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Identification of QTLs for grain yield and some agro-morphological traits in sunflower (*Helianthus annuus* L.) using SSR and SNP markers

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Abstract
Many agriculturally important traits are complex, affected by many genes and the environment. Quantitative trait loci (QTL) mapping is a key tool for studying the genetic structure of complex traits in plants. In the present study QTLs associated with yield and agronomical traits such as leaf number, leaf length, leaf width, plant height, stem and head diameter were identified by using 70 recombinant inbred lines (RILs) from the cross (♀) PAC2 × RHA266(♂). RILs and their parents were evaluated in a rectangular 8×9 lattice design with two replications. High genetic variability and transgressive segregation were observed in all studied traits. Genetic gain representing the difference between 10% of selected RILs and their parents was significant for most of the studied traits. Positive and significant genotypic and phenotypic correlations were observed among the studied traits. QTL analysis was performed using a recently developed SSR and SNP sunflower linkage map. The map consists of 210 SSRs and 11 SNP markers placed in 17 linkage groups (LGs). The total map length is 1,653.1 cM with a mean density of 1 marker per 7.44 cM. Composite interval mapping (CIM) procedure detected 21 QTLs involved in genetic control of studied traits. The phenotypic variance explained by the identified QTLs varied from 1.13 to 73.70%. QTLs such as HMBPP associated with the expression of more than one trait could increase the efficiency of marker-assisted selection (MAS) and genetic progress in sunflower.

Keywords*:* Genetic variation, linkage map, molecular markers, QTL mapping, sunflower, yield-related traits.

Abbreviations: AFLP: amplified fragment length polymorphism, BIO: total dry mater, CIM: composite interval mapping, DNA: deoxyribonucleic acid, DHs: doubled haploids, F_2: second filial generation, Gb: giga base pairs = 1,000,000,000 bp, GLM: general linear model, GYP: grain yield per plant, HD: head diameter, INDEL: Insertion-deletion polymorphisms as genetic markers in natural populations, LAF: leaf area at flowering, LAD: leaf area duration, LL: leaf length, LGs: linkage groups, LOD: logarithm (base 10) of odds, LW: leaf width, NL: number of leaf, PL: petiole length, PH: plant height, OP: osmotic potential; QTL: quantitative trait loci, RAPD: random amplified polymorphic DNA, RFLP: restriction fragment length polymorphism, RILs: recombinant inbred lines, SD: stem diameter, SF: days from sowing to flowering, SSR: simple sequence repeat, SNP: single nucleotide polymorphism, TRAP: target region amplification polymorphism.

Introduction

Sunflower is an annual and diploid (2n =34) species from North America with an estimated genome size of 3.5 Gb (Baack *et al.* 2005). The crop is grown worldwide and performs well in most temperate climates of the world (Hu *et al.* 2010). It is the second largest hybrid crop and the fifth largest edible oilseed crop worldwide (Hu *et al.* 2010). Between 2000/01 and 2010/11, sun-flower has been cultivated on 19-23 million hectares in 60 countries, producing 23-33 million tons of seed and 7.4-12.2 million tons of oil. The areas planted in Iran in 2012 were 68000 hectare with production of 90000 ton grain yield (http:// faostat.fao.org/ site/567/DesktopDefault.aspx?PageID=567#ancor). Sunflower seed contains high oil content ranging from 35-48% with some types yielding up to 50% (Skoric and Marinkovic 1986), 20-27% protein (Nazir *et al.* 1994) and high percentage of poly-unsaturated fatty acids (60%) including oleic acid (16.0%) and linoleic acid (72.5%), which control cholesterol in blood (Satyabrata *et al.* 1988). The majority of edible oil requirements of Iran are met through imports. Any breeding efforts that increase oily plants production can be effective in reducing the country's dependence on incoming oil.

Traits are classified into two categories including quantitative and qualitative. Quantitative traits are naturally complex and controlled by many genes (Kearsey and Pooni 1996). Understanding the genetic structure of quantitative traits is a long-term challenge for quantitative geneticists and plant breeders, who wish to design efficient breeding programs. Conventionally, the genetic properties of traits can be deciphered by partitioning the total variation into variation components caused by specific genetic effects (Kearsey and Pooni 1996). With recent advances in molecular genotyping and high-throughput technology, unrevealing the genetic architecture of complex traits has become possible via quantitative trait loci (QTL) analysis (Collard *et al.* 2005; De Vienne 2003). Molecular markers linked to QTLs/major genes of traits are being routinely identified in many crops by utilizing genetic linkage map developed from F_2, back cross families, recombinant inbred lines (RILs) or doubled haploids (DHs) population (Collard *et al.* 2005). DHs and RILs are genetically homozygous in genetic loci and can be mostly multiplied without genetic changes. These populations provide more accurate phenotypic data by testing multiple plants per line to minimize environmental effects. The seeds of DHs and RILs can be transferred from a laboratory to another place for collaborative works. However, only additive effects of genes are estimated in DHs and RILs populations (Kearsey and Pooni 1996; Darvishzadeh *et al.* 2007). A DH population is produced by doubling the gametes of F_1 or F_2 population. RILs are developed by single-seed selections from individual plants of an F_2 population. DH population is quicker to generate than RILs but the production of DHs is only possible for species with well established protocols for pollen grains culture and chromosome doubling.

In sunflower, several molecular genetic linkage maps have been constructed on F_2 or RIL mapping populations using restriction fragment length polymerphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), insertion / deletion (INDELs) polymorphism, single nucleotide polymorphism (SNP) and target region amplification polymorphism (TRAP) markers (Berry *et al.* 1995; Gentzbittel *et al.* 1995, 1998; Jan *et al.* 1998; Flores-Berrios *et al.* 2000; Burke *et al.* 2002; Mokrani *et al.* 2002; Bert *et al.* 2003, 2004; Langar *et al.* 2003; Yu *et al.* 2003; Rachid Al-Chaarani *et al.* 2004; Lai *et al.* 2005; Hu *et al.* 2007; Poormohammad Kiani *et al.* 2007a; Yue *et al.* 2008).

Recently microsatellites or simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers have gained substantial attention in sunflower studies (Tang *et al.* 2002). SSR are tandem repeats of short (1 to 6 bp) DNA sequences. The desirable features of SSR markers include their easy use, high information content, codominant inheritance pattern, even distribution along chromosomes, reproducibility, and locus specificity (Kalia *et al.* 2011). SNPs widely distributed in the genome are the most abundant type of DNA variation currently used as a genetic marker (Brooks 1999). Compared to markers based on size discrimination or hybridization, SNPs directly interrogate sequence variation and can reduce genotyping errors (Oliver *et al.* 2011).

Up to date, several QTL mapping studies were published in sunflower (Poormohammad Kiani and Sarrafi 2010). An RFLP/isoezymes F_2:F_3 mapping population (162 F3 plants) from a cross between two inbred lines (GH and PAC2) developed by INRA-France with 82 markers was used for mapping days from sowing to flowering, and three QTLs were detected on linkage group LG A, LG G and LG L (Mestries *et al.* 1998). The three QTLs explained 30% of the total phenotypic variation (R^2) and the type of gene action observed was consistent with partial dominance on LG L and overdominance on LG A and LG G. For the QTLs on LG A and LG G, the parent, GH, contributed to positive alleles and for the QTL on LG L, PAC2 alleles increased the trait. Leon *et al.* (1995) used a genetic map of 201 RFLP markers and identified six QTLs associated with 57% of genetic variation for oil content among the F_2 progeny. Rachid Al-Chaarani *et al.* (2004) identified five QTLs for each of plant height and shoot diameter, and four QTLs for head diameter on different linkage groups in the RIL population of sunflower. LOD scores for these QTLs ranged from 4.35 to 13.66 and total phenotypic variances explained by the QTLs were 71% for plant height, 50% for shoot diameter and 25% for head diameter. Identification of molecular markers associated with genes controlling traits facilitates pyramiding multiple QTL alleles in the same genotype background and also progeny selection in segregation population via marker-assisted selection (MAS) (Sarrafi and Gentzbittel 2004; Mohler and

Singrun, 2004). The objective of the present research was to identify the chromosomal regions involved in genetic variation of different agro-morphological traits using a linkage map developed by SSR and SNP markers on RILs population from the cross (♀) PAC2×RHA266(♂). This public RILs population has been widely used for genetic analysis of complex traits in sunflower (Rachid Al-Chaarani *et al.* 2004, 2005; Abou Al Fadil *et al.* 2007; Darvishzadeh *et al.* 2007; Poor-mohammad Kiani *et al.* 2007a, 2007b, 2008, 2009).

Materials and methods

Plant materials and experimental design

Seventy RILs developed via single seed descent (SSD) method from the cross (♀) PAC2 × RHA266 (♂) was used for QTL analysis (Flores Berrios *et al.* 2000). RHA266 is an American inbred line developed from a across between the wild species *H. annuus* and peredovik. PAC2 is an INRA-France inbred line developed from a cross between *H. petiolaris* and HA61 (Gentzbittel *et al.* 1995). RHA266 is a branched and high yielding line in comparison with PAC2 (Rachid Al-Chaarani *et al.* 2004). PAC2 is a line with high total dry matter per plant and head weight per plant compared to RHA266. PAC2 showed high net photosynthesis and relative water content in water stressed condition compared to RHA266.

The experiment was conducted in 2011-2012 at the research farm of Shahid Beheshti Agricultural high school with the latitude and longitude of 37°/32' north and 45°/5' east and the height of 1313 m above sea level. Climate of the region was cold and semidry and the average rainfall and temperature according to 16 years statistics were 184 mm and 12°C, respectively. Seeds of RILs and their two parents, kindly provided by INRA (France), were cultivated in rectangular 8×9 lattice design with two replications. Each plot comprised 3 lines 3 m long spaced 65 cm. Plants spaced 25 cm in each raw. Irrigations were carried out when an amount of evaporated water (from Class 'A pan' evaporation) reached to 60 mm (Akbari *et al.* 2008). Weeds were mechanically controlled. 100 kg per hectare urea was distributed between rows at 8 leaf growth stage and irrigation was performed immediately. Any particular pest or disease was not seen during vegetative and reproductive growth stages. In order to prevent sparrows' damage during seed filling stage, the heads were covered by white envelopes. Seven traits including plant height (PH; cm), stem diameter (SD; cm), head diameter (HD; cm), number of leaf (NL), leaf length (LL; cm), leaf width (LW; cm) and petiole length (PL; cm) were measured on 5 plants per plot selected randomly at flowering stage. Grain yield per plant (GYP; g) was measured at maturity stage by harvesting five plants per plot.

Statistical and QTL analyses

Normality test were done by Proc Univariate in the SAS 9.2 software (SAS Institute Inc., Cary, N.C.). Variance analysis of phenotypic data was

performed with Proc GLM in the SAS software. Estimation of phenotypic and genotypic correlations and their standard errors were realized by using multivariate restricted maximum likelihood with SAS Proc MIXED (Holland 2006). QTL analysis was performed using a recently developed sunflower linkage map (Haddadi *et al.* 2012; Amouzadeh *et al.* 2013) via composite interval mapping (CIM) in Windows QTL Cartographer Version 2.5 (Wang *et al.* 2005). The map consists of 210 SSRs and 11 SNP markers placed in 17 linkage groups (LGs). The total map length is 1,653.1 cM with a mean density of 1 marker per 7.44 cM. Significant LOD for each trait was determined by permutation test (n=1,000 permutations) (Churchill and Doerge 1994). 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 that was identified by the module Srmapqtl (model 6) of QTL Cartographer. The percentage of phenotypic variance (R^2) explained by each QTL was estimated at the peak of the LOD curve in Windows QTL Cartographer. MapChart 2.2 (Voorrips 2002) was used to draw a graphical presentation of linkage groups and the map of QTLs.

Results

Phenotypic variation

The results of ANOVA for rectangular lattice design showed that inter-block variance was smaller than residual variance in all studied traits, then these two variances were pooled (Table 1). Analysis of variance revealed significant differences among genotypes for studied traits (Table 1). The genetic parameters and phenotypic variation observed among RILs and their parents are presented in Table 2. The performance of RHA266 was better than that of PAC2 in GYP and PH. The difference between the mean of RILs ($\overline{X}_{\text{RILs}}$) and their parents ($\overline{X}$ p) was only significant for GYP, PH and PL (Table 2). Genetic gain, calculated as difference between the mean values of 10% selected RILs ($\overline{X}_{\text{10\%bestRILs}}$) and the mean of parents ($\overline{X}$ p) (Rachid Al-Chaarani *et al.* 2004; Poormohammad Kiani *et al.* 2007a, b), was significant for all the studied traits (Table 2). Frequency distribution of RILs and their parents for most of the studied traits showed continuous patterns, suggesting that studied agro-morphological traits were controlled by a polygenic system (Figure 1, Table 2). The amounts of studied traits in some RILs were less than their parents, whereas in some others, the values were higher than their parents (Figure 1).

Table1. Mean squares of yield and some agro-morphological traits in sunflower recombinant inbred lines (RILs) and their two parents.

Source of variation	LN		LL		PL		[1]PH		LW		SD		HD		[1]GYP	
	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
Rectangular lattice design																
Replication	1	34.00^{**}	1	27.74^{**}	1	3.02^{*}	1	1.24^{**}	1	12.04^{**}	1	8.80^{*}	1	17.47^{**}	1	2.26^{ns}
Genotype	70	17.79^{**}	68	18.43^{**}	68	12.08^{**}	71	1.06^{**}	68	26.40^{**}	71	26.48^{**}	69	32.87^{**}	68	3.31^{**}
Block/ Replication	16	1.49^{ns}	16	0.29^{ns}	16	0.10^{ns}	16	0.05^{ns}	16	0.25^{ns}	16	1.11^{ns}	16	2.629^{ns}	16	0.54^{ns}
Error	49	2.21	46	0.53	47	0.06	50	0.06	47	0.36	47	1.90	47	2.623	45	1.22
Normality test on residuals																
BDT		0.99^{ns}		0.99^{ns}		0.99^{ns}		0.95^{**}		0.99^{ns}		0.99^{ns}		0.98^{ns}		0.95^{**}
ADT		-		-		-		0.07^{ns}		-		-		-		0.05^{ns}
%CV		11.42		4.16		2.89		2.45		4.21		7.42		10.58		23.42
Pooling																
Replication	1	34.0^{**}	1	27.74^{**}	1	3.02^{**}	1	1.24^{**}	1	12.04^{**}	1	8.80^{*}	1	17.47^{**}	1	2.26^{**}
Genotype	70	17.79^{**}	68	18.43^{**}	68	12.08^{**}	71	1.06^{**}	68	26.40^{**}	71	26.48^{**}	69	32.87^{**}	68	3.31^{**}
Error	65	2.08	62	0.47	63	0.07	66	0.06	63	0.33	63	1.71	63	2.62	61	1.04

[1]Square root transformation, BDT: before data transformation, ADT: after data transformation, CV: coefficient of variation, df: degree of freedom, MS: mean of square, LN: leaf number, LL: leaf length, PL: petiole length, PH: plant height, LW: leaf width, SD: stem diameter, HD: head diameter, GYP: grain yield per plant, ns: non significant, * and ** significant at 0.05 and 0.01 probability level, respectively.

Table 2. Genetic parameters and gain for traits in sunflower recombinant inbred lines (RILs) and their two parents.

Item	Traits							
	GYP	**HD**	**SD**	**LW**	**PH**	**PL**	**LL**	**LN**
PAC2 (P1)	10.56	12.33	19.17	14.76	95.56	7.48	18.22	14.53
RHA266 (P2)	16.40	15.50	17.99	12.84	106.84	7.48	16.59	13.73
P1- P2	-5.84*	-3.17^{ns}	1.18^{ns}	1.93*	-11.28*	0^{ns}	1.63*	0.80^{ns}
$\overline{X}$ **P**	13.48	13.92	18.58	13.8	101.20	7.48	17.4	14.13
Max	98.56	24.87	28.57	26.52	152.27	16.1	24.91	22.47
Min	5.13	6.72	10.42	7.74	87.52	3.84	11.44	7.51
$\overline{X}$ **RIL**	21.10	15.40	18.57	14.40	115.82	8.83	17.60	13.08
$\overline{X}$ **RIL-** $\overline{X}$ **P**	7.61*	1.48^{ns}	-0.01^{ns}	0.60^{ns}	14.62*	1.35*	0.20^{ns}	-1.05^{ns}
$\overline{X}$ **10% best RIL**	58.91	22.98	25.21	21.48	143.28	13.74	23.10	19.06
$\overline{X}$ **10% best RIL-** $\overline{X}$ **P**	45.43*	9.06*	6.63*	7.68*	42.08*	6.26*	5.7*	4.93*
STDEV	1.47	3.84	4.43	3.76	0.74	2.45	3.10	2.96
LSD	2.21	3.24	2.76	1.20	0.49	0.49	1.46	2.97
Normality test on original data	0.15**	0.06^{ns}	0.04^{ns}	0.10**	0.06^{ns}	0.09**	0.12**	0.12**

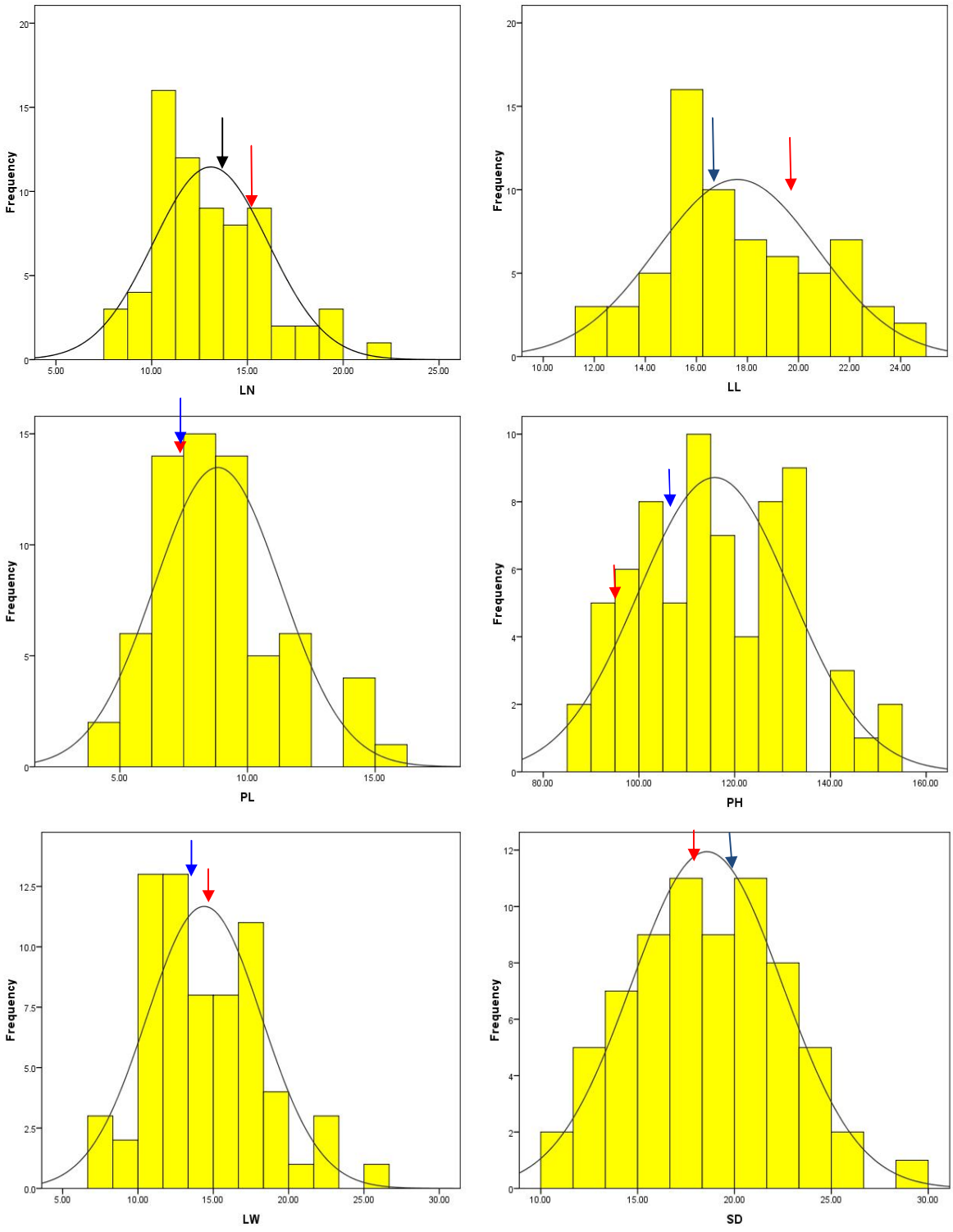
$\overline{X}$ P: mean of parents; $\overline{X}$ RILs: mean of recombinant inbred lines; 10%SRILs: mean of the 10% of selected recombinant inbred lines; GG10%: genetic gain when the mean of 10% of selected recombinant inbred lines are compared with the mean of parents; STDEV: standard deviation. LN: leaf number; LL: leaf length; PL: petiole length; PH: plant height; LW: leaf width; SD: stem diameter; HD: head diameter; GYP: grain yield per plant.

Correlation analysis

Genotypic and phenotypic correlations among studied traits are summarized in Table 3. The highest genotypic correlation coefficients were observed between LL and LW (0.91±0.02), SD and LW (0.78± 0.05), LL and SD (0.76±0.05), LW and PL (0.75±0.05) and GYP and HD (0.72±0.05). Significantly positive genotypic correlations were observed between LL, PL, LW, HD and SD with GYP (Table 3).

QTLs

The map and the characteristics of QTLs associated with the studied traits are presented in Table 4. QTL names were constructed using the trait abbreviations suffixed with numbers presenting the linkage group and the order of QTL on the linkage group. For an easier overview of overlapping QTLs between traits, an image of QTL regions was presented in Figure 2. A total number of 21 QTLs was identified for the studied traits.


Frequency
LN
LL
PL
PH
LW
SD

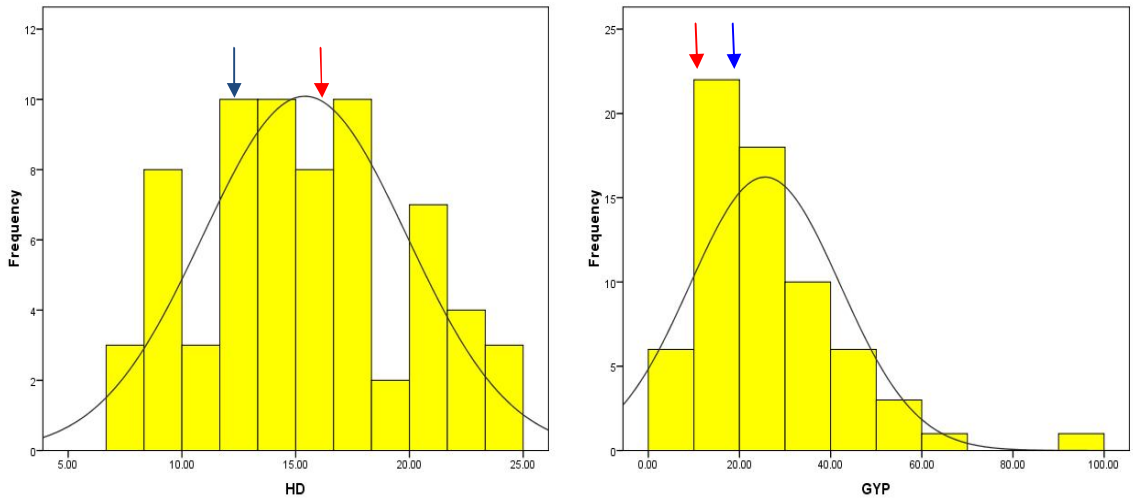


Figure 1. The frequency distribution of sunflower recombinant inbred lines (RILs) and their parents for agro-morphological traits. Red and blue arrows show the phenotypic value of PAC2 (maternal line) and RHA266 (paternal line). LN: leaf number; LL: leaf length; PL: petiole length; PH: plant height; LW: leaf width; SD: stem diameter; HD: head diameter; GYP: grain yield per plant.

Table 3. Correlation among traits in sunflower recombinant inbred lines (RILs).

Variable	LN	LL	PL	PH	LW	HD	SD
Genotypic correlation							
LL	0.15±0.11						
PL	0.28±0.11	0.65±0.07					
PH	0.21±0.11	0.48±0.09	0.32±0.11				
LW	0.25±0.11	0.91±0.02	0.75±0.05	0.41±0.10			
HD	0.23±0.11	0.54±0.08	0.60±0.08	0.27±0.11	0.63±0.07		
SD	0.40±0.09	0.76±0.05	0.71±0.06	0.54±0.08	0.78±0.05	0.66±0.06	
GYP	0.22±0.10	0.32±0.10	0.41±0.09	0.13±0.10	0.42±0.09	0.72±0.05	0.44±0.09
Phenotypic correlation							
LL	0.17±0.13						
PL	0.32±0.12	0.67±0.07					
PH	0.22±0.13	0.49±0.10	0.34±0.11				
LW	0.28±0.12	0.94±0.02	0.77±0.05	0.41±0.11			
HD	0.25±0.14	0.60±0.09	0.64±0.08	0.31±0.12	0.70±0.07		
SD	0.50±0.11	0.82±0.05	0.76±0.06	0.61±0.09	0.85±0.04	0.75±0.07	
GYP	0.26±0.18	0.53±0.14	0.58±0.13	0.19±0.16	0.66±0.12	0.89±0.07	0.59±0.12

LN: leaf number; LL: leaf length; PL: petiole length; PH: plant height; LW: leaf width; SD: stem diameter; HD: head diameter; GYP: grain yield per plant. $r \geq |\pm 0.232|$ is significant at 0.05 probability level (http://www.gifted.uconn.edu/siegle/research/correlation/corrchrt.htm).

The QTLs corresponding to various traits were located throughout the genome except on linkage groups 1, 3, 4, 7, 9, 13 and 16 (Figure 2). LOD thresholds determined after 1,000-permutation LR tests ranged from 2.85

to 4.73 depending on the trait with the mean of 3.55. Individual QTLs explained 1.13 to 73.70% of phenotypic variation of the studied traits. The sign of additive gene effects showed that favorable alleles for the studied traits come from both parental lines. Overlapping QTLs were found on linkage groups 6, 11, 12 and 17.

Table 4. Map position and effect of QTLs detected for agro-morphological traits in sunflower recombinant inbred lines (RILs) population.

Traits	QTL	LG	Position (cM)	LOD	Additive effects	R^2	Traits	QTL	LG	Position (cM)	LOD	Additive Effects	R^2 (%)
LN	NL.8.1	8	102.71	3.78	-0.81	11.53	SD	SD.2.1	2	10.01	3.27	-1.14	13.13
	NL.12.1	12	64.01	3.08	-0.22	1.85		SD.2.3	2	41.01	3.50	-1.16	10.15
LL	LL.11.1	11	10.01	3.48	-3.30	25.31		SD.5.1	5	16.01	3.16	-1.02	10.05
PL	PL.11.1	11	0.01	3.33	-1.05	11.37		SD.5.2	5	49.01	3.14	1.65	18.91
	PL.14.1	14	36.01	3.39	1.85	4.99		SD.6.1	6	28.01	3.77	1.25	53.88
LW	LW.12.1	12	67.01	3.67	0.95	2.11		SD.6.2	6	47.01	3.16	1.32	51.45
PH	PH.6.1	6	23.01	4.30	8.29	73.70		SD.10.1	10	12.01	3.50	-1.61	1.62
	PH.17.1	17	29.01	4.21	7.81	1.80		SD.11.1	11	12.01	3.64	-3.85	17.65
HD	HD.8.1	8	53.21	2.87	-4.90	60.86		SD.15.1	15	106.01	4.16	1.59	7.65
	HD.11.1	11	0.01	2.85	-1.88	13.19		SD.17.1	17	29.01	3.60	1.07	11.54
GYP	GYP.17.1	17	11.01	4.73	2.35	1.13							

cM: centi Morgan; LG: linkage group; LOD: log10 likelihood ratio (likelihood that the effect occurs by linkage/likelihood that the effect occurs by chance); QTL: quantitative trait loci; R^2: percentage of phenotypic variance explained by the individual QTLs. The positive additive effect shows that PAC2 allele increase the trait and negative value shows that RHA266 allele increases the trait. LN: leaf number; LL: leaf length; PL: petiole length; PH: plant height; LW: leaf width; SD: stem diameter; HD: head diameter; GYP: grain yield per plant. Ns: non significant; *, ** significant at 0.05, 0.01 probability level. QTLs with LOD $\geq$ 3 and $R^2 \geq$ 10% are considered major QTLs. The relatively high R^2 values found for some QTL with lower LOD scores may be influenced by large distances between flanking markers (Balyejusa Kizito et al., 2007).

For PH, 2 QTLs were identified on linkage groups 6 and 17. The phenotypic variance explained by PH.6.1 QTL (LOD=4.30) was 73.70% and the positive alleles came from the maternal line 'PAC2'. The phenotypic variance explained by PH.17.1 QTL (LOD=4.21) was 1.80% and the positive alleles came from the paternal line 'RHA266'. Two QTLs were identified for HD, on linkage groups 8 and 11, accounting for 60.86 and 13.19% of phenotypic variation, respectively. The positive alleles for these QTLs came from PAC2. Ten QTLs were identified for SD. The phenotypic variance explained by QTLs ranged from 1.62 to 53.88%. The positive alleles for the identified QTLs came from both parental lines. One QTL (LOD=4.73) was identified for GYP on linkage group

17 that explained 1.13% of the phenotypic variance. The positive allele for detected QTL came from RHA266. Two QTLs were identified for NL on linkage groups 8 and 12, accounting for 1.85 and 11.53% of phenotypic variance, respectively. The positive allele for QTL located on linkage group 8 (R^2 =11.53%) derived from RHA266, and for other the positive allele came from PAC2. Two QTLs were identified for PL on linkage groups 11 and 14. The phenotypic variance explained by PL QTLs was 4.99 and 11.37%. The positive alleles for the detected QTLs came from both parental lines. One QTL (LOD=3.67) was detected for LW that explained 2.11% of phenotypic variance of trait and the positive allele came from RH266. A QTL (LOD=3.48) was detected for LL on linkage group 11 accounting for 25.31% of phenotypic variance. Positive allele for QTL came from PAC2. QTLs with LOD ≥ 3 and R^2 ≥ 10% are considered major QTLs. The relatively high R^2 values found for some QTL with lower LOD scores may be influenced by large distances between flanking markers (Balyejusa Kizito *et al.* 2007).

Discussion

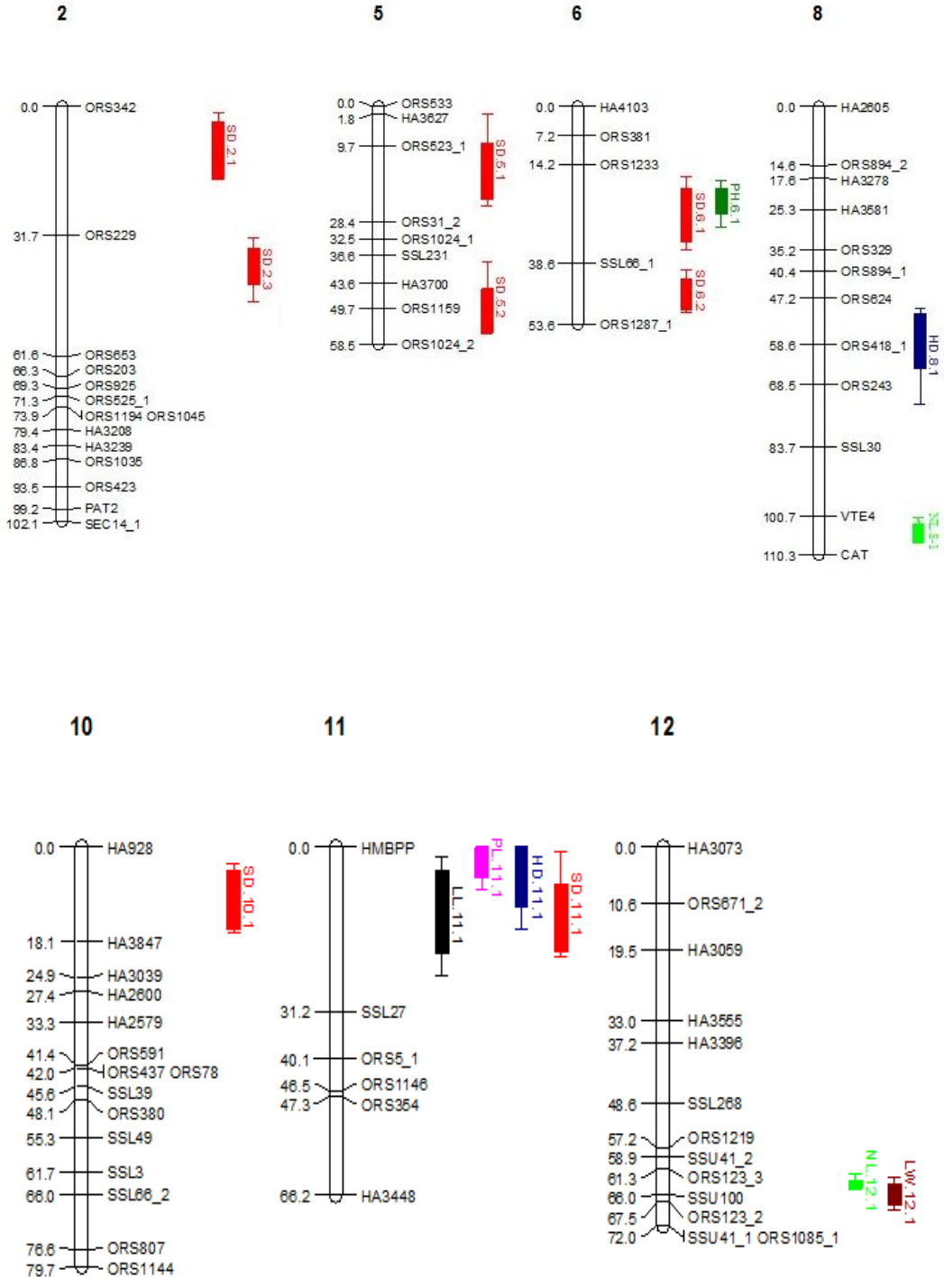
Phenotypic variation

Significant differences among genotypes for the studied traits provide necessary genetic variation for QTL analysis. Any significant differences between the means of RILs and their parents for most of the studied traits indicate that RILs used in this study were representative of possible recombination of the cross 'PAC2×RHA266' (Rachid Al-Chaarani *et al*. 2004; Poormohammad Kiani *et al.* 2007a). The frequency distribution of RILs and their parents for most of the studied traits showed a continuous pattern suggesting polygenic control. Continuous variation was also observed for early seedling vigour traits in sunflower (Davar *et al.* 2011). Transgressive segregation that would be the result of the accumulation of positive alleles from both parental lines was observed for most of the studied traits. The positive and negative signs of additive effect at different loci indicate the contribution of both parental lines and confirm the transgressive segregation observed at the phenotypic level. Transgressive segregation for morphological and agronomical traits as well as for water status traits under well-watered and water-stressed conditions has been also reported by Rachid Al-Chaarani *et al.* (2004) and Poormohammad Kiani *et al.* (2007a, b) in sunflower.

Positive and significant genotypic correlations were observed among the studied traits (Table 3).

Genotypic correlation analysis indicated that leaf length, petiole length, head diameter, leaf width and stem diameter positively influenced the grain yield of sunflower. A strong correlation between yield and agro-morphological traits has been reported in Rachid Al-Chaarani *et al.* (2004) research on sunflower.

Significant correlations among traits may be resulted from either pleiotropic effect of single gene or tight linkage of several genes that individually influence specific traits (Veldboom *et al.* 1994).


2
0.0 ORS342
31.7 ORS229
61.6 ORS653
66.3 ORS203
69.3 ORS925
71.3 ORS525_1
73.9 ORS1194 ORS1045
79.4 HA3208
83.4 HA3239
86.8 ORS1035
93.5 ORS423
99.2 PAT2
102.1 SEC14_1
SD.2.1
SD.2.3
5
0.0 ORS533
1.8 HA3627
9.7 ORS523_1
28.4 ORS31_2
32.5 ORS1024_1
36.6 SSL231
43.6 HA3700
49.7 ORS1159
58.5 ORS1024_2
SD.5.1
SD.5.2
6
0.0 HA4103
7.2 ORS381
14.2 ORS1233
38.6 SSL66_1
53.6 ORS1287_1
SD.6.1
SD.6.2
PH.6.1
8
0.0 HA2605
14.6 ORS894_2
17.6 HA3278
25.3 HA3581
35.2 ORS329
40.4 ORS894_1
47.2 ORS624
58.6 ORS418_1
68.5 ORS243
83.7 SSL30
100.7 VTE4
110.3 CAT
HD.8.1
10
0.0 HA928
18.1 HA3847
24.9 HA3039
27.4 HA2600
33.3 HA2579
41.4 ORS591
42.0 ORS437 ORS78
45.6 SSL39
48.1 ORS380
55.3 SSL49
61.7 SSL3
66.0 SSL66_2
76.6 ORS807
79.7 ORS1144
SD.10.1
11
0.0 HMBPP
31.2 SSL27
40.1 ORS5_1
46.5 ORS1146
47.3 ORS354
66.2 HA3448
LL.11.1
PL.11.1
HD.11.1
SD.11.1
12
0.0 HA3073
10.6 ORS671_2
19.5 HA3059
33.0 HA3555
37.2 HA3396
48.6 SSL268
57.2 ORS1219
58.9 SSU41_2
61.3 ORS123_3
66.0 SSU100
67.5 ORS123_2
72.0 SSU41_1 ORS1085_1
NL.12.1
LW.12.1

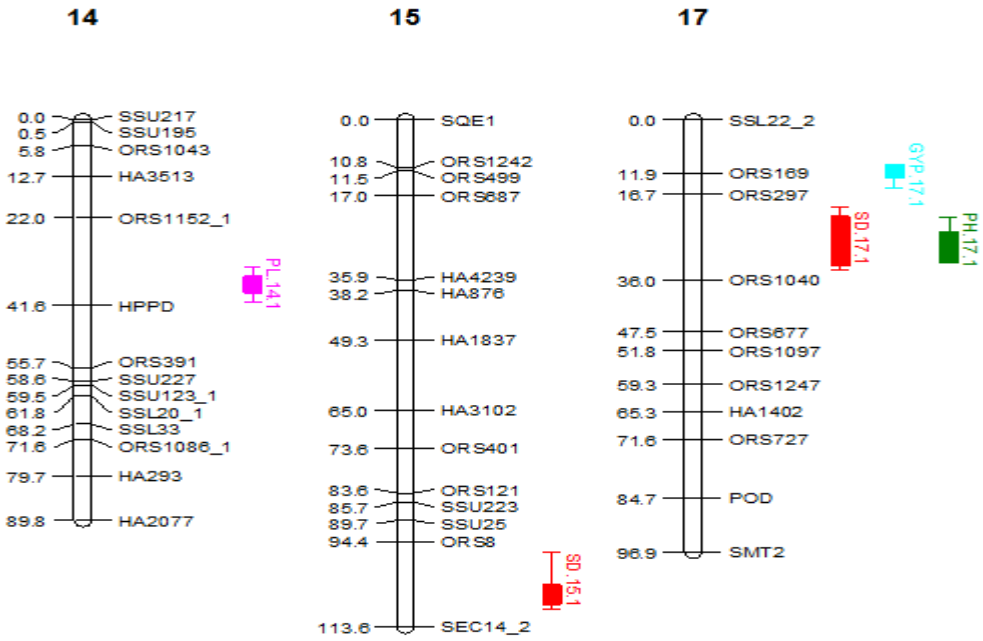


Figure 2. Sunflower linkage groups and QTLs for agro-morphological characteristics in recombinant inbred lines (RILs) population. Linkage groups are named as 1 to 17 according to reference linkage map of sunflower (Tang *et al.* 2002). LN: leaf number; LL: leaf length; PL: petiole length; PH: plant height; LW: leaf width; SD: stem diameter; HD: head diameter; GYP: grain yield per plant.

Co-localization of identified QTLs for some traits confirms the observed significant correlations among traits. Yield and yield associated traits are complex quantitative traits controlled by multiple genes and are highly influenced by environmental conditions (Shi *et al.* 2009). Yield associated traits that are less environmentally sensitive and have higher heritabilities than grain yield (Cuthbert *et al.* 2008) could be crucial for sustained sunflower improvement.

The range of identified QTLs for the studied traits was from 1 to 10. The number of QTLs detected in a given study depends on different factors, including type and size of mapping population used, trait investigated, the number of environments used for phenotyping, and genome coverage. The larger the environmental effect on the character (low heritability), the less likely a QTL will be detected. The low number of identified QTLs for some studied traits such as GYP could be improved by replicate phenotyping in different environments in order to controlling environmental error.

Individual QTLs explained 1.13 (GYP.17.1) to 73.70% (PH.6.1) of phenotypic variation of the studied traits. As anticipated, because of the quantitative and complex nature of GYP, the magnitude of phenotypic variance explained by identified QTL was low, and low phenotypic variation is consistent with results also from other crop species.

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

QTL analysis identified several putative genomic regions involved in the expression of the studied traits. Some identified QTLs associated with the expression of more than one trait. For example, PH.6.1 (LOD= 4.30) and SD.6.1 (LOD= 3.77) co-localized on the linkage group 6 at 23.01 and 28.01 cM distances involved in plant height and stem diameter variations; LL.11.1 (LOD=3.48), PL.11.1 (LOD=3.33), HD.11.1 (LOD=2.85) and SD.11.1 (LOD=3.64) co-localized on linkage group 11 at 0.01-12.01 cM interval associated with leaf length, petiole length, head diameter and stem diameter phenotypes; NL.12.1 (LOD=3.08) and LW.12.1 (LOD=3.67) co-located on the linkage group 12 at 64.01 and 67.01 cM associate with number of leaf and leaf width phenotypes. QTLs: PH.17.1 (LOD=4.21) and SD.17.1 (LOD=3.60) co-localized on the linkage group 17 at 11.01- 29.01 cM associate with plant height and stem diameter phenotypes (Table 4, Figure 2). An important QTL was detected on linkage group 11 in interval 0.01-12.01 near marker HMBPP for head diameter, stem diameter, petiole length and leaf length traits. The positive allele for this QTL comes from PAC2. The findings were supported by the results of correlation analysis. Identification of co-localized QTLs could be the reason for high correlation coefficients among the traits. The co-locality of QTLs for different traits implies the presence of pleiotropic or the close linkage of QTLs. Co-localized QTLs significantly increases the efficiency of selection in breeding programs (Tuberosa *et al*. 2002a; Hittalmani *et al.* 2003).

Some QTLs identified herein for agro-morphological traits showed co-locality with QTLs identified for water status traits in Poormohammad Kiani *et al.* (2007a; 2009) studies (Table 5). For example, the petiole length QTL on linkage group 14 (PL.14.1, LOD=3.39) was found in the same interval for the osmotic potential QTL under water-stressed condition in Poormohammad Kiani *et al.* (2007a, 2009) studies (Table 5). QTL identified herein for stem diameter on linkage group 10 co-localized with QTL identified for total dry mater (BIO) and leaf area duration (LAD) in Poormohammad Kiani *et al.* (2007a) studies (Table 5). One of the identified QTLs for stem diameter on linkage group 2 was co-localized with QTL identified for grain yield per plant (GYP) (Poormohammad Kiani *et al.* 2009) (Table 5).

Table 5. Co-localized QTLs for agro-morphological traits as compared with published QTLs in sunflower.

Linkage Group	In present study	In published works	Overlapped QTLs
LG2	SD	GYP (Poormohammd Kiani *et al.* 2009)	SD.2.1, GYPN.2.1
LG5	SD	LN, GYP (Poormohammd Kiani *et al.* 2009)	SD.5.1, LND.5.1, GYPI.5.1
LG8	LN	HD (Poormohammd Kiani *et al.* 2009)	LN.8.1, HD-W-8-1
LG10	SD	BIO, LAD (Poormohammd Kiani *et al.* 2007a, 2009)	SD.10.1, BIOW.10.1, LADW.10.2
LG12	PH	LAF (Poormohammd Kiani *et al.* 2007a, 2009)	PH.12.1, LAF-W-12-1
LG14	PL	OP (Poormohammd Kiani *et al.* 2007a)	PL.14.1, OP.WW.14.1
LG17	PH, SD	OP (Poormohammd Kiani *et al.* 2007a)	NAW.17.1, OPF.WS.17.1

In conclusion, we have detected several specific and nonspecific QTLs for agro-morphological traits. The detection of QTLs influencing various traits such as HMBPP on LG 11 could increase the efficiency of marker-assisted selection and genetic progress.

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شناسایی QTL های کنترل‌کننده عملکرد دانه و برخی صفات آگرومورفولوژیک آفتابگردان (*Helianthus annuus* L.) با استفاده از نشانگرهای SSR و SNP

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۱- دانش آموخته کارشناسی ارشد اصلاح نباتات، گروه اصلاح و بیوتکنولوژی گیاهی دانشکده کشاورزی دانشگاه ارومیه

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چکیده

بسیاری از صفات مهم زراعی کمی بوده و تحت تاثیر تعداد زیادی ژن و محیط قرار می‌گیرند. مکان‌یابی ژنی صفات کمی (QTL) یک ابزار کلیدی برای مطالعات ساختار ژنتیکی صفات کمی در گیاهان است. در این مطالعه QTLهای مرتبط با عملکرد و صفات زراعی مثل تعداد برگ، طول برگ، عرض برگ، ارتفاع بوته، قطر ساقه و قطر طبق با استفاده از ۷۰ لاین خالص نوترکیب (RILs) حاصل از تلاقی (♂)PAC2×RHA266 (♀) شناسایی شدند. RILها و والدین آنها در قالب طرح لاتیس مستطیل ۹×۸ با دو تکرار مورد ارزیابی قرار گرفتند. تنوع بالا و تفکیک متجاوز در تمام صفات مورد مطالعه مشاهده گردید. سودژنتیکی که نشان‌دهنده تفاوت بین میانگین ۱۰٪ از بهترین RILها و میانگین والدین است، برای بیشتر صفات معنی‌دار بود. همبستگی ژنوتیپی و فنوتیپی مثبت و معنی‌داری بین صفات مورد مطالعه مشاهده شد. تجزیه و تحلیل QTL با استفاده از نقشه جدید آفتابگردان که توسط نشانگرهای SSR و SNP توسعه یافته است انجام گرفت. نقشه متشکل از ۲۱۰ نشانگر SSR و ۱۱ نشانگر SNP در ۱۷ گروه لینکاژی می‌باشد. طول کل نقشه ۶۵۳/۱ سانتی مورگان با میانگین تراکم یک نشانگر در ۷/۴۴ سانتی مورگان است. با مکان‌یابی به روش فاصله‌ای مرکب تعداد ۲۱ QTL درگیر در کنترل ژنتیکی صفات مورد مطالعه شناسایی شد. واریانس فنوتیپی توجیه شده توسط QTL های شناسایی شده بین ۱/۱۳ تا ۷۳/۷۰ متغیر بود. QTLهایی نظیر HMBPP که در کنترل بیش از یک صفت درگیرند می‌توانند با افزایش بهره‌وری انتخاب به کمک نشانگر پیشرفت ژنتیکی در آفتابگردان را ارتقاء دهند.

کلمات کلیدی: تنوع ژنتیکی، نقشه پیوستگی، نشانگرهای مولکولی، نقشه QTL، آفتابگردان، صفات مرتبط با عملکرد.