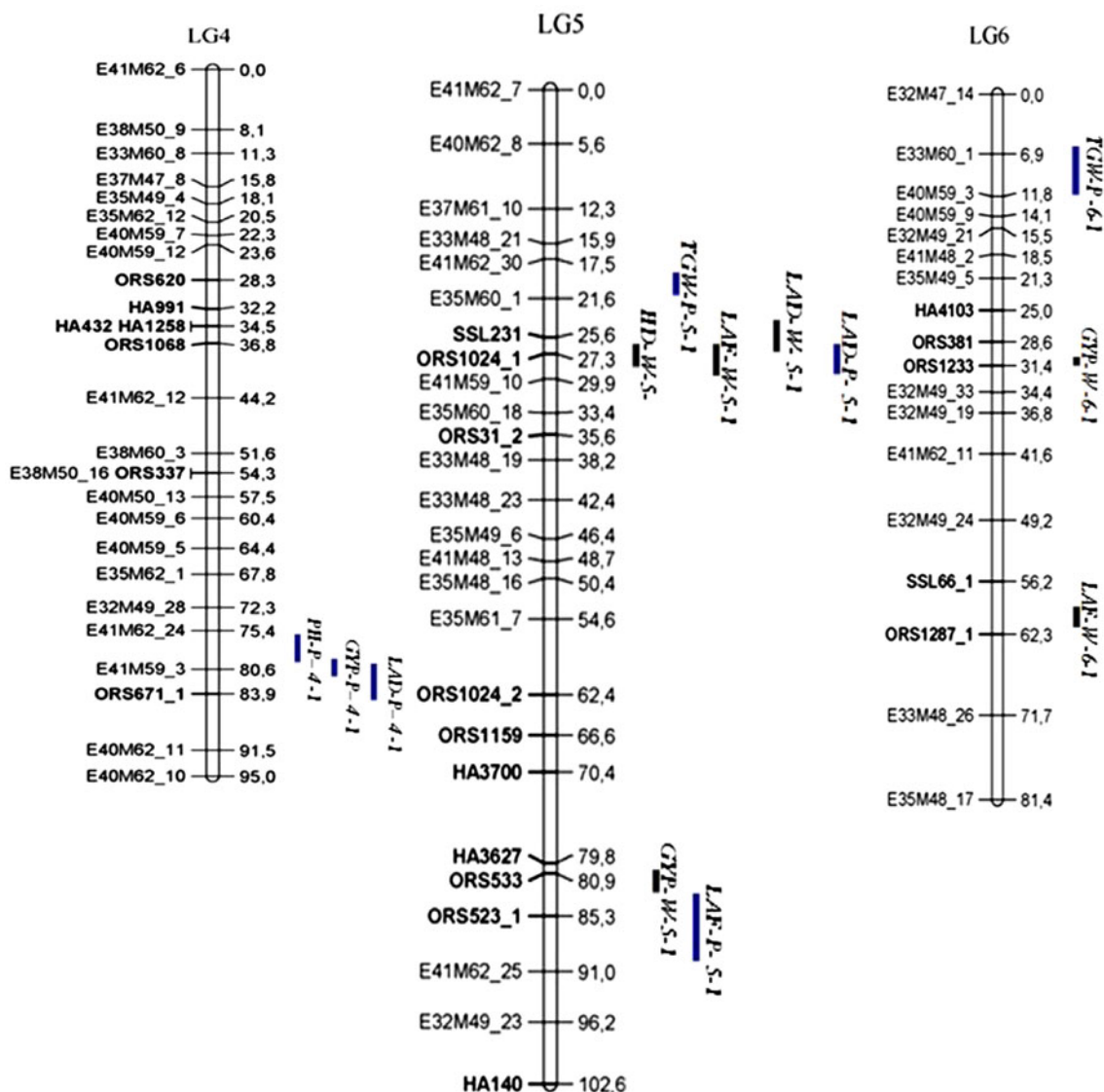


Fig. 1 continued

carried out in order to determine the main effect of RILs for the studied traits. Genetic correlations between the traits were also determined.

A sunflower map recently constructed by Poormohammad Kiani et al. (2007) was used for identification of QTLs. The chromosomal locations of QTLs for the studied traits were resolved by composite interval mapping (CIM), using Win QTL Cartographer, version 2.5, using mean values of three replications for each RIL in each conditions (Wang et al. 2005). The control marker number and the window size were 15 and 15 cM, respectively. The

percentage of phenotypic variance (R^2) explained by each QTL and the percentage of phenotypic variance (TR^2) explained by the QTLs given all the covariants were also estimated by Win QTL Cartographer.

Results

Results of analysis of variance for the agronomic traits are presented in Table 1. Results showed significant genotypic effect among RILs for all the studied traits in both well-

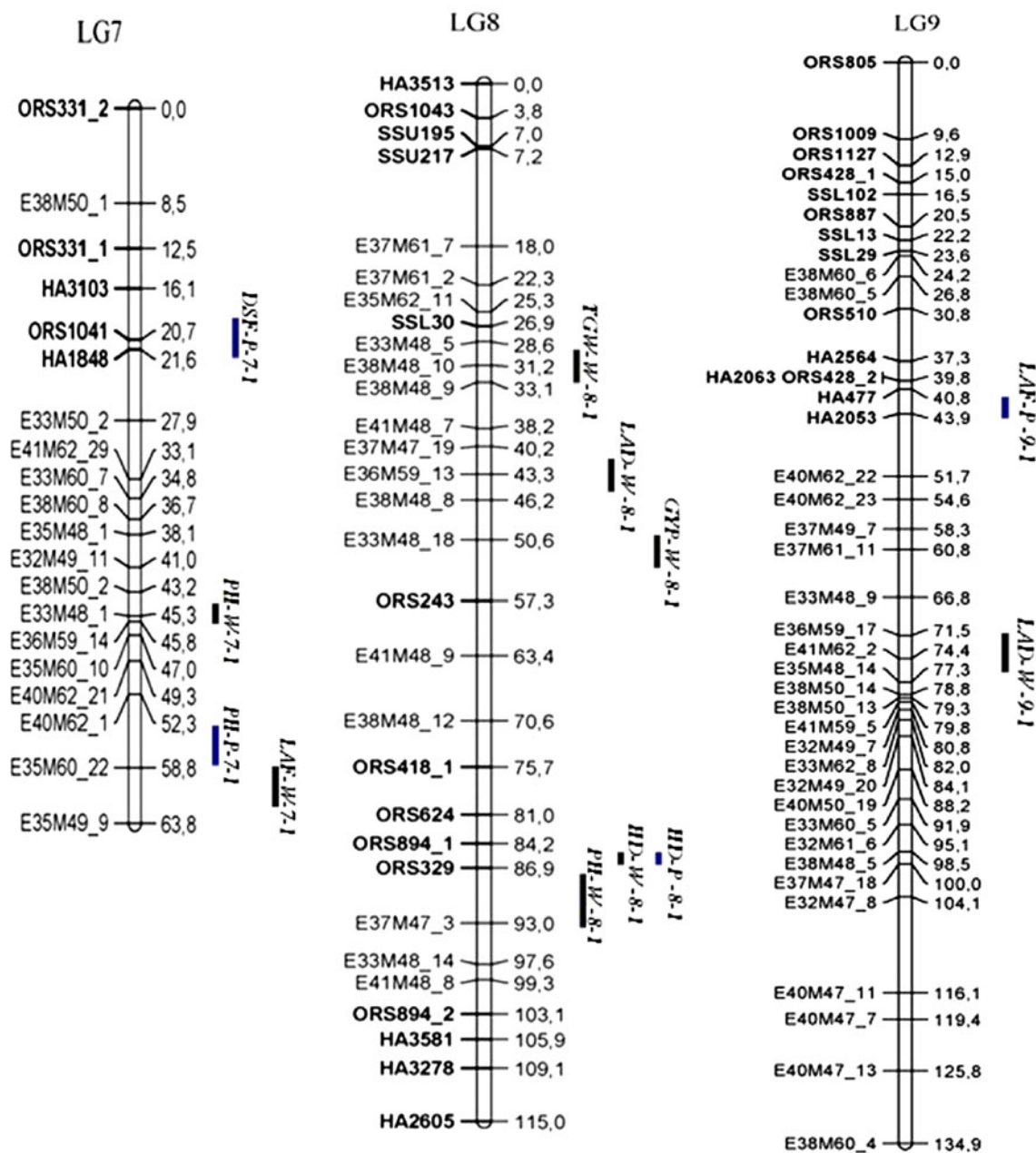


Fig. 1 continued

irrigated and partially irrigated conditions. The effect of blocks on GYP was significant under both conditions whereas it was significant for TGW and DSF under the

well-irrigated condition. Correlations among the studied traits are shown in Table 2. Positive and significant correlations are observed between LAF and LAD as well as

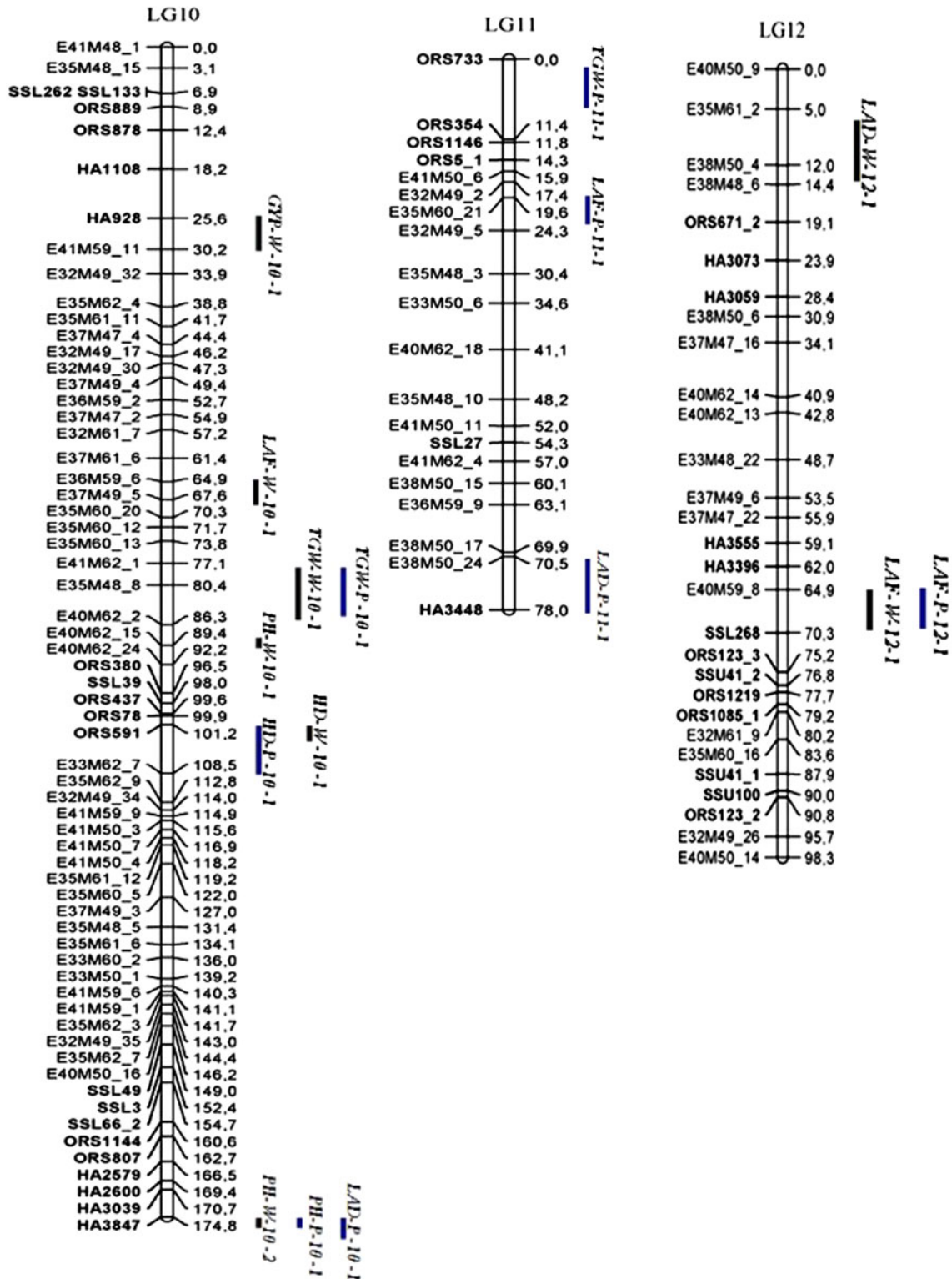


Fig. 1 continued

between yield components in both well-irrigated and partially irrigated conditions. Head diameter (HD) is positively correlated with TGW, GYP, LAF and LAD in both conditions (Table 2). Highly significant and positive correlation between TGW and GYP is observed in both well-irrigated and partially irrigated conditions (Table 2). Under the well-irrigated condition, the correlation between DSP and LAF is significant. Grain yield per plant (GYP) is also positively correlated with LAF and LAD in both conditions

(Table 2). The characteristic of the linkage map has been explained in detail in a previous study (Poormohammad Kiani et al. 2007). This map contains 304 AFLP and 191 SSR markers which are placed in 17 groups (Fig. 1). The total map length is 1,824.6 cM with a mean density of 1 marker per 3.7 cM, which has a density close to the sunflower reference map (3.1) presented by Tang et al. (2002). The map position and characteristics of QTLs associated with the studied traits under well-irrigated and partially

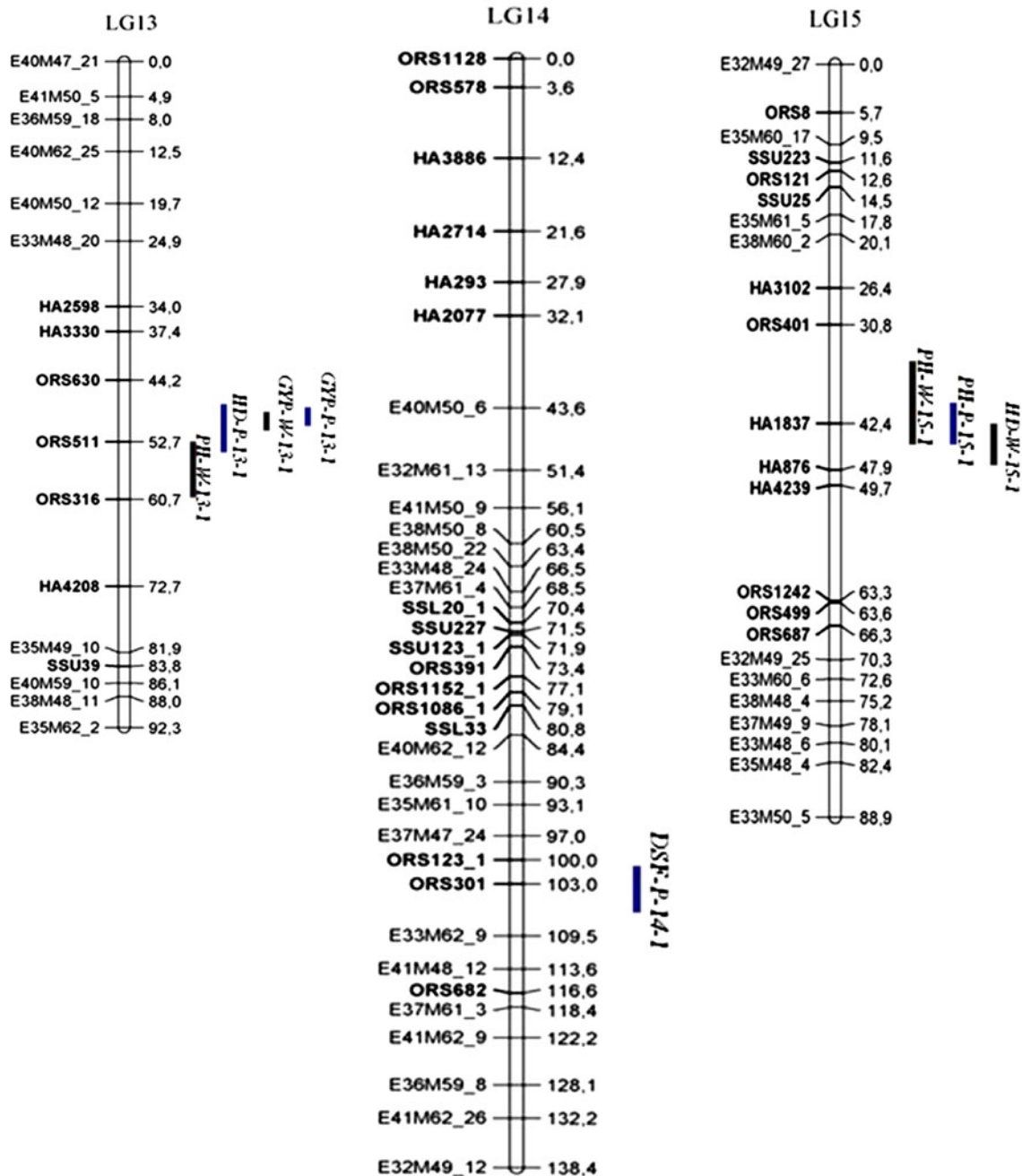


Fig. 1 continued

irrigated conditions are presented in Tables 3 and 4, respectively. The bold QTLs are stable across both well-irrigated and partially irrigated conditions for each trait. The QTLs are designated as the abbreviation of the trait followed by 'W' or 'P' for well irrigated or partially irrigated. The corresponding linkage group and the number of QTLs in the group are also indicated for each QTL. A large phenotypic variation was also explained when considering cofactors TR^2 (Tables 3 and 4). Both parental lines

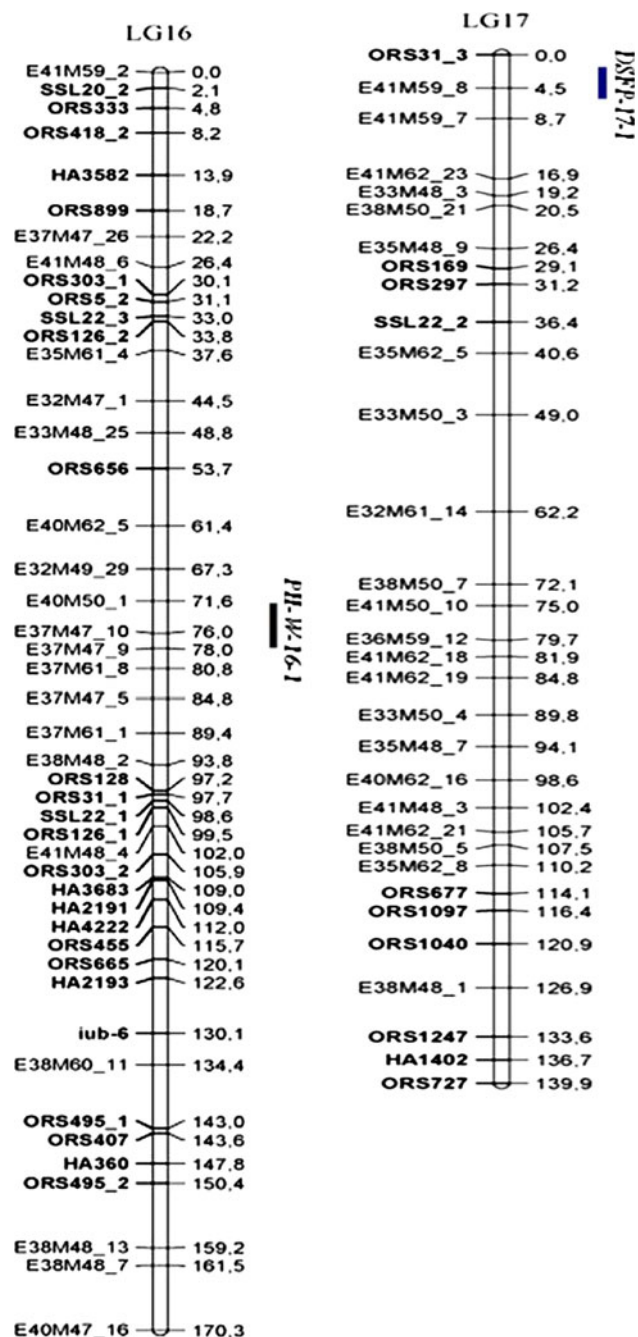


Fig. 1 continued

contributed to the expression of the different target traits. The percentage of phenotypic variance explained by QTLs of DSF ranged from 5 to 38%. The most important QTL for DSF (*DSF-P-14-1*) is found on linkage group 14 and explained 38% of the phenotypic variance. The positive alleles for this QTL come from RHA266 (Table 4). A total of 11 QTLs are found for PH in well-irrigated and partially irrigated conditions and the phenotypic variance varies from 4 to 27%. The most important QTL for PH is *PH-W-16-1*, which is located on linkage group 16 at 75.61 cM. The individual effect of this QTL on the expression of R^2 is 27%. Two common QTLs are identified for PH in both conditions on linkage groups 10 and 15 (*PH-W-10-1* and *PH-P-10-1*; *PH-W-15-1* and *PH-P-15-1*). Three co-localized QTLs for leaf-related traits (LAF and LAD), are detected on linkage groups 2, 5 and 12. Both parents (PAC2 and RHA266) contributed in controlling the QTLs of the two traits mentioned in both conditions. Three to six QTLs are found for each yield-related trait in well-irrigated and partially irrigated conditions. QTLs explained from 4 to 30% and 4 to 49% of the total phenotypic variance of yield-related traits (R^2) in well-irrigated and partially irrigated conditions, respectively. The major QTL for HD (*HD-P-13-1*) is found on linkage group 13 and explained 37% of the phenotypic variance. The positive alleles for this QTL come from PAC2 (Table 4). A total of nine QTLs are identified for grain yield per plant under both conditions. The most important is *GYP-P-13-1*, in the partially irrigated condition, which is positioned on linkage group 13 at 48.21 cM. The LOD score is 7.7 and the individual effect of this QTL on the expression of R^2 is 49%, whereas the TR^2 is 91% (Table 4). The major QTL for 1,000-grain weight (*TGW-P-11-1*) is identified on linkage group 11. For yield-related traits, the favorable alleles of identified QTLs come from both parents.

Discussion

Detection of genomic regions associated with leaf-related traits and yield components under well-irrigated and partially irrigated conditions will be useful for marker-based approaches to improve the aforementioned traits in sunflower. In our experiments, variation was observed for all studied traits in both well-irrigated and partially irrigated conditions. Genetic variation for some agronomic traits have also been reported by Rachid Al-Chaarani et al. (2004). The QTLs detected in our research revealed that several putative genomic regions are involved in the expression of the studied agronomic traits under well-irrigated and partially irrigated conditions. The percentage of phenotypic variance explained by the QTLs (R^2) ranged from 4 to 49%. The positive and negative signs of additive

Table 3 QTLs detected for days from sowing to flowering (*DSF*), plant height (*PH*), head diameter (*HD*), 1,000-grain weight (*TGW*), grain yield per plant (*GYP*), leaf area at flowering (*LAF*) and leaf area duration (*LAD*) under the well-irrigated condition

Trait	QTL	Linkage group	Marker On the left of LOD peak	Position (cM)	LOD	Additive effect	R^{2a}	TR ^{2b}
DSF	<i>DSF-W-1-1</i>	1	ORS959	68.21	3.7	-1.06	0.07	0.45
	<i>DSF-W-1-2</i>	1	E32M61_10	43.01	3.1	1.26	0.08	0.53
	<i>DSF-W-2-1</i>	2	E40M62_17	65.81	5.5	1.75	0.17	0.54
PH	<i>PH-W-7-1</i>	7	E38M50_2	45.21	6.6	-7.72	0.13	0.63
	<i>PH-W-8-1</i>	8	ORS329	86.91	4.3	-5.72	0.06	0.64
	<i>PH-W-10-1</i>	10	E40M62_15	91.41	5	8.38	0.11	0.67
	<i>PH-W-10-2</i>	10	HA3039	172.71	3.7	-7.39	0.07	0.6
	<i>PH-W-13-1</i>	13	ORS511	54.71	10.1	10.6	0.16	0.6
	<i>PH-W-15-1</i>	15	ORS401	34.81	4.9	6.46	0.06	0.67
	<i>PH-W-16-1</i>	16	E40M50_1	75.61	6.7	-25.33	0.27	0.84
	<i>PH-W-16-1</i>	16	E40M50_1	75.61	6.7	-25.33	0.27	0.84
HD	<i>HD-W-2-1</i>	2	E35M60_4	100.61	4.1	-0.88	0.04	0.63
	<i>HD-W-5-1</i>	5	SSL231	25.61	5.8	1.41	0.1	0.6
	<i>HD-W-8-1</i>	8	ORS894_1	86.21	3.8	-1.13	0.04	0.64
	<i>HD-W-10-1</i>	10	ORS591	101.21	3.7	-1.43	0.05	0.63
	<i>HD-W-15-1</i>	15	HA1837	46.41	3	-1.08	0.04	0.64
TGW	<i>TGW-W-2-1</i>	2	E35M60_4	100.61	11	-1.03	0.18	0.85
	<i>TGW-W-8-1</i>	8	E33M48_5	30.61	6.5	-0.67	0.07	0.74
	<i>TGW-W-10-1</i>	10	E35M48_8	80.41	6	-1.02	0.1	0.73
GYP	<i>GYP-W-3-1</i>	3	ORS657	54.31	5.6	6.18	0.04	0.46
	<i>GYP-W-5-1</i>	5	ORS533	84.91	7.7	6.12	0.04	0.48
	<i>GYP-W-6-1</i>	6	ORS381	30.61	6	3.56	0.04	0.46
	<i>GYP-W-8-1</i>	8	ORS243	57.31	5.9	-6.9	0.04	0.49
	<i>GYP-W-10-1</i>	10	HA928	24.21	8.8	-16.62	0.3	0.53
	<i>GYP-W-13-1</i>	13	ORS630	51.91	6.4	12.76	0.14	0.58
LAF	<i>LAF-W-1-1</i>	1	ORS509	57.91	4	-1.84	0.11	0.45
	<i>LAF-W-2-1</i>	2	ORS229	80.11	5	-0.39	0.04	0.52
	<i>LAF-W-5-1</i>	5	ORS1024_1	27.31	6.5	1.6	0.04	0.41
	<i>LAF-W-6-1</i>	6	SSL66_1	62.21	3.9	-0.55	0.04	0.39
	<i>LAF-W-7-1</i>	7	E35M60_22	60.81	4.8	-0.8	0.11	0.41
	<i>LAF-W-10-1</i>	10	E37M49_5	69.61	3.8	-0.66	0.07	0.33
	<i>LAF-W-12-1</i>	12	E40M59_8	68.9	4.5	0.8	0.11	0.61
LAD	<i>LAD-W-2-1</i>	2	E33M48_4	94.51	4.1	-0.33	0.04	0.58
	<i>LAD-W-5-1</i>	5	SSL231	25.61	6.7	0.64	0.05	0.57
	<i>LAD-W-8-1</i>	8	E36M59_13	43.31	6.7	-1.04	0.1	0.6
	<i>LAD-W-9-1</i>	9	E36M59_17	73.51	6.4	0.91	0.1	0.58
	<i>LAD-W-11-1</i>	11	E38M50_24	72.51	3.9	-0.7	0.06	0.57
	<i>LAD-W-12-1</i>	12	E35M61_2	5.01	4.3	0.92	0.09	0.59

The QTLs are designated as the abbreviation of the trait followed by 'W' for well-irrigated. The positive additive effect shows that PAC2 alleles increase the trait and negative additive effect shows that RHA266 alleles increase it. The bold QTLs, presented as stable QTLs, are common across both well-irrigated and partially irrigated conditions for each trait

^a Percentage of individual phenotypic variance explained. Value determined by Win QTL Cart., version 2.5

^b Percentage of phenotypic variance explained by the QTLs given all the covariants

effect at the different loci indicate the contribution of both parental lines and confirm the transgressive segregation observed at the phenotypic level (Tables 3 and 4). Transgressive segregation would be the result of the

accumulation of favourable alleles coming from different parental lines. Transgressive segregation has also been reported for other traits in the same population (Poormohammad Kiani et al. 2007; Ebrahimi et al. 2008).

Table 4 QTLs detected for days from sowing to flowering (*DSF*), plant height (*PH*), head diameter (*HD*), 1,000-grain weight (*TGW*), grain yield per plant (*GYP*), leaf area at flowering (*LAF*) and leaf area duration (*LAD*) under the partially irrigated condition

Trait	QTL	Linkage group	Marker On the left of LOD peak	Position (cM)	LOD	Additive effect	R^{2a}	TR ^{2b}
DSF	<i>DSF-P-1-1</i>	1	ORS959	68.21	4.5	-1.34	0.05	0.53
	<i>DSF-P-7-1</i>	7	ORS1041	20.71	4	-1.17	0.06	0.5
	<i>DSF-P-14-1</i>	14	E37M47_24	101.01	8.1	-2.6	0.38	0.67
	<i>DSF-P-17-1</i>	17	ORS31_3	2.01	4.1	1.13	0.08	0.59
PH	<i>PH-P-4-1</i>	4	E41M62_24	77.41	4	-6.6	0.1	0.53
	<i>PH-P-7-1</i>	7	E40M62_1	56.31	3	-4.33	0.04	0.42
	<i>PH-P-10-1</i>	10	HA3039	172.71	4.3	-4.82	0.04	0.41
	<i>PH-P-15-1</i>	15	ORS401	40.81	4.6	4.6	0.05	0.43
HD	<i>HD-P-3-1</i>	3	ORS432	57.81	3.6	1.14	0.06	0.5
	<i>HD-P-8-1</i>	8	ORS894_1	86.81	5.2	-1.05	0.04	0.55
	<i>HD-P-10-1</i>	10	ORS591	101.21	5.1	-1.59	0.08	0.52
	<i>HD-P-13-1</i>	13	ORS630	48.21	5	3.29	0.37	0.79
TGW	<i>TGW-P-2-1</i>	2	E35M60_4	100.61	5.3	-1.03	0.07	0.74
	<i>TGW-P-3-1</i>	3	E33M48_15	70.11	5.8	1	0.1	0.71
	<i>TGW-P-5-1</i>	5	E41M62_30	19.51	3.9	0.72	0.06	0.71
	<i>TGW-P-6-1</i>	6	E33M60_1	6.91	4.4	-0.93	0.08	0.72
	<i>TGW-P-10-1</i>	10	E35M48_8	82.41	3.6	-0.99	0.09	0.74
GYP	<i>TGW-P-11-1</i>	11	ORS733	8.01	6.4	-1.62	0.22	0.93
	<i>GYP-P-2-1</i>	2	E35M60_4	98.61	8.4	-6.31	0.04	0.54
	<i>GYP-P-4-1</i>	4	E41M59_3	80.61	8	-4.07	0.04	0.54
LAF	<i>GYP-P-13-1</i>	13	ORS630	48.21	7.7	18	0.49	0.91
	<i>LAF-P-5-1</i>	5	ORS523_1	85.31	5	0.7	0.13	0.59
	<i>LAF-P-9-1</i>	9	HA477	42.81	5.4	0.5	0.1	0.61
	<i>LAF-P-11-1</i>	11	E35M60_21	19.61	3.4	-0.58	0.08	0.54
LAD	<i>LAF-P-12-1</i>	12	E40M59_8	68.91	5.1	1.01	0.16	0.63
	<i>LAD-P-1-1</i>	1	E35M60_11	53.01	3.1	0.51	0.04	0.57
	<i>LAD-P-2-1</i>	2	E33M48_4	93.51	3.5	-0.7	0.04	0.56
	<i>LAD-P-4-1</i>	4	E41M59_3	82.61	4.6	-0.62	0.06	0.55
	<i>LAD-P-5-1</i>	5	SSL231	27.01	4	0.3	0.04	0.54
	<i>LAD-P-10-1</i>	10	HA3039	170.71	6.2	-1.20	0.15	0.55

The QTLs are designated as the abbreviation of the trait followed by 'P' for partial-irrigated. The positive additive effect shows that PAC2 alleles increase the trait and negative additive effect shows that RHA266 alleles increase it. The bold QTLs, presented as stable QTLs, are common across both well-irrigated and partially irrigated conditions for each trait

^a Percentage of individual phenotypic variance explained. Value determined by Win QTL Cart., version 2.5

^b Percentage of phenotypic variance explained by the QTLs given all the covariants

In several cases, one QTL was identified to be associated with more than one trait. Under the well-irrigated condition, co-localization occurs for QTLs of HD and TGW on linkage group 2 (*HD-W-2-1* and *TGW-W-2-1*). Significant and positive correlation between HD and TGW (Table 2), is justified by the effects of their co-localized QTLs (Table 3). Co-localized QTLs for HD and LAD are identified on linkage group 5 (*HD-W-5-1* and *LAD-W-5-1*). Under the partially irrigated condition, co-localized QTLs are also identified on linkage group 2 for TGW and GYP (*TGW-P-2-1* and *GYP-P-2-1*), linkage group 4 for LAD

and GYP (*LAD-P-4-1* and *GYP-P-4-1*), linkage group 10 for PH and LAD (*PH-P-10-1* and *LAD-P-10-1*) and linkage group 13 for HD and GYP (*HD-P-13-1* and *GYP-P-13-1*) (Table 4). Identification of co-localized QTLs, influencing several traits shows that genes controlling the traits are in the same genomic region. The correlation among different traits as well as their observed co-localization is relevant to effort for manipulating multiple traits simultaneously (Poormohammad Kiani 2007). The locations of QTLs identified in the present research for different traits, when compared with those controlling some agronomic traits

reported by Rachid Al-Chaarani et al. (2004), revealed three co-localized QTLs. The co-localized QTLs are observed on linkage group 5 (*TGW-P-5-1* and 1,000-grain weight), linkage group 7 (*PH-P-7-1* and stem diameter; *LAF-W-7-1* and sowing-to-flowering date).

Several QTLs for LAF and LAD are detected on different linkage groups (Fig. 1). The effect of leaf area duration (LAD) on grain yield per plant is more important than leaf area at flowering (LAF) (Poormohammad Kiani 2007). This indicates that maintaining green leaf area longer after anthesis is important for a high yield production under both water treatments. It has been reported that maintaining green leaf area and consequently a longer duration of photosynthetic activity has contributed to increased yield in most major crops (Russel 1991; Evans 1993). Genetic differences in photosynthetic duration have been also associated with a longer grain filling duration and higher yield in maize (Richards 2000).

The QTL detected for grain yield per plant (*GYP-W-3-1*) co-localized with a QTL controlling stearic acid content (*4.SA.3.1*) identified by Ebrahimi et al. (2008). Some QTLs detected in our research co-localized with QTLs controlling germination and seedling development identified by Rachid Al-Chaarani et al. (2005). The co-localized QTLs are identified on linkage group 1 (*DSF-W-1-2* and shoot dry weight), linkage group 5 (*TGW-P-5-1* and percentage or normal seedlings), linkage group 6 (*LAF-W-6-1* and shoot length), and linkage group 16 (*PH-W-16-1* and root length).

Some QTLs detected in our research co-localized with QTLs controlling plant water status and osmotic adjustment in RILs of sunflower under two water treatments in greenhouse conditions reported by Poormohammad Kiani et al. (2007). The co-localized QTLs are also identified on linkage group 7 (*LAF-W-7-1* with leaf water potential and relative water content), on linkage group 10 (*LAF-W-10-1* with relative water content), and on linkage group 11 (*LAD-W-11-1* with osmotic potential).

Eleven QTLs are common across two well-irrigated and partially irrigated conditions for the studied traits. Stable QTLs contain genes controlling the trait in both conditions. Our results do highlight the importance of the role of linkage groups 9 and 12 for controlling leaf-related traits. The most important are on linkage groups 9 (*LAF-P-9-1* and *LAD-W-9-1*) and 12 (*LAD-W-12-1*, *LAF-W-12-1* and *LAF-P-12-1*). Detailed characterization of these genomic regions through the development and evaluation of near-isogenic lines will lead to an improved understanding and might set the stage for the positional cloning of genes related to them. The relatively low number of RILs used in the current research may have a negative influence on the accuracy of the calculated QTL effects and the ability to detect QTLs with small effects, and R^2 may be overestimation (Beavis 1994;

Bachlava et al. 2008). This was, to some degree, compensated for by the higher precision of the phenotyping and the use of our saturated map.

References

- Alza JO, Fernandez-Martinez JM (1997) Genetic analysis of yield and related traits in sunflower (*Helianthus annuus* L.) in dryland and irrigated environments. *Euphytica* 95:243–251
- Bachlava E, Dewey RE, Auclair J, Wang S, Burton JW, Cardinal AJ (2008) Mapping genes encoding microsomal ω -6 desaturase enzymes and their cosegregation with QTL affecting oleate content in soybean. *Crop Sci* 48:640–650
- Beavis WD (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies, pp 250–266. In: Wilkinson DB (ed) 49th Annual corn and sorghum Research Conference, Chicago. American Seed Trade Association, Alexandria
- Bert PF, Dechamp-Guillaume G, Serre F, Jouan I, de Labrouhe DT, Nicolas P, Vear F (2004) Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.). 3. Characterisation of QTL involved in resistance to *Sclerotinia sclerotiorum* and *Phoma macdonaldii*. *Theor Appl Genet* 109:865–874
- Ebrahimi A, Maury P, Berger M, Poormohammad Kiani S, Nabipour A, Shariati F, Grieu P, Sarrafi A (2008) QTL mapping of seed-quality traits in sunflower recombinant inbred lines under different water regimes. *Genome* 51:599–615
- Evans LE (1993) Crop evolution, adaptation and yield. Cambridge University Press, New York
- Flagella Z, Rotunno T, Tarantino E, Di Caterina R, De Caro A (2002) Changes in seed yield and fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime. *Eur J Agron* 17:221–230
- Flores Berrios E, Gentzbittel L, Kayyal H, Alibert G, Sarrafi A (2000a) AFLP mapping of QTLs for in vitro organogenesis traits using recombinant inbred lines in sunflower (*Helianthus annuus* L.). *Theor Appl Genet* 101:1299–1306
- Flores Berrios E, Sarrafi A, Fabre F, Alibert G, Gentzbittel L (2000b) Genotypic variation and chromosomal location of QTLs for somatic embryogenesis revealed by epidermal layers culture of recombinant inbred lines in the sunflower (*Helianthus annuus* L.). *Theor Appl Genet* 101:1307–1312
- Flores Berrios E, Gentzbittel L, Mokrani L, Alibert G, Sarrafi A (2000c) Genetic control of early events in protoplast division and regeneration pathways in sunflower. *Theor Appl Genet* 101:606–612
- Gentzbittel L, Vear F, Zhang Y-X, Bervillé A, Nicolas P (1995) Development of a consensus linkage RFLP map of cultivated sunflower (*Helianthus annuus* L.). *Theor Appl Genet* 90:1079–1086
- Gimenez C, Fereres E (1986) Genetic variability in sunflower cultivars under drought. II. Growth and water relations. *Aust J Agr Res* 37:583–597
- Hervé D, Fabre F, Flores Berrios E, Leroux N, Al Chaarani G, Planchon C, Sarrafi A, Gentzbittel L (2001) QTL analysis of photosynthesis and water status traits in sunflower (*Helianthus annuus* L.) under greenhouse conditions. *J Exp Bot* 52:1857–1864
- Hugo A, DaMatta Fabio Pinheiro M, Chaves Aginaldo RM, Fontes Elizabeth PB, Loureiro Marcelo E (2004) Drought tolerance in relation to protection against oxidative stress in clone of *Coffea*

- canephora* subjected to long-term drought. *Plant Sci* 167: 1307–1314
- Leon AJ, Andrade FH, Lee M (2003) Genetic analysis of seed oil percentage across generations and environments in sunflower (*Helianthus annuus* L.). *Crop Sci* 43:135–140
- Maury P, Berger M, Mojayad F, Planchon C (2000) Leaf water characteristics and drought acclimation in sunflower genotypes. *Plant Soil* 223:153–160
- Mestries E, Gentzbittel L, Tourvieille de Labrouhe D, Nicolas P, Vear F (1998) Analysis of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflower (*Helianthus annuus* L.) using molecular markers. *Mol Breeding* 4:215–226
- Micic Z, Hahn V, Bauer E, Schon CC, Melchinger AE (2005) QTL mapping of resistance to *Sclerotinia* midstalk-rot in RIL of sunflower population NDBLOSSel × CM625. *Theor Appl Genet* 110:1490–1498
- Mokrani L, Gentzbittel L, Azanza F, Fitamant L, Al-Chaarani G, Sarrafi A (2002) Mapping and analysis of quantitative trait loci for grain oil and agronomic traits using AFLP and SSR in sunflower (*Helianthus annuus* L.). *Theor Appl Genet* 106: 149–156
- Pankovic D, Sakac Z, Kevresan S, Plesnicar M (1999) Acclimation to longterm water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. *J Exp Bot* 330:127–138
- Poormohammad Kiani S (2007) Analyse génétique des réponses physiologiques du tournesol (*Helianthus annuus* L.) soumis à la sécheresse. PhD thesis, l'Institut National Polytechnique de Toulouse
- Poormohammad Kiani S, Talia P, Maury P, Grieu P, Heinz R, Perrault A, Nishinakamasu V, Hopp E, Gentzbittel L, Paniego N, Sarrafi A (2007) Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. *Plant Sci* 172:773–787
- Prieto Iosada H (1992) Response to drought conditions of sunflower genotypes differing in yield potential and length of cycle. PhD thesis, University of Cordoba (in Spanish)
- Rachid Al-Chaarani G, Roustae L, Gentzbittel L, Mokrani L, Barrault G, Dechamp-Guillaume G, Sarrafi A (2002) A QTL analysis of sunflower partial resistance to downy mildew (*Plasmopara halstedii*) and black stem (*Phoma macdonaldii*) by the use of recombinant inbred lines (RILs). *Theor Appl Genet* 104:490–496
- Rachid Al-Chaarani G, Gentzbittel L, Huang X, Sarrafi A (2004) Genotypic variation and identification of QTLs for agronomic traits using AFLP and SSR in recombinant inbred lines of sunflower (*Helianthus annuus* L.). *Theor Appl Genet* 109: 1353–1360
- Rachid Al-Chaarani G, Gentzbittel L, Wedzony M, Sarrafi A (2005) Identification of QTLs for germination and seedling development in sunflower (*Helianthus annuus* L.). *Plant Sci* 169: 221–227
- Richards RA (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. *J Exp Bot* 51:447–458
- Russel WA (1991) Genetic improvement of maize yields. *Adv Agron* 46:245–298
- SAS Institute (1996) SAS/STAT user's guide, v.6, 4th edn, vols 1 and 2. SAS Institute, Cary
- Tang S, Yu JK, Slabaugh MB, Shintani DK, Knapp SJ (2002) Simple sequence repeat map of the sunflower genome. *Theor Appl Genet* 105:1124–1136
- Tezara W, Mitchall V, Driscoll SP, Lawlor DW (2002) Effects of water deficit and its interaction with CO₂ supply on the biochemistry and physiology of photosynthesis in sunflower. *J Exp Bot* 375:1781–1791
- Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S (2002) Mapping morpho-physiological traits and yield: case studies, shortcoming and perspectives in drought-stressed maize. *Ann Bot* 89:941–963
- Wang S, Basten CJ, Zeng ZB (2005) Windows QTL Cartographer V2.5. Department of Statistics, North Carolina State University, Raleigh. Available from <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>