



HAL
open science

Identification of quantitative trait loci for relative water content and chlorophyll concentration traits in recombinant inbred lines of sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions

Nishtman N. Abdi, Reza R. Darvishzadeh, Hamid H. Hatami Maleki, Parham Haddadi Haddadi, Ahmad A. Sarrafi

► To cite this version:

Nishtman N. Abdi, Reza R. Darvishzadeh, Hamid H. Hatami Maleki, Parham Haddadi Haddadi, Ahmad A. Sarrafi. Identification of quantitative trait loci for relative water content and chlorophyll concentration traits in recombinant inbred lines of sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. *Zemdirbyste-Agriculture*, 2013, 100 (2), pp.159 - 166. 10.13080/z-a.2013.100.020 . hal-01001065

HAL Id: hal-01001065

<https://hal.science/hal-01001065>

Submitted on 29 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 100, No. 2 (2013), p. 159–166

DOI 10.13080/z-a.2013.100.020

Identification of quantitative trait loci for relative water content and chlorophyll concentration traits in recombinant inbred lines of sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions

Nishtman ABDI¹, Reza DARVISHZADEH¹, Hamid HATAMI MALEKI², Parham HADDADI³, Ahmad SARRAFI⁴

¹Urmia University
Urmia, Iran
E-mail: r.darvishzadeh@urmia.ac.ir

²University of Maragheh
Maragheh, Iran

³Institut National de la Recherche Agronomique (INRA), Unité Mixte de Recherche (UMR1318)
Institut Jean-Pierre Bourgin
78026 Versailles Cedex, France

⁴INP-Ecole Nationale Supérieure d'Agronomie de Toulouse,
Laboratoire Ecologie Fonctionnelle et Environnement
18 chemin de Borde Rouge, BP 32607 Auzeville, 31326 Castanet-Tolosan, France

Abstract

The goal of the present research work was to identify quantitative trait loci (QTLs) involved in the genetic variation of relative water content and chlorophyll concentration in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. 70 recombinant inbred lines (RILs) out of 123 from the cross PAC2 × RHA266 and their parental lines were evaluated in a rectangular 8 × 9 lattice design with two replications under well-watered and water-stressed conditions. High genetic variability and transgressive segregation was observed among RILs for evaluated traits in both water treatment conditions. QTLs were mapped using an updated high-density simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) linkage map. The map consisted of 210 SSR and 11 genes placed in 17 linkage groups. The total map length is 1,653.1 cM (centimorgan) with a mean density of 1 marker per 7.44 cM. Under well-watered state, 3 and 6 QTLs were identified for chlorophyll concentration and relative water content, respectively. In water-stressed condition 7 and 2 QTLs were identified. The percentage of phenotypic variance (R^2) explained by QTLs ranged from 0.39% to 52.48%. QTLs for chlorophyll concentration and relative water content on linkage group 10 and 16 were overlapped. Common QTLs for different traits in both water treatment conditions on linkage groups 10 seem to be more important as it gives a constitutive performance for the traits without being affected by water treatment.

Key words: *Helianthus annuus*, linkage map, photosynthetic traits, plant water-status, QTL mapping, transgressive segregation.

Introduction

Drought is the major factor limiting crop productivity worldwide (Chimenti et al., 2006). Crops with improved tolerance level to drought stress appear to be crucial in areas where dry seasons are common (Andrew et al., 2000). Drought tolerance is not a unique and easily quantifiable plant attribute; it is a complex character that can be evaluated with relative water content (RWC), relative water loss, chlorophyll fluorescence, stomatal resistance, cell membrane stability, free proline accumulation and many other characters (Zarei et al., 2007). Among the traits used for evaluating plant water-status, RWC gives best measure of the level of the water deficit in the plant at a specific time-point. As RWC is related to cell volume, it may closely reflect the balance between water supply to the leaf and transpiration rate (Sinclair, Ludlow, 1986). Decreased RWC and leaf water

potential (ψ_w) inhibit the photosynthesis capacity in sunflower (Tezara et al., 2002). It was reported that among the photosynthetic traits, chlorophyll concentration is an index for estimating environmental stress effects on growth and yield, since this trait was closely correlated with the rate of carbon exchange (Guo, Li, 2000; Fracheboud et al., 2004). Chlorophyll concentration as one of the major chloroplast components has a positive relationship with photosynthetic rate (Guo, Li, 1996). So, this parameter can be used as a reliable indicator to evaluate the energetic/metabolic imbalance of photosynthesis and yield performance across genotypes under water deficit (Araus et al., 1998).

In the past, progress in improving drought tolerance via conventional breeding methods has been slow, due to genotype × environment interactions, poor

definition of the target environment, the complexity and difficulty of drought-evaluation procedures, and the inconsistency of morpho-physiological traits as selection criteria for drought tolerance (Ceccarelli et al., 2004; 2007). In the last decade, molecular marker technologies have been successfully used to identify quantitative trait loci (QTLs) controlling important traits in various crop plants in responses to drought stress (Verma et al., 2004; Abdi et al., 2012). Marker assisted selection may solve problems associated with genotype × environment interactions, improve the selection efficiency and facilitate pyramiding different genes into a single genotype background. Although sunflower is moderately tolerant to water stress, its distribution and production are greatly influenced by drought stress. Several researches were conducted to identify putative QTLs related to osmotic adjustment (Jamaux et al., 1997; Poormohammad Kiani et al., 2007 a), agronomic traits (Poormohammad Kiani et al., 2009; Haddadi et al., 2012), photosynthesis parameters (Herve et al., 2001; Poormohammad Kiani et al., 2008), whereas, a few studies have been carried out to determine the QTLs linked to RWC (Herve et al., 2001; Poormohammad Kiani et al., 2007 a) and chlorophyll concentration (Herve et al., 2001) in sunflower. Herve et al. (2001) reported 4 QTLs for chlorophyll concentration and 1 QTL for RWC which explained 53% and 9.8% of their phenotypic variation, respectively. Poormohammad Kiani et al. (2007 a) have identified 6 QTLs for RWC whose phenotypic variation ranged from 7% to 25%.

In the present study, we aimed to identify QTLs involved in genetic variation of chlorophyll concentration and relative water content in RILs of sunflower under well-watered and water-stressed conditions using an integrated and high density genetic-linkage map based on SSR and SNP markers.

Material and methods

Plant materials and experimental methodology.

Recombinant inbred lines (RILs) used in this research were developed through single seed descent from F_2 plants, derived from the cross between PAC2 and RHA266 (Flores Berrios et al., 2000). Seeds of 70 RILs and their two parents kindly provided by National Institute for Agricultural Research (INRA, France) were evaluated in both well-watered and water-stressed conditions using a rectangular 8 × 9 lattice design with two replications. The experiment was arranged in research farm of Urmia University, Iran in 2010. Each plot comprised 1 line 8 m long, with a spacing of 75 × 25 cm between lines and plants, respectively. The distance between well-watered and water-stressed experiments was considered 5 m. From the beginning of planting time until the complete establishment of sunflower plants (eight-leaf (V8) stage), irrigation in both well-watered and water-stressed experiments was carried out when the amount of evaporated water (from class 'A pan' evaporation) reached to 60 mm (Pourtaghi et al., 2011). After V8 stage, the irrigation was continued in the same manner (60 mm evaporation from class 'A pan') in well-watered experiment but it was carried out in the water-stressed experiment when the amount of evaporated water (from class 'A pan' evaporation) reached to 180 mm (Pourtaghi et al., 2011).

Traits measurement. Chlorophyll concentration.

Leaf chlorophyll concentration was determined using a chlorophyll meter SPAD-502 ("Minolta", Japan). During filling grain stage, chlorophyll concentration on the youngest fully expanded leaves of five plants from each

genotype per replicate were measured before irrigation in both well-watered and water-stressed experiments. Three measurements were made at random locations in the middle of the leaf for each plant and the average was used for the analysis.

Relative water content (RWC). The youngest fully expanded leaf of four plants per genotype per replicate was sampled in both well-watered and water-stressed conditions. Leaf discs of 4 cm² area in rectangle shape per plant were prepared and the fresh weight (Wf) was measured. Leaf discs were then dipped in the glass vials containing 20 ml deionized water. These vials were left for four hours at room temperature (about 25°C) with no illumination. After four hours, leaf discs were blotted and their turgid weight (Wt) was recorded. Leaf discs were oven dried in 80°C for 24 h and weighed (Wd). RWC was calculated using the following formula:

$$RWC(\%) = \left[\frac{Wf - Wd}{Wt - Wd} \right] \times 100.$$

Map construction and quantitative trait loci (QTL) mapping. Simple sequence repeat (SSR) mapping.

The genomic DNA of RILs and their parents (PAC2, RHA266) were extracted according to the method of extraction and purification presented by Porebski et al. (1997). We used Picogreen fluorescent stain (Quanti-iTTM Picogreen®, Invitrogen) to quantify DNA concentration with the BioTek FL600 Fluorescence Microplate Reader (BioTek Instruments Inc., USA). One hundred and fourteen SSRs were studied. All SSR markers are public and can be provided upon request. We used multiplex polymerase chain reaction (PCR) method, in which several SSR markers are simultaneously amplified in the same reaction (we used 4 SSR markers in the same reaction thanks to their size). PCR is done using a forward primer with a nucleotide extension at its 5'-end, identical to the sequence of an M13 sequencing primer and a standard length reverse primer and fluorescently (6-FAM, NED, VIC and PET) labelled M13 primer. During PCR, the SSR product is fluorescently labelled following participation of the M13 primer after the first few cycles of amplification. Therefore, instead of synthesizing one specific fluorescently labelled primer for each SSR marker, only a dye labelled M13 primer is needed. PCR products were diluted with ultrapure water (2 µl of each PCR product in 20 µl water) and 2 µl of diluted PCR products mixed with 7.94 µl Formamide HiDiTM and 0.06µl GeneScanTM 500 LIZTM size standard (Applied Biosystems, USA). After denaturing at 94°C for 5 min, we used sequencer ABI3730 and fragments were sized using the *GeneMapper*® software, version 4 (Applied Biosystems). Chi-square-tests were performed for segregation distortion of each locus. All new SSRs are mapped to our previous map (Haddadi et al., 2012) by *Carthagène* (De Givry et al., 2005) and *Mapmaker* (Lander et al., 1987).

Candidate genes (CGs) mapping. Some important tocopherol and phytosterol pathway-related genes, enzymatic antioxidant-related genes, drought-responsive genes and *Arabidopsis* Sec14 homologue genes were selected to introduce in our map. Respective sequence data for CGs coding for these proteins were obtained from the *Arabidopsis* information resource (www.arabidopsis.org). In order to seek the *Helianthus* homolog sequences to the *Arabidopsis* genes, we used the *Compositae* expressed sequence tag (EST) assembly clusters, available at the *Helianthus*-devoted bioinformatics portal HeliaGene (www.heliagene.org).

The *Helianthus* EST clusters presenting the reciprocal blast with the highest score and lowest E value with regarding to the original *Arabidopsis* genes were chosen for our studies. All primers were designed by *MATLAB*. Forward primers were tailed at 5' with M13-Fwd tail (5' CACGACGTTGTAAAACGAC3') and reverse primers were tailed at 5' with M13-Rev tail (5' ACAGGAAACAGCTATGAC3'). Between 2 to 4 various primer combinations per each candidate gene were tested on agarose gel. The PCR program was: 4 min at 94°C followed by 35 cycles; 30 s at 94°C, 30 s at 55°C or 58°C, 1 min at 72°C and final extension of 5 min at 72°C. One PCR fragment per gene was sequenced using M13-Fwd and M13-Rev primers. After sequencing, SNP-PHAGE (SNP discovery Pipeline with additional features for identification of common haplotypes within a sequence tagged site (Haplotype Analysis) and GenBank (-dbSNP) submissions), through the website (www.heliagene.org), was applied for analyzing sequence traces from both parents to identify SNPs. Several types of markers such as dominant, co-dominant, HRM: high resolution melting, InDel (short insertions and deletions), and SNP-based cleaved amplified polymorphic sequences (CAPS) markers are developed for genotyping of the studied candidate genes.

QTL mapping. QTL mapping of the studied traits was performed by composite interval mapping (CIM), using *Win QTL Cartographer*, version 2.5 (Wang et al., 2012). The genome was scanned at 2 cM intervals with a window size of 15 cM and up to 15 background markers were used as cofactors in the CIM analysis identified by the *SMapqtl* program (model 6). LOD (\log_{10} likelihood ratio: likelihood that the effect occurs by linkage/likelihood that the effect occurs by chance) thresholds resulting from permutation tests were used for identifying significant QTLs. Additive effects of the detected QTLs were estimated with *Zmapqtl* program. The percentage of phenotypic variance (R^2) explained by the QTLs was estimated at the peak of curve by *Win QTL Cartographer*. *MapChart 2.2* was used to draw a graphical presentation of linkage groups and map position of QTLs.

Statistical analysis. Analysis of variance was performed using *PROC GLM* procedure in the *SAS* software (SAS/STAT® 9.2, 2009). Phenotypic correlations between studied traits in water treatment states were determined using *PROC CORR* in the *SAS* software.

Results and discussion

Phenotypic variation among inbred lines.

Analysis of variance indicated the presence of genetic variation and possibility of mapping the genes involved in the inheritance of studied drought tolerance criteria (chlorophyll concentration and relative water content) in sunflower (Table 1). The effects of water treatment condition, genotype, and the interaction between genotype and water treatment were significant (Table 1). The presence of significant genotype \times water treatment interaction on the studied traits means that the changes occur in relative performance of genotypes across water treatment conditions. So, a genotype showing promise in one water treatment condition may not be exceptional in another condition. Slicing significant interaction effects for relative water content trait revealed the significant difference among genotypes in each one of water treatment conditions, while concerning chlorophyll concentration there were significant difference among genotypes only in well-watered condition (Table 1).

Table 1. Mean squares (MS) of chlorophyll (Chl) concentration and relative water content (RWC%) in sunflower recombinant inbred lines (RILs) and their two parents under two water treatment conditions

Source of variation	df	MS	
		Chl	RWC%
Water treatment	1	516.66***	857.59***
Replication (water treatment)	2	465.86***	586.05***
Genotype	71	37.75**	92.25*
Genotype \times water treatment	71	33.83*	101.87**
Block (water treatment \times replication)	32	21.86 ^{ns}	170.24**
Residual	110	21.47	58.46
Genotype \times water treatment effect sliced by water treatment for genotype			
Water-stressed	71	29.25*	70.80*
Well-watered	71	31.11*	84.47 ^{ns}
CV%		24.63	11.03

CV% – coefficient of variation, df – degree of freedom; *, **, *** – significant at 0.05, 0.01 and 0.001 probability level

Phenotypic performance of RILs and their parents for chlorophyll concentration and relative water content under the two water treatment conditions are summarized in Table 2. Parental lines showed different chlorophyll concentration under water-stressed condition (Table 2). The differences between the mean of RILs (\bar{X}_{RIL}) and the mean of their parents (\bar{X}_p) were not significant for studied traits (Table 2). This non-significant difference shows that the RILs used in this study are representative of possible recombination of the cross PAC2 \times RHA266. The genetic gain calculated as difference between the mean values of 10% selected best RILs ($\bar{X}_{10\%bestRILs}$) and the mean of parents (\bar{X}_p) was significant for the studied traits in both water treatment conditions except for chlorophyll concentration in water-stressed condition (Table 2). High standard deviation was observed for studied traits in both water treatment conditions. Transgressive segregation that would be the result of the accumulation of positive alleles from both parental lines was observed for both of the studied traits (Table 2). Transgressive segregation for osmotic adjustment, photosynthesis and plant water status traits under well-watered and water-stressed conditions as well as for physiological traits associated to low temperature growth under early sowing conditions has been also reported by Herve et al. (2001), Poormohammad Kiani et al. (2007 a; b) and Allinne et al. (2009) in sunflower. Results revealed that under water-stressed condition, the mean value of chlorophyll concentration increased and the mean value of relative water content decreased compared with the well-watered condition.

In this study, chlorophyll concentration was significantly and positively correlated with relative water content under water-stressed condition (Table 3). Therefore, changes in water content are not directly related to the chlorophyll concentration in all types of environments. Furthermore, highly significant correlations between relative water content in two water treatments were observed that show the phenotypic value under well-watered condition explain a large proportion of the variation for performance under water-stressed condition (Table 3). This result suggests that selection under well-watered conditions could partly be effective to improve relative water content under water-stressed condition.

Table 2. Genetic parameters and gain for chlorophyll (Chl) concentration and relative water (RWC%) content in sunflower recombinant inbred lines (RILs) and their two parents under two water treatment conditions

Conditions	Well-watered		Water stressed	
	Chl	RWC%	Chl	RWC%
PAC2 (P1)	15.19	73.32	69.3	75.75
RHA266 (P2)	19.10	63.86	67.30	80.21
P1-P2	-3.91	9.46	-10.93	-4.46
\bar{X}_p	17.14	68.59	68.3	77.98
Max	34.74	87.90	25.93	88.84
Min	10.50	44.59	6.74	52.04
\bar{X}_{RIL}	18.86	67.40	15.97	70.98
$\bar{X}_{RIL} - \bar{X}_p$	1.70	-1.17	-4.43	-6.90
$\bar{X}_{10\%bestRIL}$	26.72	80.61	22.30	83.44
GG10%= $\bar{X}_{10\%bestRIL} - \bar{X}_p$	17.60*	19.31*	5.46 ^{ns}	10.86*
STDEV	4.24	7.84	3.75	7.21
LSD-I _{0.05}	6.51	12.56	5.72	8.87
LSD-C _{0.05}	9.08	14.99		

Notes. \bar{X}_p – mean of parents, \bar{X}_{RILs} – mean of all RILs, $\bar{X}_{10\%bestRIL}$ – 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-I_{0.05} – least significant differences calculated using *t* 0.05 and error mean square of each experiment. LSD-C_{0.05} – least significant differences calculated using *t* 0.05 and error mean square of combined analysis of experiments.

Table 3. Correlation among traits in sunflower recombinant inbred lines (RILs) under well-watered and water-stressed conditions

Trait	Chl	RWC%
well-watered condition		
Chl	1	
RWC%	-0.25 ^{ns}	1
water-stressed condition		
Chl	1	
RWC%	0.34*	1
well-watered with water-stressed		
	0.11 ^{ns}	0.32*

Chl – chlorophyll content, RWC% – relative water content; ^{ns} – non significant; * – significant at 0.05 probability level, respectively

Linkage map. Of 114 SSR primer pairs tested, 23 SSR primers (20.2%) produced clear polymorphisms between the parental lines which segregated in a Mendelian manner. These new SSR markers together with 5 new candidate genes identified with the code HuCL were assigned to the previously reported linkage map (Haddadi et al., 2012). New SSR can be found in linkage groups 1, 2, 3, 4, 5, 6, 8, 10, 13, 14, 15 and 17 and new 5 candidate genes (HuCLs) in linkage groups 2, 8 and 16. The updated map consisted of 210 SSR markers and 11 genes placed in 17 linkage groups. Linkage groups were named as 1 to 17 according to the reference linkage map of sunflower (Tang et al., 2002). The total map length is 1,653.1 cM with a mean density of 1 marker per 7.44 cM (Fig.). The number of markers per linkage group ranged from 5 to 26. Linkage group 14 is the largest in term of cM size (197.6 cM) while linkage group 4 (32 cM) is the smallest (Fig.).

Quantitative trait loci (QTL) analysis. QTL mapping showed the presence of 16 QTLs on 9 linkage groups in the expression of chlorophyll concentration and relative water content under both water treatment conditions (Table 4). The number of detected QTLs for each trait varied from 2 to 7 depending on water treatments. The phenotypic variance explained by QTLs (*R*²) ranged from 0.39% to 52.48%, and the sign of additive gene effects showed that both parental lines contributed to the expression of studied traits. For chlorophyll concentration, 3 QTLs were detected under well-watered condition on linkage groups 10 and 16 with the phenotypic variance ranging from 0.56% to 42.19% (Table 4, Fig.).

Table 4. Map position and effect of quantitative trait loci (QTL) detected for chlorophyll (Chl) concentration and relative water content (RWC) in sunflower recombinant inbred lines (RILs) in well-watered and water-stressed conditions

Trait	QTL	LG	Position cM	LOD	Additive effects	R ² %
Well-watered condition						
Chl	ChlW.10.1	10	18.31	2.81	2.69	42.19
	ChlW.10.2	10	25.41	3.57	2.79	0.56
	ChlW.16.1	16	43.01	3.29	2.47	30.29
RWC	RWCW.11.1	11	40.51	3.36	-3.00	21.18
	RWCW.12.1	12	66.01	3.51	0.19	0.93
	RWCW.16.1	16	47.01	3.37	-1.14	1.99
	RWCW.17.1	17	12.91	3.26	1.58	6.79
Water-stressed condition						
Chl	ChlS.3.1	3	29.01	4.90	-1.41	21.10
	ChlS.8.1	8	35.21	4.19	-1.95	52.48
	ChlS.10.1	10	95.61	3.77	-0.09	0.39
	ChlS.14.1	14	195.31	4.30	-1.05	15.38
	ChlS.15.1	15	29.01	4.20	-0.62	2.18
	ChlS.16.1	16	109.01	3.73	-1.06	14.51
	ChlS.16.2	16	143.01	4.32	-0.73	7.01
RWC	RWCS.10.1	10	17.31	2.51	-2.88	23.00
	RWCS.10.2	10	119.61	2.62	4.30	29.48

Notes. QTL names were constructed using the trait abbreviation name and 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. The positive additive effect shows that PAC2 allele increase the trait and negative value shows that RHA266 allele increases the trait. LG – linkage groups; cM – centimorgan; LOD – log₁₀ likelihood ratio; likelihood that the effect occurs by linkage/likelihood that the effect occurs by chance; % – percentage of phenotypic variance explained by the individual QTLs.

The positive alleles for these QTLs come from PAC2. The major QTL for chlorophyll concentration (ChlW.10.1) is found on linkage group 10 (Table 4). This QTL was also identified for relative water content, with an *R*² value of 23.00% in water-stressed condition (Table 4, Fig.). The most important QTL detected for chlorophyll concentration under well-watered condition (ChlW.10.1) is also reported for leaf area duration under well-watered condition by Poormohammad Kiani et al. (2008). Under water-stressed condition, 7 QTLs were identified for chlorophyll concentration on linkage groups 3, 8, 10, 14, 15 and 16 with the phenotypic variance ranging from 0.39% to 52.48% (Table 4, Fig.). RHA266

contributed positive alleles for all detected QTLs. In this study, one identified QTL for chlorophyll concentration under water-stressed condition (ChlS.3.1) was also reported for leaf area duration under both water-treatment conditions by Poormohammad Kiani et al. (2009) and for 1000 kernel weight and palmitic acid content under water-stressed condition by Ebrahimi et al. (2008; 2009). Four QTLs were detected for relative water content, on linkage groups 11, 12, 16 and 17 under well-watered condition, and 2 QTLs on linkage group 10 under water-stressed condition (Table 4, Fig.). These putative QTLs explained 0.93% to 21.18% and 23.00% to 29.48% of the total phenotypic variance of target trait in each one of the conditions, respectively (Table 4). The identified QTL on linkage group 10 (RWCS.10.2) under water-stressed condition, was also reported by Ebrahimi et al. (2008; 2009) for percentage of protein, 1000 kernel weight, oil content and palmitic acid content under well-watered condition. Results revealed that PAC2 and RHA266 contributed equally to QTLs controlling relative water content.

QTLs overlapped among studied traits and water treatments. Results showed that some detected QTLs are associated with two traits and some others are specific for only one trait or a given water treatment (Table 4, Fig.). Co-localization of QTLs for different traits implies likely the presence of pleiotropic or closed linkage between QTLs controlling traits (Tuberosa et al., 2002). For instance, 2 QTLs including ChlW.16.1 and RWCW.16.1 for chlorophyll concentration and relative water content under well-watered condition were overlapped on linkage group 16 (Fig.). These QTLs were also reported for stomatal conductance and plant dry weight by Herve et al. (2001) and for linoleic acid content by Haddadi et al. (2010). According to simple correlation (Table 3), negative correlation between relative water content and chlorophyll concentration is justified by opposite additive effects of their overlapped QTLs. These phenomena possess potential challenges to breeders for simultaneous improvement of both traits. However, independent segregation of QTLs for studied traits at genetic level provides opportunity for simultaneous improvement of these traits in sunflower breeding programs. QTLs common for both water treatments seem to be more important as it gives a constitutive performance for the traits without being affected by water treatment. For instance, overlapped QTLs between two water treatment regimes was observed in interval 17–18 cM on linkage group 10 near markers HA928 and ORS456 for chlorophyll concentration and relative water content (ChlW.10.1 and RWCS.10.1). However, majority of identified QTLs were specific for each or both water treatment conditions, which could be of interest for marker-assisted selection (MAS) when favourable alleles are selected. Overlapping QTLs involved in studied traits show that the plant water status and chlorophyll concentration are genetically related, and should be considered together when used as the selection criteria for drought tolerance improvement in sunflower. Therefore, this region may increase yield under stress through drought avoidance by increasing water uptake and mineral nutrients resulting in better growth and photosynthesis.

Conclusions

1. Quantitative trait loci (QTL) analysis for water status and photosynthetic traits using our improved map with 210 simple sequence repeats (SSRs) and 11 genes enabled us to investigate with greater precision the genetic basis of trait association by looking for co-location of corresponding QTLs on the genetic map.

2. The identification of genomic regions associated with water status and chlorophyll concentration under well-watered and water-stressed conditions will be useful for marker-based approaches to improve drought tolerance in sunflower.

3. In addition, our map complements the public SSR and SSR/SNP maps (Tang et al., 2002; Lai et al., 2005) as a genetic framework for quantitative and qualitative trait analysis for sunflower (*Helianthus annuus* L.)

Acknowledgments

We would like to thank the Institute of Biotechnology, Urmia University, Urmia, Iran, for financial support of this work.

Received 31 08 2012

Accepted 08 01 2013

References

- Abdi N., Darvishzadeh R., Jafari M., Pirzad A., Haddadi P. 2012. Genetic analysis and QTL mapping of agromorphological traits in sunflower (*Helianthus annuus* L.) under two contrasting water treatment conditions. *Plant Omics Journal*, 5: 1491–158
- Allinne C., Maury P., Sarrafi A., Grieu P. 2009. Genetic control of physiological traits associated to low temperature growth in sunflower under early sowing conditions. *Plant Science*, 177: 349–359
<http://dx.doi.org/10.1016/j.plantsci.2009.07.002>
- Andrew K. B., Hammer G. L., Henzell R. G. 2000. Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. *Crop Science*, 40: 1037–1048
<http://dx.doi.org/10.2135/cropsci2000.4041037x>
- Araus J. L., Amaro T., Voltas J., Nakkoul H., Nachit M. M. 1998. Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions. *Field Crops Research*, 55: 209–223
[http://dx.doi.org/10.1016/S0378-4290\(97\)00079-8](http://dx.doi.org/10.1016/S0378-4290(97)00079-8)
- Ceccarelli S., Grando S., Baum M., Udupa S. M. 2004. Breeding for drought resistance in a changing climate. Challenges and strategies for dryland agriculture. Rao S. C., Ryan J. (eds). *CSSA Special Publication No. 32*. Madison, USA, p. 167–190
- Ceccarelli S., Grando S., Baum M. 2007. Participatory plant breeding in water-limited environments. *Experimental Agriculture*, 43: 411–435
<http://dx.doi.org/10.1017/S0014479707005327>
- Chimenti C. A., Marcantonio M., Hall A. J. 2006. Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. *Field Crop Research*, 95: 305–315 <http://dx.doi.org/10.1016/j.fcr.2005.04.003>
- De Givry S., Bouchez M., Chabrier P., Milan D., Schiex T. 2005. Carthage: multi-population integrated genetic and

- radiation hybrid mapping. *Bioinformatics*, 21: 1703–1704
<http://dx.doi.org/10.1093/bioinformatics/bti222>
- 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 <http://dx.doi.org/10.1139/G08-038>
- Ebrahimi A., Maury P., Berger M., Calmon A., Grieu P., Sarrafi A. 2009. QTL mapping of protein content and seed characteristics under water-stress conditions in sunflower. *Genome*, 52: 419–430 <http://dx.doi.org/10.1139/G09-020>
- Flores Berrios E., Gentzbittel L., Kayyal N., Alibert G., Sarrafi A. 2000. AFLP mapping of QTLs for in vitro organogenesis traits using recombinant inbred lines in sunflower (*Helianthus annuus* L.). *Theoretical Applied Genetics*, 101: 1299–1306
<http://dx.doi.org/10.1007/s001220051610>
- Fracheboud Y., Jompuk C., Ribaut J. M., Stamp P., Leipner J. 2004. Genetic analysis of cold-tolerance of photosynthesis in maize. *Plant Molecular Biology*, 56: 241–253
<http://dx.doi.org/10.1007/s11103-004-3353-6>
- Guo P., Li M. 1996. Studies on photosynthetic characteristics in rice hybrid progenies and their parents I. chlorophyll content, chlorophyll-protein complex and chlorophyll fluorescence kinetics. *Journal of Tropical and Subtropical Botany*, 4: 60–65
- Guo P., Li R. 2000. Effects of high nocturnal temperature on photosynthetic organization in rice leaves. *Acta Botanica Sinica*, 42: 13–18
- Haddadi P., Yazdi-samadi B., Langlade N. B., Naghavi M. R., Berger M., Kalantari A., Calmon A., Maury P., Vincourt P., Sarrafi A. 2010. Genetic control of protein, oil and fatty acids content under partial drought stress and late sowing conditions in sunflower (*Helianthus annuus* L.). *African Journal of Biotechnology*, 9: 6768–6782
<http://dx.doi.org/10.1007/s11032-011-9585-7>
- Haddadi P., Ebrahimi A., Langlade N. B., Yazdi-samadi B., Berger M., Calmon A., Naghavi M. R., Vincourt P., Sarrafi A. 2012. Genetic dissection of tocopherol and phytosterol in recombinant inbred lines of sunflower through quantitative trait locus analysis and the candidate gene approach. *Molecular Breeding*, 29: 717–729
- Herve D., Fabre F., Berrios E. F., Leroux N., Chaarani G. A., Planchon C., Sarrafi A., Gentzbittel L. 2001. QTL analysis of photosynthesis and water status traits in sunflower (*Helianthus annuus* L.) under greenhouse conditions. *Journal of Experimental Botany*, 52: 1857–1864
<http://dx.doi.org/10.1093/jexbot/52.362.1857>
- Jamaux I., Steinmetz A., Belhassen E. 1997. Looking for molecular and physiological markers of osmotic adjustment in sunflower. *New Phytologist*, 137: 117–127
<http://dx.doi.org/10.1046/j.1469-8137.1997.00817.x>
- Lai Z., Livingstone K., Zou Y., Church S. A., Knapp S. J., Andrews J., Rieseberg L. H. 2005. Identification and mapping of SNPs from ESTs in sunflower. *Theoretical Applied Genetics*, 111: 1532–1544
<http://dx.doi.org/10.1007/s00122-005-0082-4>
- Lander E. S., Green P., Abrahamson J., Barlow A., Daly M. J., Lincoln S. E., Newberg L. A. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, 1: 174–181
[http://dx.doi.org/10.1016/0888-7543\(87\)90010-3](http://dx.doi.org/10.1016/0888-7543(87)90010-3)
- Poormohammad Kiani S., Talia P., Maury P. 2007 (a). Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. *Plant Science*, 172: 773–787
<http://dx.doi.org/10.1016/j.plantsci.2006.12.007>
- Poormohammad Kiani S., Grieu P., Maury P., Hwezi T., Gentzbittel L., Sarrafi A. 2007 (b). Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L.). *Theoretical Applied Genetics*, 114: 193–207
<http://dx.doi.org/10.1007/s00122-006-0419-7>
- Poormohammad Kiani S., Maury P., Sarrafi A., Grieu P. 2008. QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. *Plant Science*, 175: 565–573
<http://dx.doi.org/10.1016/j.plantsci.2008.06.002>
- Poormohammad Kiani S., Maury P., Nouri L., Ykhlef N., Grieu P., Sarrafi A. 2009. QTL analysis of yield-related traits in sunflower under different water treatments. *Plant Breeding*, 128: 363–373
<http://dx.doi.org/10.1111/j.1439-0523.2009.01628.x>
- Porebski S., Bailey L., Baum B. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter*, 15: 8–15
<http://dx.doi.org/10.1007/BF02772108>
- Pourtaghi A., Darvish F., Habibi D., Nourmohammadi G., Daneshian J. 2011. Effect of irrigation water deficit on antioxidant activity and yield of some sunflower hybrids. *Australian Journal Crop Science*, 5: 197–204
- SAS/STAT® 9.2. 2009. User's guide, 2nd ed. SAS Institute Inc. Cary, USA
- Sinclair T. R., Ludlow M. M. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. *Australian Journal of Plant Physiology*, 13: 329–341
<http://dx.doi.org/10.1071/PP9860329>
- Tang S., Yu J. K., Slabaugh M. B., Shintani D. K., Knapp S. J. 2002. Simple sequence repeat map of the sunflower genome. *Theoretical Applied Genetics*, 105: 1124–1136
<http://dx.doi.org/10.1007/s00122-002-0989-y>
- Tezara W., Mitchall V., Driscoll S. P., Lawlor D. W. 2002. Effects of water deficit and its interaction with CO₂ supply on the biochemistry and physiology of photosynthesis in sunflower. *Journal of Experimental Botany*, 375: 1781–1791
<http://dx.doi.org/10.1093/jxb/erf021>
- Tuberosa R., Salvi S., Sanguineti M. C., Landi P., Maccaferri M., Conti S. 2002. Mapping QTLs regulating morpho-physiological traits and yield in drought-stressed maize: case studies, shortcomings and perspectives. *Annals of Botany*, 89: 941–963
<http://dx.doi.org/10.1093/aob/mcf134>
- Verma V., Foulkes M. J., Worland A. J., Sylvester-Bradley R., Caligari P. D. S., Snape J. W. 2004. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. *Euphytica*, 135: 255–263
<http://dx.doi.org/10.1023/B:EUPH.0000013255.31618.14>
- Wang S., Basten C. J., Zeng Z.-B. 2012. *Windows QTL cartographer 2.5*. Department of Statistics, North Carolina State University, Raleigh, USA. <<http://statgen.ncsu.edu/qtllcart/WQTLCart.htm>> [accessed 31 08 2012]
- Zarei L., Farshadfar E., Haghparast R., Rajabi R., Mohammadi M. 2007. Evaluation of some indirect traits and indices to identify drought tolerance in bread wheat. *Asian Journal of Plant Sciences*, 6: 1204–1210
<http://dx.doi.org/10.3923/ajps.2007.1204.1210>

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 100, No. 2 (2013), p. 159–166

DOI 10.13080/z-a.2013.100.020

Kiekybinių požymių lokusų (KPL) nustatymas santykinio drėgmės kiekio ir chlorofilo koncentracijos požymiams tikrosios saulėgražos (*Helianthus annuus L.*) rekombinantinėse inbreditinėse linijose lietinant ir esant drėgmės stresui

N. Abdi¹, R. Darvishzadeh¹, H. Hatami Maleki², P. Haddadi³, A. Sarrafi⁴¹Urmijos universitetas, Iranas²Maragheh universitetas, Iranas³Prancūzijos nacionalinis žemės ūkio mokslų tyrimo Jean-Pierre Bourgin institutas⁴Ecologie Fonctionnelle laboratorija, Prancūzija

Santrauka

Tyrimo tikslas – identifikuoti kiekybinių požymių lokusus (KPL), lemiančius tikrosios saulėgražos (*Helianthus annuus L.*) santykinio drėgmės kiekio ir chlorofilo koncentracijos įvairovę lietinant ir esant drėgmės sukeltam stresui. Septyniasdešimt rekombinantinių inbreditinių linijų (RIL) iš 123, gautų sukryžminus PAC2 × RHA266, ir jų tėvinės linijos vertintos atsitiktine tvarka išdėstytoje 8 × 9 bandymo schemoje dviem pakartojimais lietinant ir drėgmės sukkelto streso sąlygomis. Lyginant vertinamus požymius, didelis genetinis kintamumas ir transgresinė segregacija nustatyta tarp RIL esant abiem drėgmės sąlygoms. Kiekybiniai požymių lokusai nustatyti naudojant atnaujintą didelio tankio paprastųjų pasikartojančių sekų ir vieno nukleotido pakitimų genolapį. Genolapį sudarė 210 paprastųjų pasikartojančių sekų ir 11 genų, išsidėsčiusių 17 sankibos grupių. Bendras genolapio ilgis yra 1653,1 cM su vidutiniu tankiu 1 žymeklis per 7,44 cM. Lietinant 3 ir 6 KPL buvo identifikuoti atitinkamai chlorofilo koncentracijai ir santykiniam drėgmės kiekiui. Drėgmės sukkelto streso sąlygomis buvo identifikuoti atitinkamai 7 ir 2 KPL. Fenotipinės variacijos (R^2) dalis, paaiškinama KPL, svyravo nuo 0,39 iki 52,48 %. KPL chlorofilo koncentracijai ir santykiniam drėgmės kiekiui 10 ir 16 sankibos grupėse buvo persidengusios. Bendri KPL įvairiems požymiams, esant abiem drėgmės sąlygoms, sankibos grupėse 10, atrodo, yra svarbesni, nes pasižymi pastovumu nepriklausomai nuo lietinimo sąlygų.

Reikšminiai žodžiai: augalų aprūpinimo drėgme būklė, fotosintezės požymiai, genolapis, *Helianthus annuus*, KPL lokalizavimas, transgresinė segregacija.