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With 2 figures and 4 tables

Abstract

A set of sunflower recombinant inbred lines (RILs) was used to study agronomical traits under greenhouse and field conditions each with two water treatments and three replications. The difference among RILs was significant for all the traits studied in all conditions; and water treatment × RILs interaction was also observed for most of the traits in both field and greenhouse conditions. Because of the low rate of drought stress, this part of field data are not informative. Several quantitative trait loci (QTLs) were identified for yield-related traits with the percentage of phenotypic variance explained by QTLs (R^2) ranging from 4% to 40%. Several QTLs for grain yield per plant (GYP) under four water treatments were identified on different linkage groups, among which two were specific to a single treatment (*GYPN.4.1*, *GYPI.7.1*). Three QTLs for GYP were overlapped with several QTLs for drought-adaptative traits detected in our previous study (Poormohammad Kiani et al. 2007b). The whole results do highlight interesting genomic regions for marker-based breeding programmes for drought tolerance in sunflower.

Key words: sunflower — water stress — grain yield — QTL — yield-related traits

Sunflower (*Helianthus annuus* L.) is one of the most important sources of vegetable oil in the world. Identification of genetic factors affecting agronomic and economically important traits in sunflower could help to improve breeding methods. Yield components as well as other quantitative traits are controlled by several genetic loci with additive and non-additive gene actions, and genotype × environment interactions are important components of variance decreasing their heritability (Fick 1978, Fick and Miller 1997).

Progress in increasing yield and its stability through a direct selection has been hampered by the low heritability of yield, particularly under drought and by its large genotype × environment interaction (Blum 1988, Ceccarelli and Grando 1996, Tuberosa et al. 2002). As an alternative to the direct selection for yield under drought conditions, morpho-physiological traits genetically correlated with yield, have been targeted in selection programmes pursued in collaboration between physiologists and breeders (Blum 1988, Chimenti et al. 2002, Tuberosa et al. 2002). Correlation coefficients have been used by many researchers in determining interrelationships between seed yield and other characters in sunflower under both well-watered and drought conditions (Fereres et al. 1986, Alza and Fernandez-Martinez 1997, Chimenti et al. 2002, Flagella et al. 2002).

Fereres et al. (1986) showed that physiological traits responsible for drought tolerance were not correlated with yield potential in sunflower, indicating that both can be combined in improved cultivars. Chimenti et al. (2002) demonstrated that osmotic adjustment (OA), a parameter directly related to drought tolerance, contributes to yield maintenance of sunflower under pre-anthesis drought conditions. However, the capacity of sunflower for OA was different depending on genotype (Maury et al. 1996). 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 (BIO) (Alza and Fernandez-Martinez 1997). However, these phenotypic relationships have not been shown at molecular level, i.e. by mapping quantitative trait loci (QTLs) for yield and morpho-physiological traits in the same mapping population.

Progress in plant genome analysis has made it possible to examine naturally occurring allelic variation underlying complex traits such as yield. There are several reports, especially over the last years that deal with drought tolerance on the physiological and molecular levels in sunflower (Poormohammad Kiani et al. 2007a,b, 2008). Many studies have been undertaken to find genetic variation in agronomical traits in sunflower, and QTLs controlling yield components and morphological traits have been identified in sunflower RILs (Rachid Al-Chaarani et al. 2004) or F_2/F_3 populations (Mokrani et al. 2002, Bert et al. 2003). However, the studies mentioned above have been conducted under well-watered conditions and to our knowledge QTL mapping of agronomical traits and yield in sunflower under drought conditions has not been reported in literature.

The objectives of the present study were to identify QTLs in a population of RILs for yield-related traits by using our recently saturated simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) linkage map (Poormohammad Kiani et al. 2007b), and to compare QTLs controlling these traits in controlled (greenhouse) and natural (field) well-watered and water-stressed conditions.

Materials and Methods

Plant materials and genetic linkage map: The characteristic of mapping population (RILs) and their parents (PAC2 and RHA266) used in the present study has been explained in detail in our previous study (Poormohammad Kiani et al. 2007b). Briefly, the mapping population was developed through single seed descent from F_2 plants derived from a cross between PAC2 and RHA266 and a map was constructed with 304 AFLP and 191 SSRs. Both parental lines (PAC2 and RHA266) are

public inbred lines of sunflower (Zhang et al. 2005). RHA266 is a branched restorer inbred line, obtained from a cross between wild *H. annuus* and *Peredovik* by USDA and PAC2 is a non-branched restorer inbred line obtained from a cross between *H. petiolaris* and 'HA61' by INRA-France (Gentzbittel et al. 1994, 1995). RHA266 is a branched line with higher values for yield, 1000-grain weight and oil percentage compared with PAC2 (Gentzbittel et al. 1995, Rachid Al-Chaarani et al. 2004). The recent map from the cross PAC2 × R-HA266 (Poormohammad Kiani et al. 2007b), was used in the present work for identification of QTLs for yield-related traits under different water treatments.

Greenhouse experiment: A population of 78 RILs were randomly selected and grown together with their parents (PAC2 and RHA266) in the greenhouse under controlled conditions. Plants were individually grown in plastic pots (4.0 l) containing a mixture of 40% soil, 40% compost and 20% sand. Temperature was maintained at 25/18 ± 2°C (day/night) and relative humidity was about 65–85 ± 5%. Supplementary light giving an approximately 16-h light and 8-h dark period was maintained during experiment.

A split-plot experimental design with three blocks was used with water treatments (well-watered and water-stressed) as the main plot factor and genotypes (RILs and parental lines) as sub-plot factor. The RILs and their two parents were randomized within each treatment–block combination. To simulate water deficit conditions similar to field, a progressive water stress was imposed at stage near flower bud formation (R1, Schneiter and Miller 1981) by decreasing progressively the irrigation to 30% field capacity during 12 days. Both well-watered and water-stressed plants were weighed and water lost replaced carefully. Well-watered (control) plants received sufficient water to maintain soil water content close to field capacity. Water-stressed plants were subjected to a progressive water stress and irrigated with a water volume of 60%, 50%, 40% and 30% of field capacity (each for 3 days) during 12 days. Water-stressed plants were then irrigated at 30% of field capacity until harvest.

Field experiment: Two experiments were undertaken in the field conditions during April–September in 2005 with irrigated and non-irrigated (rain fed) water treatments and three replications per each water treatment. Both irrigated and non-irrigated experiments were located in the same field to have the same condition with a distance sufficient enough to not allow water to reach non-irrigated field. The experiment consisted of a split-plot design with three replications. The main plot contains water treatments (irrigated and non-irrigated) and genotypes (RILs and parental lines) were considered as sub-plot. A population of 100 RILs comprising 78 RILs used in the greenhouse experiment, together with their parents was sown in each water treatment with three replications. Each replication consisted of two rows 4.6-m long, with 50 cm between rows and 25 cm between plants in rows, giving a total number of about 32 plants per plot. The so-called 'irrigated' field was irrigated two times at two critical stages, just before flowering (33 mm) and at about grain filling (44 mm), according to sunflower irrigation programme determined by INRA-France for the region (Fig. 1). A sprinkler irrigation system was used because of the uniformity of water application. The 'rainfed' experiment was not irrigated. Three plants per genotype per water treatments were randomly chosen for evaluation of the studied traits.

Trait measurements: Days from sowing to flowering (DSF) were recorded when individual plants in greenhouse and 50% of the plants per plot in the field were at anthesis. Leaf number per plants (LN), plant height (PH) and leaf area were measured at flowering stage. Leaf length (L) and width (W) of all green leaves were measured in both well-watered and water-stressed conditions at flowering stage, and total leaf area at flowering (LAF) was calculated with the formula: $\mathrm{LAF} = \Sigma\, 0.7L \times W$ (Alza and Fernandez-Martinez 1997). Green leaf area of the plants was determined weekly from flowering to harvest to evaluate green leaf area with respect to time. An integral of weekly leaf

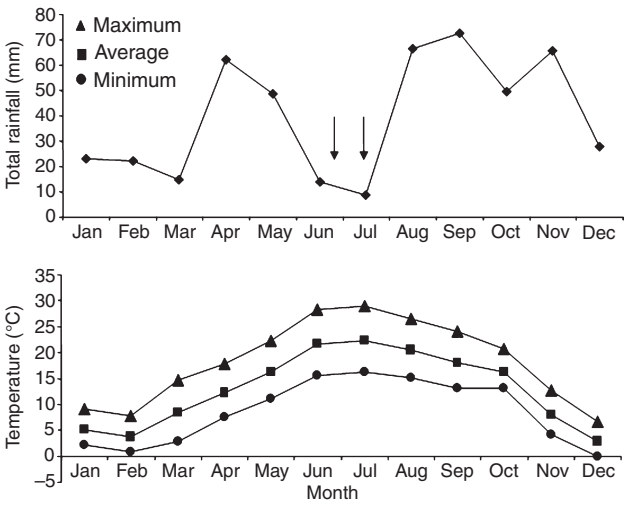

Fig. 1: Total monthly rainfall and the minimum, maximum and average of temperature for the year 2005 at INRA experimental field – Toulouse, France. The arrows show the date of first (33 mm) and second (44 mm) supplementary irrigation for 'irrigated' experiment determined by INRA. Sprinkler irrigation systems was used for irrigation because of the uniformity of water application

area was considered as being an estimate of leaf area duration (LAD, cm^2 days). At maturity, total dry matter 'BIO' per plant and head weight per plant (HW) were determined for three plants per genotype per water treatment. All plants of plots were also harvested at maturity and grain yield per plant (GYP) was determined using whole plot weight divided by the number of plants per plot for all genotypes in each replication and water treatment.

Statistical analysis and QTL mapping: Normality of the different traits was assessed according to the Shapiro and Wilk test (SAS PROC UNIVARIATE). The data were analysed using the SAS PROC GLM as a split-plot experimental design (SAS Institute Inc. 2002). A mixed model with water treatment (main plot factor) as fixed effect and genotypes (sub-plot factor) as random effect, was used for analysis of the data in both greenhouse and field conditions. Correlations between GYP and other traits in each of the four conditions and between genotypes for the same traits across water treatments were determined using SAS PROC CORR (SAS Institute Inc. 2002).

Quantitative trait loci mapping of the studied traits was performed by composite interval mapping (CIM) conducted with QTL Cartographer, version 1.16 (Basten et al. 2002) using mean values of three replications for each RIL in each water treatment and growth condition (field or greenhouse). 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 identified with the programme module Srmapqtl (model 6; Basten et al. 2002). A LOD threshold of 3.0 was used for considering a QTL significant (Rachid Al-Chaarani et al. 2004). QTLs for different traits were compared on the base of overlapping support intervals: a decrease in the LOD score of 1.0, determined the end point of support interval for each QTL (Lander and Botestein 1989). Additive effects of the detected QTLs were estimated with the Zmapqtl programme (Basten et al. 2002). The percentage of phenotypic variance (R^2) explained by each QTL was estimated by QTL Cartographer (Basten et al. 2002).

Results

Phenotypic variation and effect of water stress

The yield-related traits except GYP and HW under water-stressed condition in the greenhouse did not show deviation from normal distribution. As normalizing data through

transformation may misrepresent differences among individuals by pulling skewed tails toward the centre of the distribution (Doerge and Churchill 1994, Mutschler et al. 1996), all phenotypic analyses were performed on untransformed data.

Considering all the traits studied in different growth conditions, a high transgressive segregation was observed. Phenotypic variation of RILs and their parents (PAC2 and RHA266) in the greenhouse and in the field under two water treatments are shown in Table 1. Under greenhouse conditions, significant water treatment effect was observed for all of the studied traits except for DSF and the number of leaf per plant (LN). Under field conditions, water stress had significant effect on only HW per plant and GYP. Because of low water stress effect under field conditions, the results obtained from non-irrigated field are similar to those obtained from irrigated field. Therefore, the field data are not very informative for the purpose of this study; but the QTLs identified under field conditions were compared with those identified under greenhouse conditions. The variability was significant for all the traits studied in all conditions. Water treatment × RILs interaction was observed for most of the traits in both field and greenhouse conditions. Parental lines (PAC2 and RHA266) differed significantly for LAF and LAD under both water treatments in the greenhouse and under rainfed treatment in the field. The difference between parental lines was also significant for PH, BIO and HW under both water treatments in the field.

Correlation analysis

Correlations between GYP and other studied traits together with correlations between water treatments for each trait are summarized in Table 2. High significant correlations were observed between water treatments for the studied traits under both greenhouse and field conditions. GYP was correlated with LAF, LAD, HW and BIO in all four water treatments. GYP was correlated with PH only under field conditions. DSF and the number of leaf per plant (LN) were not correlated with GYP.

QTLs mapping

The map position and characteristics of QTLs associated with the studied traits in the greenhouse and in the field, under two water treatments are summarized in Table 3. The QTLs were designated as the abbreviation of the trait followed by '*W*' or '*D*' for well-watered or water-stressed in the greenhouse, and by '*I*' and '*NI*' for irrigated and non-irrigated (rainfed) in the field. The corresponding linkage group and the number of QTLs in the linkage group were also indicated for each QTL. For an easier overview of overlapping QTLs between traits and growth conditions, an image of all QTL regions is presented as Fig. 2.

Two to seven QTLs were found depending on the trait and growth conditions. QTLs explained from 4% to 40% of the phenotypic variance of the traits (R^2), and both parental lines contributed to the expression of the different target traits. Overlapping QTLs were found for different traits on several linkage groups (Table 3 and Fig. 2).

Several QTLs were detected for DSF in four different growth conditions (Table 3 and Fig. 2) and most of them were common across at least two growth conditions. The most important QTL for DSF is located on linkage group 7 where several QTLs under different growth conditions were co-localized. The positive alleles for these overlapped QTLs come from RHA266. Seventeen QTLs were identified for leaf number per plant (LN) under four growth conditions among which, nine were common across at least two growth conditions and eight were detected under only one condition. Both parental lines contributed almost equally to QTL expression. For LAF, 21 QTLs were detected under four growth conditions, their number being from three to six depending on growth conditions. Among 21 QTLs, nine were detected in only one of the growth conditions and 12 were detected in at least two growth conditions. The phenotypic variance explained by each QTL ranged from 5% to 19%, and both parental lines contributed to positive alleles. As far as LAD is concerned, 22 QTLs were identified under four growth conditions, explaining from 4% to 17% of the total variation. Eleven QTLs were specific to single water treatment and 11 were detected in at least two water treatments. The positive alleles for 17 QTLs come from RHA266 and for five QTLs they come from PAC2. A total of 17 QTLs were detected for PH being six unique QTLs and 11 QTLs that were detected in at least two growth conditions. The QTLs explained from 5% to 23% of phenotypic variance and both parental lines contributed to trait expression. However, RHA266 contributed to positive alleles at 10 QTLs. For total dry matter 'BIO' per plant, 19 QTLs were identified, explaining from 5% to 23% of variation. The number of QTLs in four growth conditions varied from two to six; 13 were detected in only one of the growth conditions and six were common across different growth conditions. RHA266 contributed to positive alleles at 14 of 19 QTLs.

A total of 24 QTL were identified for HW per plant under four growth conditions with the phenotypic variance explained from 4% to 24%. The number of QTLs differed from four to seven depending on the growth condition. Among 24 QTLs, 16 were detected in only one of the growth conditions and eight were common across different water treatments. PAC2 contributed positive alleles at 10 QTLs and RHA266 contributed at 14 QTLs. For GYP, 20 QTLs were identified under four water treatments with the phenotypic variance explained ranging from 4% to 40%. Nine of 20 QTLs were identified in only one growth condition and the rest were common across at least two growth conditions. PAC2 and RHA266 contributed equally to QTLs controlling GYP.

Discussion

Phenotypic variation and the effect of water stress

The effect of water stress under greenhouse conditions was significant for all traits except for DSF and the number of leaf per plant (LN); whereas under field conditions, the effect of water stress was significant only for HW and GYP (Table 1), which suggests that water stress occurred earlier in the greenhouse when compared with field conditions. Under field conditions, a low drought stress rate was occurred because of a wet season in 2005, and the data from field conditions are not useful for the comparison of different water treatments, but they were used for the comparison of the QTL locations identified under greenhouse conditions. Water treatment responses were affected by significant 'genotype × water treatment' interaction for some traits, suggesting that response to water status by a given genotype in relation to other genotypes varies between water treatments (Table 1). A large genetic

Table 1: Performance of parents (PAC2 and RHA266) and recombinant inbred lines (RILs) across two water treatments, grown under greenhouse (well-watered and water-stressed) and field (irrigated and rainfed) conditions

		Well-watered (greenhouse)/Irrigated (field)				Water-stressed (greenhouse)/Rainfed (field)				
				RILs				RILs		
Condition	Trait	PAC2	RHA266	Mean	Range	PAC2	RHA266	Mean	Range	Effect
Greenhouse	DSF	75.6	77.3	76.9± 4.7	65.5–95.1	74.8	72.1	77.7± 4.3	63.7–96.7	W^{NS}, R^{***}, I^{**}
	LN	20.7	23.3	23.8± 1.8	17.0–34.6	20.0	24.0	23.2± 1.7	17.3–33.3	W^{NS}, R^{***}, I^{NS}
	LAF	2855[1]	3615	3122± 493	1585–4388	1978[1]	3121	2293 ± 401	1156–3368	W^{***}, R^{***}, I^{NS}
	LAD	83511[1]	126486	117451 ± 28892	35981–277706	36634[1]	64464	67653 ± 18948	10906–192630	W^{*}, R^{***}, I^{**}
	PH	121.0[1]	112.7	116.1± 9.0	77.7–136.0	97.7	94.7	91.8± 8.1	60.7–118.3	W^{*}, R^{***}, I^{***}
	BIO	55.6	50.6	58.6± 10.9	37.6–82.9	36.8	36.8	34.0± 5.4	25.8–52.8	W^{**}, R^{***}, I^{**}
	HW	14.9	14.4	17.5± 5.2	7.3–33.5	9.7	8.7	8.9± 2.4	4.6–20.5	W^{**}, R^{***}, I^{NS}
	GYP	6.7	6.6	7.7± 2.8	0.6–19.3	4.4	3.0	2.9 ± 1.5	0.0–8.6	W^{**}, R^{***}, I^{***}
Field	DSF	79.7	74.7	82.7± 2.2	74.7–94.7	79.7	74.7	82.7± 1.9	74.7–94.7	W^{NS}, R^{***}, I^{NS}
	LN	23.3	23.0	27.1± 2.3	17.3–37.7	22.3	22.7	26.5± 2.6	17.0–38.0	W^{NS}, R^{***}, I^{NS}
	LAF	4380	4501	4317± 952	1988–7739	4174[1]	2556	3932± 821	2479–6949	W^{NS}, R^{***}, I^{**}
	LAD	107404[1]	116188	95506± 29168	40753–196143	96108[1]	67760	92733 ± 26892	43384–185865	W^{NS}, R^{***}, I^{*}
	PH	125.7[1]	102.3	125.0± 11.6	74.3–177.7	119.7[1]	99.3	122.8± 16.6	83.3–158.7	W^{NS}, R^{***}, I^{NS}
	BIO	131.7[1]	103.9	125.9± 33.3	48.5–255.4	117.3[1]	58.9	111.0± 32.1	37.9–227.2	W^{NS}, R^{***}, I^{***}
	HW	67.1[1]	48.7	55.3± 2.2	13.8–107.0	43.7[1]	31.4	48.2 ± 3.7	12.0–117.0	W^{*}, R^{***}, I^{***}
	GYP	26.3	27.6	26.9± 2.1	2.3–58.2	16.3	17.4	22.4± 1.7	2.7–69.1	W^{**}, R^{***}, I^{***}

NS, non-significant.
***,**,*Significant at 0.001, 0.01 and 0.05 probability level.
[1]The significant differences between parental lines.
The significance is indicated for water treatment (W), RILs (R) and Water treatment × RILs interaction (I) effects. The traits are: days from sowing to flowering (DSF), leaf number per plant (LN), leaf area at flowering (LAF; cm^2), leaf area duration (LAD; cm^2 days), plant height (PH; cm), total dry matter per plant (BIO; g), head weight per plant (HW; g) and grain yield per plant (GYP; g).

Table 2: Phenotypic correlations between studied traits measured under two different water treatments, and correlations between yield and related traits under greenhouse (well-watered and water-stressed) and field (irrigated and rainfed) conditions

	Greenhouse			Field		
		Grain yield per plant with other trait			Grain yield per plant with other trait	
Trait	Well-watered with water-stressed	Well-watered	Water-stressed	Irrigated with non-irrigated	Irrigated	Non-irrigated
DSF	0.77***	NS	NS	0.99***	NS	NS
LN	0.90***	NS	NS	0.89***	NS	NS
LAF	0.72***	0.24***	0.14*	0.67***	0.45***	0.46***
LAD	0.81***	0.55***	0.41***	0.85***	0.68***	0.59***
PH	0.67***	NS	NS	0.81***	0.37***	0.30***
BIO	0.51***	0.66***	0.36***	0.57***	0.74***	0.74***
HW	0.63***	0.69***	0.69***	0.58***	0.92***	0.91***
GYP	0.65***			0.83***		

NS, non-significant.
***,**,*Significant at 0.001, 0.01 and 0.05 probability level.
The traits are: days from sowing to flowering (DSF), leaf number per plant (LN), leaf area at flowering (LAF; cm^2), leaf area duration (LAD; cm^2 days), plant height (PH; cm), total dry matter per plant (BIO; g), head weight per plant (HW; g) and grain yield per plant (GYP; g).

variation and transgressive segregation was observed for all the studied traits under different water treatments, which could be the result of the accumulation of positive alleles coming from different parental lines. Transgressive segregation has already been observed for drought adaptive traits (Poormohammad Kiani et al. 2007b).

Under greenhouse conditions, water deficit was induced in 45-day-old plants near the stage flower bud formation R1 (Schneiter and Miller 1981), with the 14th true leaf fully expanded. Although the RILs differed for plant size, the difference among RILs for the days from sowing to R1 (water stress initiating date) was not significant. The pots were weighed and water lost replaced carefully in both well-watered and water-stressed conditions to control drought stress carefully regarding each plant size. As plant sizes were taken into account during water stress and there was no significant difference among RILs for growth stage, we suggest that, plant size and/or growth stage could not have introduced experimental error during water-stress period.

Highly significant correlations between performances under two water treatments for the traits studied in both greenhouse and field conditions showed that the phenotypic value under well-watered condition explained a large proportion of the variation for performance under drought (Table 2). This result suggests that selection under well-watered and/or irrigated conditions could partly be effective to improve grain yield and other agronomical traits under water-stressed and/or non-irrigated conditions. The same results have been reported in rice RILs (Zou et al. 2005).

The correlation analysis indicated that DSF and LN were not associated with GYP in both greenhouse and field conditions, and PH was correlated with GYP under only field conditions (Table 2). Rachid Al-Chaarani et al. (2004) also reported that, DSF is not correlated with grain yield in sunflower. HW per plant and BIO per plant were the highest contributing factor to GYP, and LAD was more important than LAF in both greenhouse and field conditions. 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 of major crops (Evans 1993, Richards 2000). Genetic differences in photosynthetic duration have also been associated with a longer grain filling duration and higher yield in maize (Russel 1991).

QTLs for GYP and other traits

The QTLs identified in the present study showed that several putative genomic regions are involved in the expression of the studied traits under four growth conditions (Table 3). The percentage of phenotypic variance explained by the QTLs (R^2) ranged from 4% to 40%. Based on overlapping support intervals, the co-location of QTL for all eight traits in four growth conditions was determined. As two important examples, intervals E38M50_1-HA1848 and E41M62_29-E38M60_8 on linkage group 7 were significantly associated with various traits under different growth conditions (Fig. 2). In these two intervals, the QTLs controlling LN (under irrigated condition), LAF (under two growth conditions), LAD (under two growth conditions), PH (under two growth conditions), DSF (under three growth conditions), HW and BIO (under two growth conditions) were overlapped (Fig. 2). Similarly, several other overlapping QTLs were also observed for the studied traits. These overlapping QTLs indicate the existence of a partly common genetic base for agro-morphological traits. Several QTLs for grain yield under four water treatments were overlapped with the QTL of HW on linkage groups 2 (*GYPN.2.1*), 3 (*GYPI.3.1* and *GYPN.3.1*), 4 (*GYPD.4.1, GYPI.4.1* and *GYPI.4.2*), 5 (*GYPW.5.1*) and 10 (*GYPW.10.1, GYPN.10.1)* as well as with the QTLs controlling several other traits on linkage groups 3, 4, 5, 9, 10, 13, 14 and 16. However, two individual QTLs specific for yield were also identified on linkage groups 4 (*GYPN.4.1*) and 7 (*GYPI.7.1*) under non-irrigated and irrigated field conditions, respectively.

Identification of QTLs influencing several traits could increase the efficiency of marker-assisted selection (MAS) and enhance genetic progress (Upadyayula et al. 2006). The correlations among different traits as well as their co-localization observed are relevant to strive for manipulating multiple traits simultaneously. As we identified genetic markers linked to yield-related traits, indirect selection can be targeted at the presence or absence of markers of interest in breeding lines. However, the QTLs and related markers need to be validated in other genetic backgrounds prior to application in MAS. Some successful MAS have already been reported in rice

Table 3: QTLs detected for yield-related traits under greenhouse and field conditions under two water treatments

Well-watered (greenhouse)						Water-stressed (greenhouse)					Irrigated (field)					Non-Irrigated 'Rainfed' (field)				
Trait	QTL	Position (cM)	LOD	Additive effects	R^2	QTL	Position (cM)	LOD	Additive effects	R^2	QTL	Position (cM)	LOD	Additive effects	R^2	QTL	Position (cM)	LOD	Additive effects	R^2
DSF	*DSFW.5.1*	25.5	8.7	−2.23[1]	0.13	*DSFD.1.1*	8.0	7.2	3.02	0.15	DSFI.1.1	66.4	7.5	−1.95	0.14	*DSFN.7.1*	12.4	3.0	−1.75	0.11
	DSFW.5.2	33.3	5.1	−2.29	0.13	*DSFD.7.1*[2]	12.4	3.5	−2.24	0.07	*DSFI.7.1*	20.1	6.4	−1.59	0.10	*DSFN.7.2*	20.7	14.1	−3.22	0.37
	DSFW.6.1	75.6	5.9	−2.24	0.13	***DSFD.7.2***	20.7	6.2	−2.48	0.09	*DSFI.10.1*	152.4	5.8	1.85	0.09	*DSFN.10.1*	152.4	5.4	2.14	0.12
	DSFW.9.1	66.8	8.1	2.10	0.11	***DSFD.14.1***	105.0	5.4	−2.56	0.10	*DSFI.11.1*	15.9	8.1	−2.56	0.12	*DSFN.11.1*	19.4	9.5	−2.00	0.17
	DSFW.15.1	20.1	4.0	1.27	0.05	***DSFD.14.2***	126.1	3.4	−2.17	0.11	*DSFI.14.1*	111.4	5.6	−1.27	0.06	*DSFN.14.1*	111.4	9.9	−2.33	0.24
	DSFW.17.1	38.4	6.1	−1.91	0.09	*DSFD.16.1*	84.8	5.1	2.032	0.07	*DSFI.14.2*	126.1	3.1	−1.04	0.04	*DSFN.14.2*	126.1	5.1	−1.82	0.15
	DSFW.17.2	74.1	9.5	2.62	0.17	*DSFD.17.1*	133.6	5.8	2.56	0.10	*DSFI.17.1*	130.8	5.9	1.68	0.12					
LN	*LNW.7.1*	45.2	3.4	2.05	0.14	*LND.3.1*	46.3	3.2	0.84	0.05	*LNI.3.1*	44.3	3.1	1.11	0.05	*LNN.3.1*	44.3	5.0	2.02	0.10
	LNW.12.1	79.2	3.7	1.59	0.13	***LND.5.1***	27.3	5.1	−1.58	0.16	*LNI.7.1*	21.6	14.6	−2.64	0.24	*LNN.5.1*	25.6	25.6	−1.58	0.05
						LND.14.1	79.0	6.1	−1.28	0.11	*LNI.7.2*	33.1	8.3	−2.38	0.18	*LNN.5.2*	87.2	6.0	1.99	0.11
						LND.15.1	24.1	5.1	1.21	0.10	*LNI.14.1*	66.4	3.4	−1.16	0.04	*LNN.7.1*	60.7	8.5	−2.58	0.18
											LNI.16.1	99.5	5.7	1.57	0.09	*LNN.14.1*	66.4	8.6	−2.11	0.12
																LNN.16.1	101.9	5.1	1.46	0.07
LAF	*LAFW.3.1*	17.2	4.2	−144	0.05	*LAFD.1.1*	25.9	6.0	131	0.09	*LAFI.7.1*	20.7	4.6	−381	0.09	*LAFN.3.1*	19.2	3.6	252	0.06
	LAFW.7.1	45.1	5.9	202	0.09	*LAFD.9.1*	114.0	7.9	−175	0.14	*LAFI.11.1*	15.9	4.1	−579	0.07	*LAFN.5.1*	85.2	4.0	303	0.08
	LAFW.9.1	133.7	7.6	229	0.13	*LAFD.10.1*	103.1	10.4	−188	0.19	*LAFI.12.1*	79.2	3.4	330	0.07	*LAFN.7.1*	20.7	7.7	−416	0.15
	LAFW.12.1	79.2	8.0	212	0.11	*LAFD.12.1*	77.7	5.2	138	0.07						*LAFN.7.2*	34.7	3.3	−270	0.07
	LAFW.12.2	94.7	3.6	227	0.12	*LAFD.13.1*	0.0	7.9	−153	0.10						*LAFN.11.1*	0.0	3.6	−252	0.07
	LAFW.13.1	0.0	5.2	−175	0.07	*LAFD.17.1*	109.5	3.4	−103	0.12						*LAFN.13.1*	2.0	3.7	−297	0.10
LAD	*LADW.2.1*	33.5	6.5	−22930	0.17	*LADD.1.1*	26.5	7.3	23318	0.14	*LADI.1.1*	42.9	4.9	−9573	0.07	*LADN.1.1*	50.9	3.0	−12005	0.05
	LADW.3.1	31.0	5.0	−14681	0.10	*LADD.2.1*	38.3	3.1	−14258	0.08	*LADI.3.1*	24.0	4.6	10948	0.10	*LADN.7.1*	16.1	3.0	−9856	0.05
	LADW.10.1	16.3	7.6	24663	0.17	***LADD.3.1***	31.6	3.3	−10505	0.07	*LADI.4.1*	44.2	10.2	15529	0.16	***LADN.10.1***	49.6	3.4	−12300	0.07
	LADW.10.2	38.8	4.6	−20279	0.12	*LADD.9.1*	103.9	3.1	−12145	0.05	*LADI.6.1*	0.0	4.1	−14516	0.15	*LADN.12.1*	55.8	7.3	15768	0.16
	LADW.10.3	49.3	6.3	−25265	0.14	*LADD.16.1*	22.8	5.7	−13892	0.11	*LADI.7.1*	21.6	3.1	−7011	0.04					
	LADW.16.1	22.8	6.7	−17268	0.12	*LADD.17.1*	112.1	5.4	−13218	0.11	*LADI.10.1*	0.0	5.0	−14286	0.17					
PH	*PHW.9.1*	125.7	3.0	4.56	0.15	*PHD.1.1*	45.7	7.8	5.06	0.13	*PHI.4.1*	44.2	4.0	5.04	0.05	*PHN.7.1*	12.4	4.7	−6.63	0.12
	PHW.15.1	24.1	3.4	4.35	0.14	*PHD.2.1*	22.7	4.9	−8.13	0.20	*PHI.7.1*	14.4	3.9	−6.11	0.08	*PHN.7.2*	20.6	12.3	−9.19	0.22
						PHD.9.1	116.0	10.4	−6.74	0.20	***PHI.7.2***	20.6	10.1	−8.72	0.17	***PHN.7.3***	33.1	9.5	−8.74	0.20
						PHD.13.1	18.4	8.4	−7.16	0.23	***PHI.7.3***	34.7	4.2	−5.94	0.08	***PHN.11.1***	8.0	4.6	−5.45	0.09
						PHD.14.1	79.0	3.9	−3.90	0.08	***PHI.11.1***	11.3	3.1	−4.58	0.05	***PHN.16.1***	142.3	4.5	5.79	0.08
BIO	*BIOW.1.1*	66.4	3.3	3.18	0.07	*BIOD.1.1*	61.6	3.3	1.87	0.11	*BIOI.3.1*	0.0	4.9	−13.60	0.09	*BION.1.1*	50.9	4.8	−16.13	0.07
	BIOW.10.1	38.8	3.1	−4.03	0.09	*BIOD.13.1*	0.0	4.5	0.39	0.15	*BIOI.3.2*	21.2	6.5	14.53	0.14	*BION.7.1*	12.4	4.3	−12.04	0.08
	BIOW.10.2	49.3	9.2	−5.28	0.20						*BIOI.7.1*	20.7	4.7	−12.65	0.08	*BION.7.2*	20.1	6.8	−14.51	0.16
	BIOW.12.1	10.9	3.2	−2.89	0.06						***BIOI.7.2***	34.7	3.1	−10.49	0.05	***BION.7.3***	34.7	5.0	−12.51	0.08
	BIOW.12.2	79.2	7.3	4.45	0.16						*BIOI.8.1*	52.5	4.5	−13.74	0.08	*BION.10.1*	170.7	5.5	−16.81	0.08
	BIOW.16.1	169.5	4.1	−6.68	0.23						*BIOI.10.1*	135.9	3.0	−14.33	0.05					
HW	*HWW.5.1*	86.9	4.3	1.47	0.17	*HWD.1.1*	64.4	11.5	1.52	0.24	*HWI.3.1*	0.0	6.3	−7.53	0.10	*HWN.2.1*	100.6	4.9	−5.36	0.07
	HWW.6.1	80.0	4.3	−1.47	0.15	*HWD.4.1*	80.6	5.4	0.93	0.13	*HWI.3.2*	19.2	5.1	5.59	0.06	*HWN.3.1*	21.2	5.4	6.93	0.08
	HWW.10.1	38.8	3.1	−1.72	0.13	*HWD.6.1*	79.7	4.5	−1.26	0.17	*HWI.4.1*	44.2	9.7	8.67	0.13	*HWN.7.1*	16.1	6.0	−6.24	0.08
	HWW.15.1	26.4	3.0	1.75	0.13	*HWD.10.1*	169.4	5.6	−1.17	0.11	*HWI.8.1*	48.1	4.9	−6.26	0.06	*HWN.10.1*	49.4	3.3	−5.51	0.05
						HWD.16.1	91.3	7.8	−1.19	0.15	*HWI.10.1*	135.9	7.0	−7.69	0.10	*HWN.10.2*	170.6	5.5	−8.33	0.09
						HWD.16.2	143.0	6.9	1.15	0.14	*HWI.12.1*	19.1	6.2	−6.82	0.08	*HWN.14.1*	99.9	7.4	7.95	0.10
											HWI.14.1	118.4	4.4	5.24	0.05	*HWN.14.2*	122.1	4.0	−5.12	0.04
GYP	*GYPW.5.1*	87.5	4.0	1.87	0.20	*GYPD.4.1*	80.5	10.6	0.90	0.19	*GYPI.3.1*	21.2	4.4	3.59	0.07	*GYPN.2.1*	98.6	9.2	−5.75	0.19
	GYPW.10.1	49.3	4.9	−1.99	0.16	*GYPD.9.1*	116.1	10.0	0.90	0.19	*GYPI.4.1*	44.2	4.2	3.81	0.06	*GYPN.3.1*	19.2	3.5	2.51	0.04
	GYPW.14.1	73.8	15.6	1.68	0.40	*GYPD.13.1*	0.0	3.3	0.49	0.05	*GYPI.4.2*	83.8	4.1	3.17	0.06	*GYPN.4.1*	50.2	5.6	3.37	0.09
						GYPD.14.1	75.3	4.7	1.14	0.31	*GYPI.5.1*	29.9	13.9	−7.28	0.27	*GYPN.10.1*	46.1	11.7	−13.72	0.30
						GYPD.16.1	22.0	6.5	0.89	0.15	*GYPI.7.1*	51.2	7.3	5.93	0.07	***GYPN.14.1***	73.3	3.0	2.15	0.04
											GYPI.12.1	23.8	8.9	−6.61	0.17	*GYPN.14.2*	99.9	5.6	4.31	0.10

The traits are: days from sowing to flowering (DSF), leaf number per plant (LN), leaf area at flowering (LAF; cm^2), leaf area duration (LAD; cm^2 days), plant height (PH; cm), total dry matter per plant (BIO; g), head weight per plant (HW; g) and grain yield per plant (GYP; g). The QTLs were designated as the abbreviation of the trait followed by '*W*' or '*D*' for well-watered or water-stressed in the greenhouse, and by '*I*' and '*NI*' for irrigated and non-irrigated (rainfed) in the field.

[1]The positive additive effect shows that PAC2 allele increases the trait and negative additive effect shows that RHA266 allele increases the trait.

[2]The common QTLs across water treatments are shown as *bold-face*.

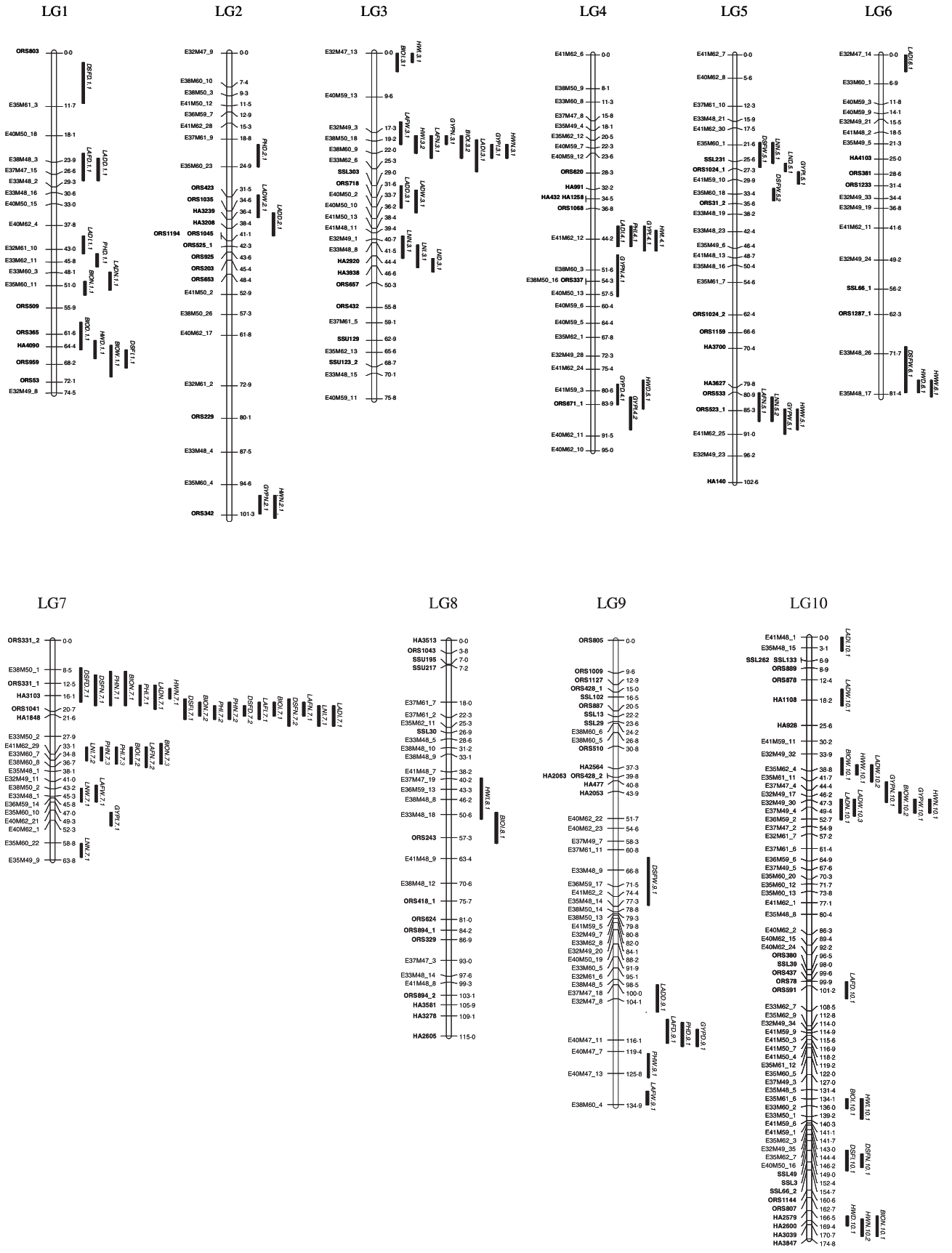

Fig. 2: Genetic linkage map and the positions of QTLs for the studied traits under different conditions. The QTLs were designated as the abbreviation of the trait followed by '*W*' or '*D*' for well-watered or water-stressed in the greenhouse, and by '*I*' and '*NI*' for irrigated and non-irrigated (rainfed) in the field. The traits are: days from sowing to flowering (DSF), leaf number per plant (LN), leaf area at flowering (LAF), leaf area duration (LAD), plant height (PH), total dry matter per plant (BIO), head weight per plant (HW) and grain yield per plant (GYP)

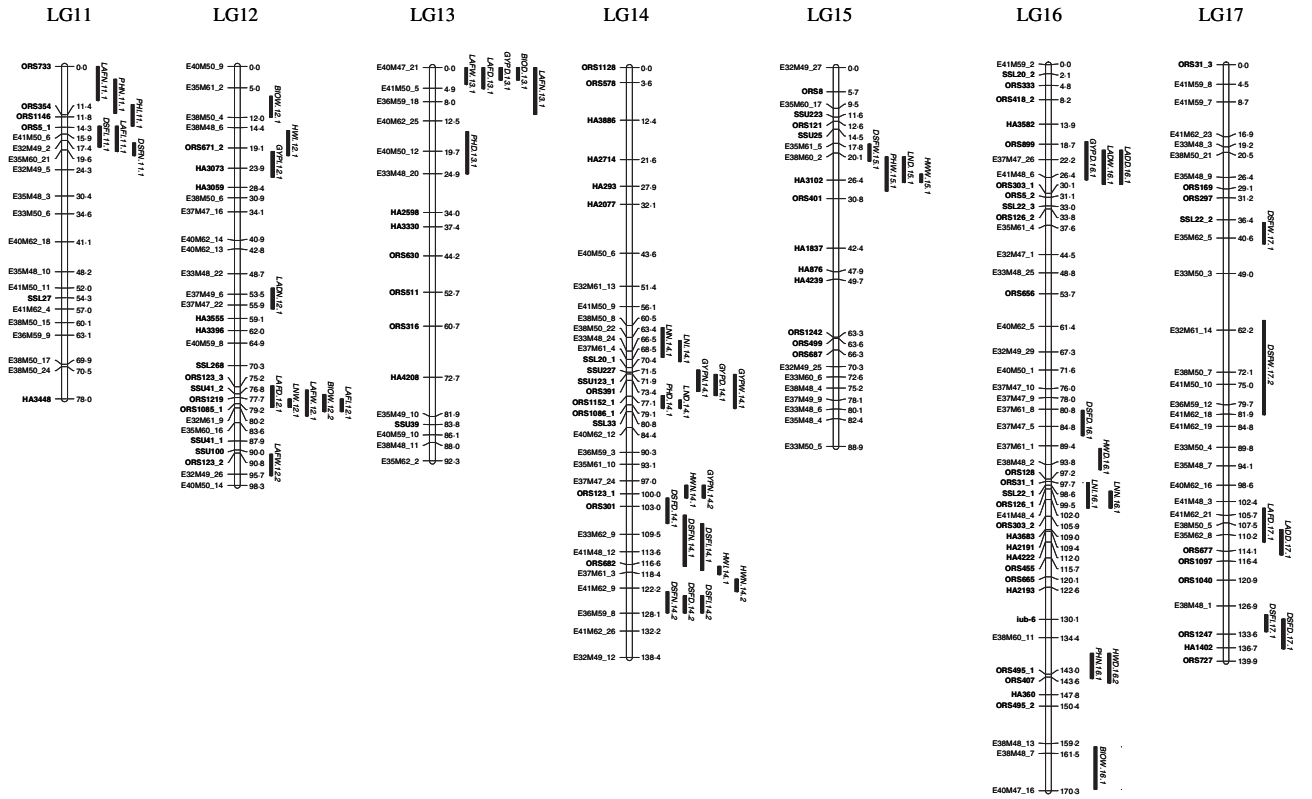

Fig. 2: Continued

breeding programmes. For example, Cho et al. (1994) used molecular markers for selection of semi-dwarf characteristic in rice. Wang et al. (2005) successfully introgressed three QTLs with large effects on spikelet fertility into near isogenic lines using marker-assisted selection. Introgression of QTLs for OA, associated with drought tolerance, has been also achieved in rice by Robin et al. (2003). However, as far as we know, MAS for drought tolerance has not been reported for sunflower in the literature.

QTLs identified for a given trait under several growth conditions

Some QTLs are associated with the same trait under different growth conditions (greenhouse and field) and/or water treatments (well-watered and water-stressed). For example, the QTLs for BIO, LAD, LAF and LN, located on linkage groups 1 (*BIOW.1.1* and *BIOD.1.1*; interval 61.6–66.4 cM), 2 (*LADW.2.1* and *LADD.2.1;* interval 33.5–38.3 cM) and 3 (*LAFW.3.2* and *LAFN.3.1*; *LNI.3.1*, *LNN.3.1* and *LND.3.1*; intervals 17.2–19.2 and 44.3–46.3 cM) were detected in more than one environment (Fig. 2). Besides these QTLs, several additional QTLs for a given trait in at least two growth conditions were detected on different linkage groups, which shows that some QTLs are detectable under multiple conditions. For GYP 11 of 20 QTLs were detected in two or three growth conditions and nine under only one of the four growth conditions. The QTLs for GYP in two or three growth conditions, are located on linkage groups 3 (*GYPI.3.1* and *GYPN.3.1*), 4 (*GYPI.4.1, GYPI.4.2, GYPD.4.1* and *GYPN.4.1*), 10 (*GYPW.10.1* and *GYPN.10.1*) and 14 (*GYPW.14.1*, *GYPD.14.1* and *GYPN.14.1*). The most consistent QTL for yield, which is linked to SSR marker ORS391, is located on linkage group 14 (interval 73.3–75.3 cM), which is a relatively major QTL explaining 40%, 31% and 4% of total phenotypic variance of yield in three growth conditions (well-watered and water-stressed treatments under greenhouse and non-irrigated treatment under field conditions, respectively). It was also overlapped with the QTLs controlling LN and PH explaining 11% and 8% of phenotypic variance, respectively, indicating a relationship between grain yield and plant architecture (Fig. 2). This finding was not supported by phenotypic correlation between yield and LN. The positive alleles for the QTLs controlling yield in this region come from PAC2 and for QTLs conferring PH and LN, the positive alleles come from RHA266. This DNA region could be important in marker-based selection for grain yield, as it was detected in three different growth conditions (Fig. 2).

In the previous study, QTLs controlling plant water status traits and OA were mapped in the same mapping population under well-watered and water-stressed conditions (Poormohammad Kiani et al. 2007b). Comparing the QTLs found in the present study for grain yield and agro-morphological traits with those previously reported, overlapping QTL indicates a physiological link between plant water status, OA and agronomical traits (Table 4). However, increasing the map resolution would be necessary to determine if this physiological link is because of pleiotropy or tight linkage. Some of the overlapped QTLs were located practically at the same positions. As an example on linkage group 5, four overlapped QTLs detected in the present study for GYP, LN and LAF, (*GYPW.5.1*, *HWW.5.1*, *LNN.5.2* and *LAFN.5.1*) has been previously detected as the most important DNA region for plant water status traits, such as relative water content (RWC) in well-watered and water-stressed conditions as well as for

Table 4: QTLs controlling agronomical traits under four growth conditions identified in the present study, which are overlapped with QTLs for plant water status and osmotic adjustment identified in our previous study (Poormohammad Kiani et al. 2007b)

Linkage group	Agronomical traits in the present study	Water status traits and osmotic adjustment (Poormohammad Kiani et al. 2007b)	Overlapped QTLs
LG1	Leaf area at flowering Leaf area duration	Turgor potential (Ψ_t), Osmotic potential (Ψ_s)	'*LAFD.1.1*','LADD.1.1' 'TP.WS.1.1', 'OP.WS.1.1'
LG5	Days from sowing to flowering	Osmotic potential (Ψ_s)	'*DSFW.5.2*', 'OP.WS.5.2'
LG5	Grain yield per plant Head weight per plant Leaf number per plant Leaf area at flowering	Osmotic adjustment (OA), Osmotic potential at full turgor (Ψ_{sFT}), Relative water content (RWC), Leaf water potential (Ψ_w)	'*YPW.5.1*', 'LNN.5.2', 'LAFNN.5.1', 'HWW.5.1', '*OA.5.2*', 'OPF.WS.5.1', RWC.WS.5.1', 'RWC.WW.5.1', 'LWP.WS.5.1'
LG6	Leaf area duration	Relative water content (RWC), Turgor potential (Ψ_t)	'*RWC.WW.6.1*','TP.WW.6.1', '*LADI.6.1*'
LG7	Several traits (Fig. 2)	Turgor potential (Ψ_t)	'*Several QTLs (Fig. 2)*', 'TP.WS.7.1'
LG7	Leaf number per plant	Leaf water potential (Ψ_w), Relative water content (RWC)	'*LWP.WS.7.1*', 'RWC.WS.7.1', 'LNN.7.1'
LG9	Days from sowing to flowering	Leaf water potential (Ψ_w)	'*DSFW.9.1*', 'LWP.WS.9.1'
LG12	Grain yield per plant Head weight per plant	Turgor potential (Ψ_t), Osmotic adjustment (OA)	'*YPI.12.1*', 'HWI.12.1', 'TP.WW.12.1', 'OA.12.1', 'OA.12.2'
LG13	Plant height	Osmotic adjustment	'*PHD.13.1*', 'OA.13.1'
LG16	Grain yield per plant Leaf area duration	Osmotic potential at full turgor (Ψ_{sFT}), Turgor potential (Ψ_t), Leaf water potential (Ψ_w), Relative water content (RWC)	'*YPD.16.1*', 'LADW.16.1', 'LADD.16.1','OPF.WS.16.1', 'OPF.WS.16.2','OPF.WW.16.1', 'TP.WS.16.1', 'LWP.WS.16.1', 'RWC.WS.16.1'
LG16	Days from sowing to flowering	Leaf water potential (Ψ_w), Relative water content (RWC), Osmotic potential (Ψ_s), Osmotic potential at full turgor (Ψ_{sFT})	'*DSFD.16.1*', 'LWP.WS.16.2', 'RWC.WS.16.2', 'OP.WS.16.1', 'OPF.WS.16.2'
LG16	Leaf number per plant	Turgor potential (Ψ_t)	'*LNI.16.2*', 'LNN.16.1'
LG16	Total dry matter per plant (biomass)	Osmotic potential (Ψ_s)	'*BIOW.16.1*', 'OP.WW.16.1'
LG17	Days from sowing to flowering	Relative water content (RWC)	'*DSFW.17.1*','RWC.WW.17.2', 'RWC.WS.17.1'

leaf water potential, osmotic potential at full turgor and OA (Poormohammad Kiani et al. 2007b).

On linkage group 7, the QTLs controlling LN (under irrigated condition), LAF (under two growth conditions), LAD (under two growth conditions), PH (under two growth conditions), DSF (under three growth conditions), HW and BIO (under two growth conditions) detected in the present study (Fig. 2, Table 4), were overlapped with the QTL controlling turgor potential identified in our previous work (Poormohammad Kiani et al. 2007b). Maintaining turgor potential under drought conditions 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 drought conditions depend on turgor potential in sunflower (Turner and Jones 1980, Morgan 1984, Maury et al. 1996, 2000). Therefore, overlapping QTLs for turgor potential and agronomical traits suggest the common genetic basis for turgor maintenance and plant growth and development in this genomic region. Although many other overlapping QTLs are observed on several linkage groups for various drought-adaptive and morphological and developmental traits (Table 4), we are especially interested in relationship between drought-adaptive and productivity QTLs. Three QTLs for GYP identified in the present study are overlapped with several QTLs for drought-adaptive traits. One of them located on linkage group 5 (*GYPW.5.1*), is overlapped with the QTLs for OA (*OA.5.2*), leaf water potential (*LWP.WS.5.1*) and RWC under both water treatments (*RWC.WW.5.1* and *RWC.WS.5.1*) (Table 4). Another QTL for GYP, located on linkage group 12 (*GYPI.12.1*), is overlapped with one QTL for turgor potential (*TP.WW.12.1*) and two QTLs for OA (*OA.12.1* and *OA.12.2*). Seemingly, the third QTL for GYP, located on linkage group 16 (*GYPD.16.1*) is overlapped with the QTLs for turgor potential (*TP.WS.16.1*), osmotic potential at full turgor (*OPF.WS.16.2* and *OPF.WW.16.1*), leaf water potential (*LWP.WS.16.1*) and RWC (*RWC.WS.16.1*) (Table 4).

We have shown a partly common genetic basis for plant water status, OA and productivity. Detailed characterization of these genomic regions through the development and evaluation of near-isogenic lines will lead to an improved understanding of drought tolerance and might set the stage for the positional cloning of drought tolerance genes. Prior results of plant water status and OA have been largely based on phenotypic association with yield under drought stress in sunflower (Chimenti et al. 2002). Overlapping QTLs for water status traits, OA and productivity has been observed in cotton (Saranja et al. 2004) and barley (Teulat et al. 1998).

In the present study, a mapping population was evaluated for agronomical traits under greenhouse and field conditions each with two water treatments. Using the same mapping population under different water regimes helped us on the identification of consistent genomic regions (QTL) from those expressed under specific conditions for several agronomical and yield-related traits. Although QTLs induced only by drought may be associated with mechanism(s) of sunflower

drought response, we suppose that the QTLs that can reduce trait difference between well-watered (irrigated) and water-stressed (non-irrigated) conditions should have an effect on drought tolerance because of their contribution to trait stability. Therefore, the QTLs, which are common across water treatment are of more interest and most useful for MAS. Regarding to these points, the most stable genomic region controlling yield is located on linkage groups 14 (SSR marker: ORS391), where three QTLs for yield under three water treatments were overlapped.

One of the major goals for plant breeders is to develop genotypes with high yield potential and the ability to be stable across environments. There are two main ways in which a cultivar can achieve stability. The first one is identification of the non-environment-specific QTLs or QTLs with minor interaction with environments (as those located on linkage group 14), which should be particularly useful in MAS for yield. The second is the development of widely adapted cultivars by pyramiding different QTLs each controlling adaptation to a different range of environments (as nine environment-specific QTLs for yield).

We compared the position of QTLs obtained in the present study with the results obtained by Rachid Al-Chaarani et al. (2004) for yield-related traits using the same mapping population (PAC2 × RHA266) under well-watered conditions. According to the authors, the most important DNA regions controlling thousand grain weight and yield are located on linkage groups 4, 6 and 9, which correspond to the linkage groups 7, 5 and 10 in the present study with public common linkage group nomenclature. One QTL for yield reported by Rachid Al-Chaarani et al. (2004) on linkage group 6 was confirmed in the present study on the corresponding linkage group 5 (*GYPI.5.1)*. Their two other QTLs are co-located with the QTLs controlling biomass on linkage groups 7 (*BION.7.3* and *BIOI.7.2*) and 10 (*BIOW.10.1*) in the present study. The latter, however, is close to two QTLs controlling GYP (*GYPN.10.1* and *GYPW.10.1*).

Another application of QTL analysis is the genetically determination of the trait association by evaluation of overlapping QTLs. In the present study we identified the genomic regions controlling productivity (BIO per plant, HW per plant and GPY), which overlapped with the QTLs previously reported for plant water status and OA (Poormohammad Kiani et al. 2007b). The results showed a partly common genetic basis for physiological traits (plant water status and OA) and grain yield in RILs. The whole results do highlight interesting genomic regions for marker-based breeding programme for drought tolerance in sunflower. Knowledge of the number and likely position of loci for drought adaptive traits and yield can provide the information required to select optimal combinations of alleles by the use of marker-assisted selection. For example, combining the major QTL for yield on linkage group 14 (nearest SSR marker ORS391) with another QTL of yield on linkage group 5 (nearest SSR marker ORS523_1), considering that the latter marker was also linked to the QTLs for several plant water status traits and OA with high phenotypic variance explained, could be beneficial for pyramiding higher grain yield and drought tolerance QTLs in the same genotype. However, like any other quantitative trait, there is a requirement to confirm the position of the QTL and carry out fine-scale mapping before MAS becomes a viable proposition.

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