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MAPPING QUANTITATIVE TRAIT LOCI (QTL) CONTROLLING SEED MORPHOLOGY AND DISK DIAMETER IN SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

Seed morphology and disk diameter are agronomically most important traits for the confection sunflower. This paper reports the results of an analysis of quantitative trait loci (QTLs) underlying 10 seed morphological traits including seed and kernel size, shape and stripe, and disk diameter in both F_2 population and $F_{2:3}$ families derived from an oilseed by confection cross. A linkage map containing 165 target region amplification polymorphism (TRAP) and 44 simple sequence repeat (SSR) markers was constructed from 120 F_2 plants. This map contained 17 linkage groups and spanned a total genetic distance of 1784.3 cM. A total of 51 QTLs were detected and 32 of them were identified in both generations. Each QTL explained 5.1-29.3% of the phenotypic variation, suggesting these traits were controlled by multiple genes. Most of the QTLs were clustered in six chromosomal regions. Two of the three QTLs identified for disk diameter were also located in two of the six regions. Moreover, alleles from the confection line at these QTLs had positive effects on these traits. Both QTL congruence and correlation analysis revealed that different genetic bases are responsible for seed shape, stripe, and other confection traits. The information generated by this study will facilitate confection sunflower breeding.

Key words: **sunflower, quantitative trait loci, mapping, seed size, kernel size, shape, disk diameter**

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INTRODUCTION

The cultivated sunflower (*Helianthus annuus* L.) is one of the staple oilseed crops of the world. There are two types of cultivated sunflowers, oilseed type and confection type. Although the oilseed is the major type of sunflower cultivated, the market and production area of confection sunflower has increased in recent years. The seeds of confection hybrids are typically gray or white in color with black or brown stripes, large-sized, and have a low kernel-to-pericarp weight ratio. In contrast, the seeds of oilseed cultivars are usually solid black, small-sized, and have a high kernel-to-pericarp weight ratio. It has been reported that the hull color is controlled by pigments in three layers:

1. the epidermis is either unpigmented, solid brown or black, or black- or brown-striped,
2. the hypodermis is either anthocyanin pigmented (black) or unpigmented, and
3. the phytomelanin layer is either present (black) or absent (Miller and Fick, 1997).

Through phenotypic analyses of mutations several hull pigment loci have been detected (reviewed by Miller and Fick, 1997). *Hyp*, a hypodermis pigment locus, has been genetically mapped (Leon *et al.,* 1996). Recently, Tang *et al.* (2006) mapped genes governing phytomelanin pigment (*P*) and hypodermal pigment (*Hyp*) on linkage groups 16 and 17, respectively. However, other seed traits including seed size and seed shape are typically quantitative traits which are controlled by multiple genes, and only limited work has been conducted on the genetic basis of these traits in sunflower.

The advent and development of molecular markers and genetic maps has provided the necessary tools to gain understanding of the genetic basis of the economically important traits and facilitate plant breeding via marker-assisted selection. To date, several linkage maps have been constructed using different molecular markers, including RFLP, RAPD, and SSR in sunflower (Berry *et al.,* 1995; Gedil *et al.,* 2001; Tang *et al.,* 2002). Recently, Lai *et al.* (2005) mapped 243 markers derived from sunflower expressed sequence tags (ESTs) to the 17 previously established linkage groups. The target region amplification polymorphism (TRAP) marker technique (Hu and Vick, 2003) takes advantage of the annotated EST information to generate PCR-based markers near the target sequences. TRAP has been successfully used in defining the linkage group ends (Hu, 2006) and in mapping a nuclear male-sterile (ms_9) gene (Chen *et al.,* 2006) and an apical branching (*b*) gene (Rojas-Barros *et al.,* 2005) in sunflower.

Quantitative trait loci (QTLs) can be identified by advanced computer software in chromosomal regions on the molecular marker linkage maps. Although confection sunflower accounts for 15 to 20% of the total US sunflower production, research activities have usually been focused on oilseed sunflower (Mestries *et al.,*

1998; Mokrani *et al.,* 2002; Bert *et al.,* 2004; Rönicke *et al.,* 2005; Micic *et al.,* 2004). Burke *et al.* (2002) first identified 14 QTLs for seed weight, seed length, and seed width. Tang *et al.* (2006) detected 34 QTLs for seven seed traits, and they also found that some QTLs with large effects on the seed traits are tightly linked to the branching and pericarp pigment loci. Recently, Wills and Burke (2007) and Baack *et al.* (2008) also identified some QTLs for seed traits and disk diameter using populations derived from crop-wild hybrids.

In this study, we analyzed the QTLs underlying 10 seed morphological traits and disk diameter in an F_2 population derived from a cross between an oilseed line, HA89, and a confection sunflower line to elucidate the genetic basis of these traits.

MATERIALS AND METHODS

Parental Lines and the Segregating Population

Two sunflower inbred lines with significant differences in some seed morphological traits were selected to develop the F_2 population used in the current study. The female was a USDA-ARS released, public oilseed sunflower inbred maintainer line, HA89 (PI 599773), and the male was a confection sunflower line (designated as LSS for its long seed size) introduced from China. The F_1 plants were grown in the greenhouse to produce F_2 seeds. One hundred and twenty F_2 plants were planted in one-gallon plastic pots in the greenhouse in the winter of 2006, with one plant per pot. Ninety-two $F_{2:3}$ families (28 plants did not produce enough seeds due to severe self incompatibility) and their parents were planted in the experimental field in Fargo, ND, USA, during the growing season of 2007 using a randomized complete block design with two replicates, with 15 to 20 plants grown in single-row plots.

Traits and Measurements

To map the self-incompatibility (*S*) gene in this population, seed set rate of each F_2 plant was investigated. Plants with two kinds of seed set rate, those with normal heads and those with sterile heads (fewer than 10 filled seeds), were observed and recorded before harvesting.

Disk diameter (DD, cm) was measured before harvesting on fully opened heads of individual F_2 plants and on each plant in every $F_{2:3}$ family row except for the two plants at both ends of the row. Ten seeds from each F_2 plant and one representative seed from each plant from each $F_{2:3}$ family row, except for the two plants at both ends of the row, were sampled to measure the seed traits after drying at 32°C for a week. Sampling and measuring of seed traits were repeated two times in the F_3 generation.

The images of the seeds from each line or family were taken using a digital camera at a fixed distance for all samples (Figure 1). The parameters of seed length (SL), and seed width (SW) were generated from the image pixels with the public

software TomatoAnalyzer, version 1.2 (Brewer *et al.,* 2006). The pixel values were then converted to centimeters by calibrating the actual length per seed (cm) measured on ten seeds from ten random plants. The same procedures were followed to obtain the measurement on kernel length (KL, cm/kernel), and kernel width (KW, cm/kernel) from the kernels manually dehulled from the seeds.

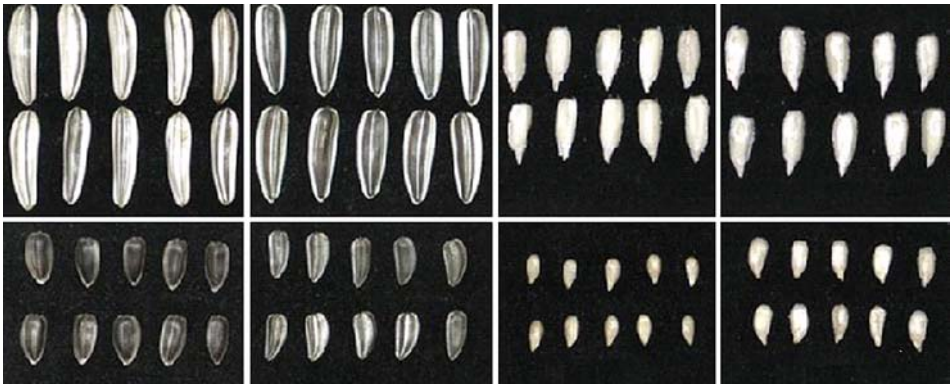

Figure 1: Sample digital images used to measure seed and kernel parameters.

The seeds and kernel from each plant were weighed, and 100-seed weight (SWT, g) and 100-kernel weight (KWT, g) were calculated. The ratios of seed length to width (SLW, %), kernel length to width (KLW, %), and kernel-to-seed weight ratio (KSR, %) were also calculated. Stripe (STR) on the hull was visibly scored from 0 (totally dark) to 3 (white with the least stripes).

DNA Preparation and Marker Generation

Total genomic DNA was isolated from about 50 mg (fresh weight) of leaf tissue sampled from individual plants of the parental lines and the F_2 population using the Qiagen DNeasy® 96 Plant Kit (Qiagen, Valencia, CA), following the manufacturer's instructions. DNA concentrations were adjusted to approximately 10 ng/μl for PCR amplification. To generate TRAP markers for map construction, 20 available fixed primers designed for related projects in our laboratory were used in the current study. These fixed primers were designed against the sequences of annotated ESTs related to chlorophyll synthesis, gibberellin synthesis, and the conserved plant telomere repeats. These sequences were obtained from the Compositae Genome Project Database (http://cgpdb.ucdavis.edu) and National Center for Biotechnology Information (http://www. ncbi.nlm.nih.gov) websites. Nine arbitrary primers labeled with either IR (infrared) 700 or IR 800 dyes were used. Table 1 lists the detailed information of all the primers. The TRAP assays followed the updated procedures described by Hu (2006). The TRAP markers were designated by the combination of the code of the fixed primer, the code of the arbitrary primer, and the fragment size in base pairs.

To align the linkage groups constructed in this study with the published SSR map, a total of 150 mapped SSR primer pairs from the 17 linkage groups (Yu *et al.,*

2003) were selected in the initial screening for polymorphisms between the two parents, and the polymorphic SSRs were used to genotype the whole F_2 population. SSR assays were carried out by following the procedures described by Tang *et al.* (2002).

Table 1: Information on fixed and arbitrary primers used in the current study

Code	Sequence ID	Sequences 5'—3'
	Fixed primer	
T98	QHA14E20	CTC CTA AAA GGT AAC CTG CT
T99	QHB26P17	GTT TTC CGT CAT ACT CGT TA
T100	QHB33I23	GAA GGG GTC AAA AAT TTA AC
T101	QHB34F17	TCC ACA CTT TTG AAG TCA TT
T103	QHK1O04	GAT ACA GGT TAT GGC AGA AA
T104	QHK7L05	TTA TGT CTA TGG CAC CAA CA
T105	QHL12I06	GCT TAC CGT CAT CAA GAA AC
T106	QHL15C24	GAA TGT CAC TTG ATT TTG CT
T107	QHL9B24	CAA TAT CCT TCC AAA CCT GA
T108	DY475501	CTC TTT GTA TGT GTT GTC CG
T110	CV987281	CAT ACA AGG TGG TCG AAA TT
T111	EC683354	AGG AAA TGT CTA TTT GGC AA
T112	QHJ12G10	ACC ACA CAA TCA TGA CTA GG
T114	QHJ4A19	TAA TAG CAA AAG CTC CAA TG
T116	QHB29B22	GCA TTA TAC TTT GGT GGA GA
T117	QHM10I05	ATT TGT TTG TTT GTT TTT GG
T64	TeloR[a]	AAC CCT AAA CCC TAA ACC
T66	TeloRT[a]	CCC TAA ACC CTA AAC CCT AAA T
T68	TeloTRG[a]	CCC AAA ACC CAA AAC CCA AAA G
T123	MAX3BR[b]	ACG TTA TGA GCC CCA TGA AGA
	Arbitrary primer	
R03	TRAP03(IR-700)	CGTAGCGCGTCAATTATG
R13	TRAP013(IR-800)	GCGCGATGATAAATTATC
R14	TRAP014(IR-800)	GTCGTACGTAGAATTCCT
R17	ODD15(IR-700)	GCGAGGATGCTACTGGTT
R18	ODD26(IR-700)	CTATCTCTCGGGACCAAAC
R19	SA12(IR-700)	TTCTAGGTAATCCAACAACA
R20	SA14(IR-700)	TTACCTTGGTCATACAACATT
R21	SA4(IR-700)	TTCTTCTTCCCTGGACACAAA
R22	GA3(IR-800)	TCATCTCAAACCATCTACAC
R23	GA5(IR-800)	GGAACCAAACACATGAAGA

[a]: from Hu (2006) and [b] from Rojas-Barros *et al.* (2005)

Map Construction and QTL Analysis

The linkage map was constructed using the computer program Mapmaker/EXP 3.0 (Lander *et al.*, 1987) (LOD>4.5). Due to the dominant nature of the TRAP

markers in this F_2 population, only interval QTL mapping was performed with both F_2 and F_3 data employing the software program Mapmaker/QTL1.1 (Lander and Botstein, 1989; Lincoln *et al.,* 1993). A LOD score of 2.4 was set for the threshold of the QTLs.

RESULTS

Phenotype Analysis

The phenotypic differences between the parents as well as the variation in the populations are summarized in Table 2. LSS had significantly higher values than HA89 for most of the seed traits including SL, KL, SLW, KLW, SWT, KWT, and STR, whereas HA89 was significantly higher than LSS for the trait of KSR in both generations (Table 2).

Table 2: The performance of seed morphological traits in the F_2 and F_3 populations

Traits [a]	HA89	LSS	$F_2/F_{2:3}$ population			
			Mean	Range	Skewness	Kurtosis
SL	1.1/1.1 [b]	3.1*/2.2*	1.6/1.7	(0.9-2.2)/(1.2-2.5)	0.2/0.7	0.1/1.3
SW	0.6/0.5	0.7/0.6	0.6/0.6	(0.3-0.8)/(0.5-0.9)	-0.2/1.1	0.3/-2.1
KL	-/0.8	-/1.4*	1.2/1.2	(0.7-1.4)/(0.9-1.5)	-0.5/0.1	1.1/0.4
KW	-/0.5	-/0.4	0.5/0.5	(0.3-0.6)/(0.4-0.6)	-0.2/0.6	0.1/1.4
SLW	2.0/2.0	4.6*/3.8*	2.8/2.8	(2.0-4.1)/(2.2-4.5)	0.8/1.4	0.8/2.5
KLW	-/1.9	-/3.5**	2.6/2.7	(2.1-3.2)/(2.1-3.5)	0.2/0.3	-0.2/0.9
SWT	-/6.1	-/12.3*	8.7/10.7	(2.9-14.8)/(5.7-21.3)	0.3/0.9	-0.5/2.1
KWT	-/4.6	-/6.4*	6.2/6.6	(2.4-10.0)/(4.0-11.2)	0.1/0.9	-0.6/1.6
KSR	-/75.9**	-/51.7	72.8/62.1	(56.2-87.3)/(48.4-77.0)	0.0/0.2	-0.4/-0.1
STR	0.0/0.0	3.0**/3.0**	1.7/1.5	(0-3)/(0-3)	-0.1/0.1	-1.0/0.2
DD	-/16.3	-/16.7	12.1/15.8	(6.5-19.0)/(13.1-26.0)	-0.2/1.3	-0.0/3.8

[a] SL=seed length, SW=seed width, KL=kernel length, KW=kernel width, SLW=seed length to width ratio, KLW=kernel length to width ratio, SWT=100-seed weight, KWT=100-kernel weight, KSR=kernel-to-seed weight ratio, STR=stripe in hull, DD=disk diameter.

[b] The values on the left side of the sign "/" are the data collected in the F_2 generation, and those on the right side were collected in F_3 generation. *, **, significant higher than the other parent at the 0.05 and 0.01 probability levels.

The difference between the two parents was not significant for SW, KW, and DD. The means for SWT, KWT, and DD in the F_3 generation were much higher than those for the F_2 generation, and it was opposite for KSR. This could be explained by the different growing environments for the two generations because the F_2 generation was grown in the greenhouse and the F_3 generation was grown in the field. This observation also indicated that these traits are greatly influenced by environmental factors, just like other quantitative traits. Transgressive segregation was observed in the population for DD, SW, KW, SWT, and KWT, while it was not obvious for the other traits. The values of skewness and kurtosis for these traits were less than or

close to 1.0 at least in one generation, indicating these traits fit a normal distribution (Table 2). Abnormal distribution for some traits in the F_3 generation might be due to the fact that only 92 lines were planted because of severe self incompatibility considering some QTLs for these traits were located in the region harboring the *S* loci in this and previous studies (Burke *et al.*, 2002; Tang *et al.*, 2006). ANOVA of the data collected in the F_3 generation indicated that variation due to genotype differences was highly significant for all the traits ($P<0.0001$).

The correlations among these traits are shown in Table 3. Six traits related to seed (kernel) size and seed (kernel) weight (SL, SW, KL, KW, SWT, and KWT) were highly intercorrelated in both generations ($0.46<r<0.96$). STR and DD were positively and significantly correlated with these traits related to seed (kernel) size and weight in some cases. As expected, SLW and SKW were positively correlated with SL and KL, but negatively correlated with SW and KW. KSR was negatively correlated with all the other traits.

Table 3: Coefficients of pairwise correlations of the traits investigated in the F_2 and F_3 generations

	SL	SW	KL	KW	SLW	KLW	SWT	KWT	KSR	STR
SW	0.57 [a]									
	0.55									
KL	0.86	0.66								
	0.86	0.55								
KW	0.44	0.89	0.61							
	0.35	0.88	0.46							
SLW	0.51	-0.40	0.22	-0.51						
	0.64	-0.28	0.49	-0.40						
KLW	0.42	-0.31	0.38	-0.50	0.85					
	0.55	-0.25	0.59	-0.44	0.87					
SWT	0.70	0.84	0.82	0.80	NS	NS				
	0.78	0.87	0.79	0.70	NS	NS				
KWT	0.61	0.83	0.80	0.85	-0.24	NS	0.96			
	0.67	0.81	0.80	0.78	NS	NS	0.91			
KSR	-0.61	-0.47	-0.48	-0.23	NS	-0.25	-0.55	-0.32		
	-0.56	-0.48	-0.35	NS	-0.21	-0.24	-0.59	-0.22		
STR	NS	NS	0.26	NS	NS	NS	0.26	NS	-0.41	
	NS	NS	NS	NS	NS	0.22	NS	NS	-0.46	
DD	0.33	0.64	0.42	0.69	-0.27	-0.30	0.62	0.62	-0.34	NS
	0.27	NS	NS	NS	0.26	0.27	NS	NS	-0.29	NS

[a] The coefficients in the first and second lines are from the F_2 and F_3 generations, respectively. Critical values at the 0.05 and 0.01 probability levels are 0.20 and 0.26, respectively, NS = not significant.

It was observed that some long seeds were not fully filled in this population. Although the correlation between seed length and kernel length is very high, a linear relationship did not exist when the seed length exceeded 1.8 cm in the greenhouse-grown F_2 population and 2.0 cm in the field-grown F_3 population (Figure 2).

Table 4: QTL for the seed morphological traits revealed using interval mapping in the F_2 and F_3 generations

Traits		QTL	LG	Interval	LOD	R^2% [a]	Direction [b]
SL	F_2	sl1	1	ORS733-1 – ORS509	2.5	6.5	L
		sl2	10	ORS595 – ORS78	6.2	14.4	L
		sl3	13	T99R21-300 – T99R21-364	5.7	14.8	L
		sl4	16	T114R13-600 – T66R22-240	4.0	8.2	L
		sl5	17	T99R21-815 – ORS811	4.4	8.3	L
	F_3	sl2	10	ORS595 – ORS78	6.1	15.9	L
		sl3	13	T99R21-300 – T99R21-364	5.3	17.3	L
		sl4	16	T114R13-600 – T66R22-240	5.5	16.3	L
		sl5	17	T99R21-815 – ORS811	2.7	5.4	L
SW	F_2	sw1	1	T99R23-115 – T66R19-755	2.7	6.8	L
		sw3	7	T100R23-260 – ORS331-7	2.5	5.6	L
		sw4	10	ORS595 – ORS78	5.9	10.7	L
		sw5	17	T66R22-550 - *S*	4.3	9.6	L
	F_3	sw1	1	T99R23-115 – T66R19-755	5.0	10.1	L
		sw2	6	T110R17-317 – T108R13-495	2.5	6.6	L
		sw4	10	ORS595 – ORS78	7.5	16.3	L
		sw5	17	T66R22-550 - *S*	3.1	6.1	L
KL	F_2	kl1	4	ORS366 – ORS337	2.7	7.1	L
		kl3	10	ORS595 – ORS78	4.8	13.1	L
		kl4	13	T99R21-300 – T99R21-364	5.4	13.5	L
	F_3	kl1	4	ORS366 – ORS337	3.0	6.6	L
		kl2	6	T100R23-205 – T64R21-365	3.1	7.5	L
		kl3	10	ORS595 – ORS78	4.5	11.2	L
		kl4	13	T99R21-300 – T99R21-364	6.5	18.4	L
		kl5	16	T114R13-600 – T66R22-240	5.0	16.7	L
KW	F_2	kw2	10	OR78 – T68R23-205	3.7	8.1	L
		kw3	17	T108R13-348 – T108R13-625	4.0	23.2	L
	F_3	kw1	5	T123R13-400 – ORS733-5	2.6	7.5	L
		kw2	10	OR78 – T68R23-205	3.3	7.8	L
		kw3	17	T108R13-348 – T108R13-625	2.9	7.5	L
SLW	F_2	slw2	6	T99R21-515 – T100R19-180	3.7	7.1	L
		slw3	9	T117R23-210 – T108R13-160	3.3	6.5	L
		slw4	16	T114R13-600 – T66R22-240	8.1	19.8	L
	F_3	slw1	3	ORS202 – T99R23-290	3.4	6.7	L
		slw4	16	T114R13-600 – T66R22-240	6.9	15.9	L
KLW	F_2	klw2	6	T99R21-515 – T100R19-180	3.9	8.6	L
		klw3	9	T117R23-210 – T108R13-160	3.5	8.1	L
		klw4	11	ORS733-11 – T108R13-680	3.7	8.2	L
		klw5	13	T99R21-300 – T99R21-364	4.3	14.4	L
		klw6	16	ORS656 – T66R19-170	6.4	14.7	L
	F_3	klw1	3	T99R23-290 – T101R19-215	4.0	8.7	L
		klw2	6	T99R21-515 – T100R19-180	4.2	8.1	L

Table 4: QTL for the seed morphological traits revealed using interval mapping in the F_2 and F_3 generations

		klw4	11	ORS733-11 – T108R13-680	4.6	10.9	L
		klw6	16	ORS656 – T66R19-170	7.5	14.2	L
SWT	F_2	swt1	1	ORS733-1 – ORS509	2.9	7.2	L
		swt2	10	ORS595 – ORS78	6.0	17.7	L
		swt3	13	T99R21-300 – T99R21-364	3.0	16.2	L
		swt4	17	T66R22-550 - *S*	6.4	15.4	L
	F_3	swt1	1	ORS733-1 – ORS509	2.9	6.3	L
		swt2	10	ORS595 – ORS78	12.3	23.3	L
		swt3	13	T99R21-300 – T99R21-364	4.2	12.6	L
KWT	F_2	kwt2	10	ORS595 – ORS78	4.4	11.6	L
		kwt3	13	T99R21-300 – T99R21-364	2.9	14.3	L
		kwt4	17	T66R22-550 - *S*	5.7	15.4	L
	F_3	kwt1	4	ORS366 – ORS337	2.8	6.5	L
		kwt2	10	ORS595 – ORS78	8.6	18.3	L
		kwt3	13	T99R21-300 – T99R21-364	4.3	14.8	L
KSR	F_2	ksr1	1	ORS733-1 – ORS509	7.2	16.2	H
		ksr2	2	T104R19-260 – T99R21-186	4.0	10.2	H
		ksr4	10	ORS537 – T100R23-380	6.4	14.8	H
		ksr5	13	T99R21-300 – T99R21-364	5.1	22.1	H
		ksr6	14	ORS398-14 – T111R18-456	4.8	11.7	H
		ksr7	16	ORS656 – T66R19-170	4.2	8.9	H
		ksr8	17	T66R22-550 - *S*	3.8	8.2	H
	F_3	ksr1	1	ORS733-1 – ORS509	8.2	16.4	H
		ksr2	2	T104R19-260 – T99R21-186	2.9	5.9	H
		ksr3	6	T68R17-890 – T110R17-317	2.6	7.8	H
		ksr4	10	ORS537 – T100R23-380	8.7	17.1	H
		ksr5	13	T99R21-300 – T99R21-364	2.7	8.7	H
		ksr6	14	ORS398-14 – T111R18-456	3.3	6.5	H
		ksr7	16	ORS656 – T66R19-170	2.5	5.1	H
STR	F_2	str1	1	T117R23-290 – T111R13-140	3.9	8.9	L
		str2	2	T99R21-186 – T108R13-202	3.3	19.4	L
		str3	14	ORS398-14 – T111R18-456	2.5	6.8	L
		str4	17	T123R13-1020 - T100R19-700	5.2	21.8	L
	F_3	str1	1	T117R23-290 – T111R13-140	4.5	10.9	L
		str2	2	T99R21-186 – T108R13-202	3.0	11.7	L
		str3	14	ORS398-14 – T111R18-456	2.7	10.7	L
		str4	17	T123R13-1020 - T100R19-700	6.6	29.3	L
DD	F_2	dd2	13	T99R21-300 – T99R21-364	2.7	7.2	L
		dd3	17	T66R22-550 - *S*	6.8	16.8	L
	F_3	dd1	9	T110R17-474 – T66R19-750	3.6	16.4	H
		dd3	17	T66R22-550 - *S*	8.3	24.7	L

[a] Amount of phenotypic variation (%) explained by the QTL

[b] The allele from LSS(L) or HA89 (H) of the locus had a positive effect on this trait

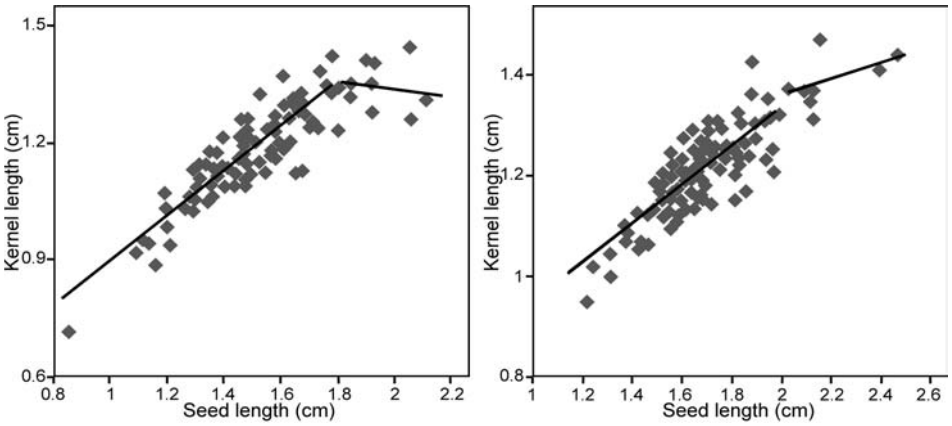

Figure 2: Scatter plots of seed length against kernel length in the F_2 (left) and F_3 (right) generations.

Molecular Genetic Linkage Map Construction

A total of 191 polymorphic fragments were generated from the 40 pairs of TRAP primer combinations (one fixed primer + one arbitrary primer labeled by IR700 or IR800). Only three of them were codominant markers. Each primer combination generated 1 to 12 TRAP markers with an average of 4.8 markers per combination. Among these TRAP markers, 179 were mapped to the 17 linkage groups and 12 were found unlinked. Of the 150 SSRs screened, only 36 (24.0%) were polymorphic between the two parents, which generated 44 SSR markers.

After a preliminary mapping test, 165 TRAP markers that were evenly distributed across the sunflower genome and the 44 SSR markers were selected to construct the linkage map for QTL analysis. The linkage map had a total length of 1784.3 cM, and the average distance between adjacent markers was 8.5 cM (Figure 3). Integration of the 44 previously mapped SSR markers to the TRAP map allowed us to align 15 of the 17 linkage groups to the previously published SSR map except for linkage groups 8 and 12 (Yu *et al.,* 2003). Each linkage group contained 1 to 6 SSR markers.

Mapping the Self-incompatibility (S) Locus

We observed self-incompatibility on the male parent when it was first grown in the greenhouse in Fargo in winter 2003. All five plants were healthy and developed normally to maturity. However, they had extremely low seed production with only a few seeds per head. Since the plants produced normal pollen, the low seed set could be attributed to self-incompatibility. This self-incompatibility was observed again in the Fargo field plot in summer 2004 when fewer than 20 seeds were obtained from each bagged head. Since seed set on the F_1 hybrids was normal, we

inferred that the male parent carries a pair of recessive alleles at the self-incompatibility (S) locus. Of the 120 F_2 plants, 28 plants expressed self-incompatibility and had very low seed set. This segregation fit the single gene ratio ($c^2_{(3:1)}$=0.17, p>0.9). Genetic mapping by molecular markers placed this self-incompatibility (S) locus on LG17 (Figure 3), anchored by SSR markers *ORS 598-17* and *ORS 811*. This *S* locus has the same proximity in map position as previously reported (Gandhi *et al.*, 2005).

QTL Analysis

A total of 51 QTLs for these traits were resolved in the two generations including 32 (62.7%) detected in both generations, and individual QTLs explained 5.1%-29.3% of phenotypic variation (Table 4). Alleles from LSS at all the QTLs for these traits, except for KSR and DD, had positive effects, and it was opposite for KSR. This was consistent with the performance of this parent for these traits.

For seed or kernel size related traits, (SL, SW, KL, and KW), a total of 18 QTLs were detected in at least one generation, and 12 of them were common in both generations. Eight QTLs were detected for SWT and KWT, with five of them identified in both generations. Furthermore, there were two QTLs for SWT, *swt2*, and *swt3*, that shared the same chromosomal locations with two QTLs for KWT, *kwt2*, and *kwt3*, respectively, and they explained 11.6% - 23.3% of phenotypic variation. The trait of KSR was governed by eight QTLs in this study, six of them were detected in both generations, and individual QTLs explained 5.1% - 22% of phenotypic variation.

A total of ten QTLs for SLW and KLW were detected in the two generations, and only four were detected in both generations. Each of them explained 8.1%-14.7% of phenotypic variation. Three QTLs were identified for DD in the F_2 or F_3 generation, and one of them, *dd3*, was detected in both generations, which alone explained 16.8% of phenotypic variation in the F_2 generation and 24.7% in the $F_{2:3}$ generation. Alleles at these loci from both parents had positive effects on disk diameter.

Congruence of QTL

The 51 QTLs identified in this study were distributed across 14 linkage groups, and there were 12 chromosomal regions that harbored more than two QTLs (Figure 3). It is interesting to note that there were six chromosomal intervals containing more than three QTLs contributing to seed or kernel sizes and weight. Two of them, *T99R21-300 – T99R21-364* on LG13 and *S –T108R13-625* on LG17 clustered 7 and 6 QTLs, respectively. Moreover, two QTLs for disk diameter, *dd2* and *dd3*, were also located in the two regions. There were three regions clustering QTLs for SLW and KLW, two regions with overlapping QTLs for STR and KSR, one region harboring QTLs for KSR and KLW, and one region harboring QTLs for KSR and SW. These results were also consistent with the correlations among these traits (Table 3). However, some clustering QTL for highly correlated seed traits might be controlled by the same gene, such as the QTL for seed (kernel) size and seed (kernel) weight, or the QTL for seed length (width) and kernel length (width).

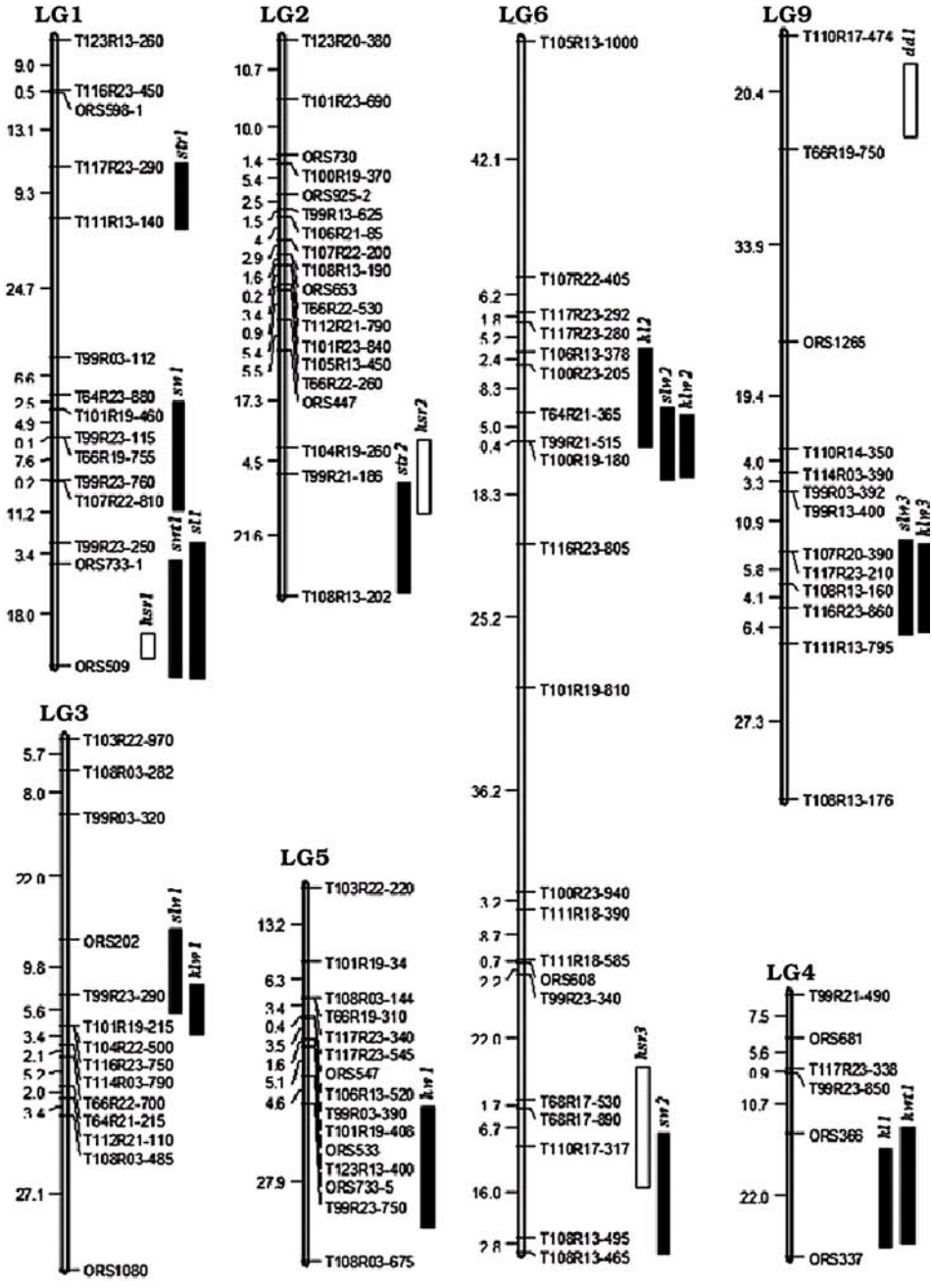

Figure 3a: The genetic linkage map and locations of the QTL detected in both generations. Designations of markers are on the right and genetic distances (cM) are on the left.

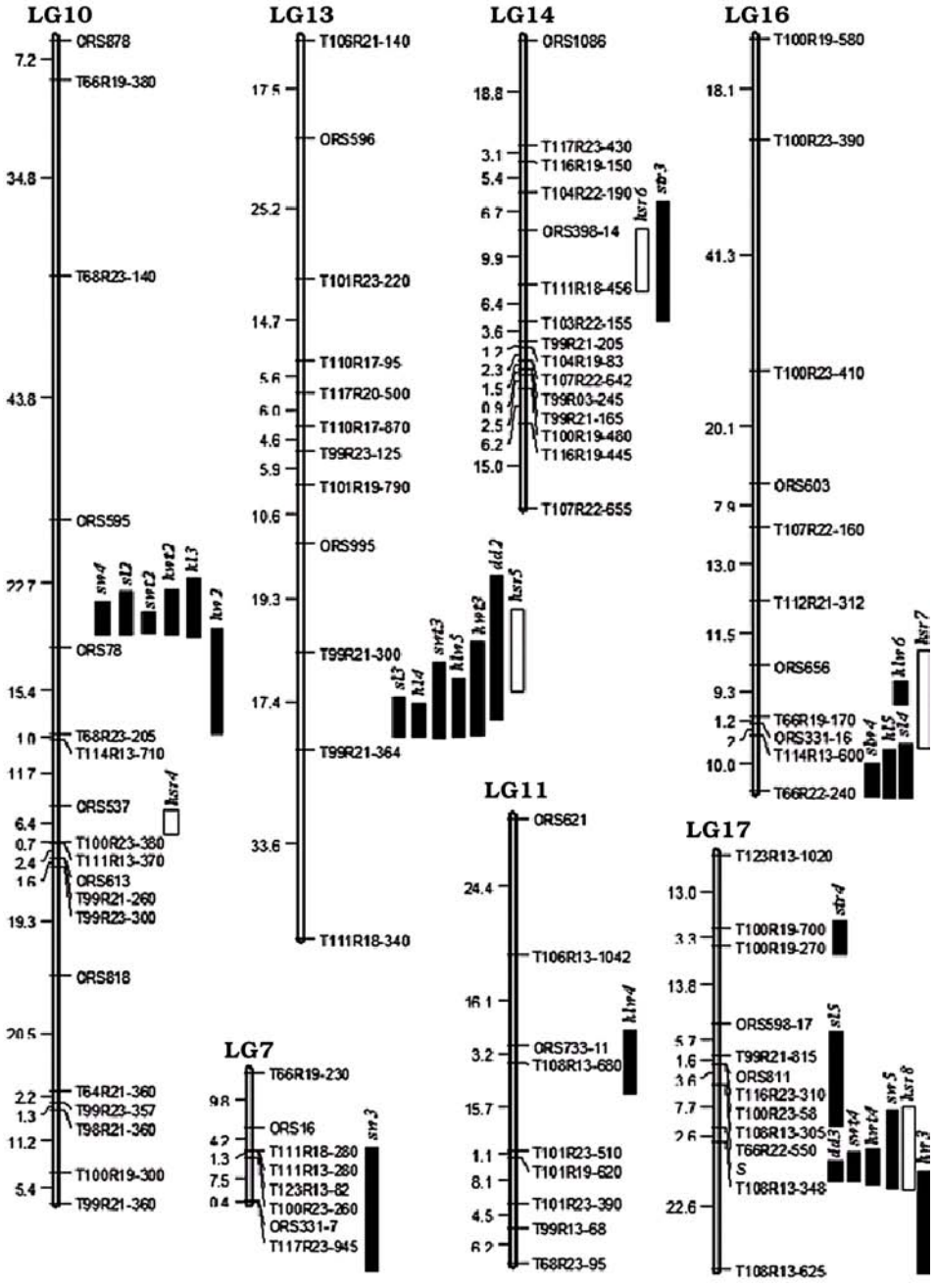

Figure 3b: The genetic linkage map and locations of the QTL detected in both generations. Designations of markers are on the right and genetic distances (cM) are on the left.

DISCUSSION

TRAP markers, in combination with SSR markers, have been used to construct linkage maps in wheat, sunflower, and common bean (Liu *et al.,* 2005; Miklas *et al.,* 2006; Hu, 2006; Chen *et al.,* 2007). Here we report the construction of a sunflower TRAP map and the application of the map to QTL analysis. This map contains 165 TRAP markers distributed across 17 linkage groups. Anchored by 44 SSR markers, 15 of the linkage groups were aligned to those of the published SSR maps (Yu *et al.,* 2003). Moreover, a total of 51 QTLs for 11 traits were identified and positioned on this map. TRAP is an efficient PCR-based marker technique for molecular mapping because it can be used to survey multiple loci for polymorphism in a single PCR reaction. For instance, each TRAP PCR reaction generated on average 9.6 polymorphic markers in the mapping population of this study, whereas only 24.0% of the SSR markers were polymorphic between the two parents and each PCR only detected 1.2 polymorphic markers. Thus, TRAP seems to be more efficient for genome mapping. Similar observations were reported in previous studies (Hu and Vick, 2003; Liu *et al.,* 2005).

The genetic basis of seed morphological traits is complex and controlled by a number of QTLs in rice and wheat (Tan *et al.,* 2000; Groos *et al.,* 2003). In sunflower, Burke *et al.* (2002) identified 14 QTLs for achene weight, achene width and achene length, each of them explaining 5.4%-17.8% of phenotypic variation. Tang *et al.* (2006) identified 34 QTLs for seed weight, seed length, seed width, seed depth, kernel weight, pericarp weight and kernel-to-pericarp weight ratio. Wills and Burke (2007) detected four QTLs for achene weight, each QTL explaining less than 19.0% of phenotypic variation. Baack *et al.* (2008) identified recently six QTLs for achene length and achene mass. In the present study, a total of 48 QTLs for 10 seed morphological traits were detected, and individual QTLs explained 5.1%-29.3% of phenotypic variation. These results indicated that seed traits were controlled by multiple genes in sunflower. Although the QTLs for seed traits were distributed across 14 linkage groups, the majority of QTLs were clustered within six chromosomal intervals (Figure 3). These regions could collectively contribute to the typical characteristics of confection type sunflower, which possesses large seed size, seed width, and lower seed to kernel ratio. Through nearby SSR markers, five of the total 19 chromosomal regions containing QTLs for seed traits in this study were previously reported to harbor QTLs underlying similar seed morphological traits. Near the branching gene on LG10 Tang *et al.* (2006) detected major QTLs for seed length, seed width, seed weight, kernel weight and kernel-to-pericarp weight ratio, which explained more than 40% of phenotypic variation. Burke *et al.* (2002) identified two QTLs for achene length and achene width. Wills and Burke (2007) and Baack *et al.* (2008) also detected a QTL for seed weight. Near the SSR marker *ORS656* on LG16, Tang *et al.* (2006) detected five QTLs for seed traits, and near the locus *S* on LG17 both Tang *et al.* (2006) and Burke *et al.* (2002) identified

QTLs related to seed traits. In the region of *ORS366* – *ORS337* on LG4, Tang *et al.* (2006) identified three QTLs for kernel-to-pericarp weight ratio, 10-pericarp weight, and seed oil concentration. In addition, the QTL for SWT LG1 in this study shares a similar position with the achene weight QTL identified by Wills and Burke (2007). These chromosomal regions that were confirmed to harbor QTLs in different studies are very useful in breeding for confection sunflower lines.

Disk diameter is an agronomically important trait in sunflower, because it is associated with seed number per head. Burke *et al.* (2002) identified three QTLs for disk diameter in sunflower, and individual QTLs explained 4.6%-6.0% of phenotypic variation. Wills and Burke (2007) found disk diameter was controlled by eight QTLs, each QTL explaining less than 13.0% of phenotypic variation. Baack *et al.* (2008) recently identified four QTLs for this trait. In the present study, three QTLs were detected in two generations and individual QTLs explained 7.2%-24.7% of phenotypic variation. This indicated that disk diameter was also controlled by multiple genes in sunflower. The QTL near SSR marker *ORS995* on LG13 in this study had a similar position to the QTL detected by Burke *et al.* (2002). Moreover, both Wills and Burke (2007) and Baack *et al.* (2008) identified a QTL for disk diameter on LG9, which might share a similar position with *dd1* which was detected in this study. However, this needs to be further confirmed since only one SSR marker was assigned on LG9 in the current linkage map. It is also interesting to note that two QTLs for disk diameter were located in two regions harboring more than five QTLs for seed traits on LG13 and LG17, and all of the favored alleles were from the confectionery line. Thus, it is possible to increase seed (kernel) size, weight, and disk diameter simultaneously by introgressing the two regions.

According to the analysis of correlation and QTL congruence, the 10 seed traits can be divided into three groups based on size (length, width, and weight), seed shape (ratio of length to width), and stripe. Among the 14 QTLs for seed shape related traits (SLW, KLW) and stripe, only three of them overlapped with the QTLs for seed (kernel) size, suggesting that they had a different genetic basis from the other confection traits. In general, seed size and seed weight are small in the branching head; however, it is unclear whether it is caused by pleiotropic or linkage effects. QTLs for seed size (weight) were detected near the branching (*b*) locus on LG10, and explained more than 40% of phenotypic variation (Tang *et al.*, 2006). With a population derived from a cross between branching and nonbranching parents, no QTL for branching was detected on LG10, but QTLs for achene length and weight were still detected in the region harboring *b*. Each QTL explained less than 12.4% of phenotypic variation (Burke *et al.*, 2002). In the present study, QTLs for seed traits were also detected in the same region on LG10 in the population without segregation for branching, and each QTL explained 7.8%-23.3% of phenotypic variation. These results suggested that the QTLs identified near the *b* locus could be caused by both the pleiotropic effects of *b* and the linkage disequilibrum between *b* and the QTL. Although four QTLs for stripe were detected in both generations in

this study, none of them co-located to the loci controlling hull color, *P* (in phytomelamin layer) and *Hyp* (in hypodermal layer), both of which were mapped in a previous report (Tang *et al.,* 2006). Thus, the difference in striping in this study might be mainly produced in the epidermal layer of the hull.

The genetic basis of kernel length is more complex than seed length because seed filling depends not only on the source (photosynthetic assimilates) and sink (seed size), but also on the source-sink relationship (Alkio and Grimm, 2003). Thus, in confection sunflower breeding, seed length should be less than two centimeters.

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ABBREVIATIONS

DD	disk diameter	KL	kernel length
KLW	kernel length to width ratio	KSR	kernel-to-seed weight ratio
KW	kernel width	KWT	100-kernel weight
QTLs	quantitative trait loci	SL	seed length
SLW	seed length to width	SSR	simple sequence repeat
STR	stripe	SW	seed width
SWT	100-seed weight	TRAP	target region amplification polymorphism

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MAPEO DE LOCI PARA CARACTERES CUANTITATIVOS (QTL) QUE CONTROLAN MORFOLOGÍA DE SEMILLAS Y DIÁMETRO DEL DISCO EN GIRASOL (*Helianthus annuus* L.)

RESUMEN

La morfología de las semillas y el diámetro del disco son los caracteres agronómicos más importantes para el girasol confitero. Este trabajo informa los resultados del análisis de los loci para caracteres cuantitativos (QTLs) que subyacen 10 caracteres morfológicos de las semillas, incluyendo tamaño de aquenio y pepita, forma y estriado y diámetro del disco en una población F_2 y las familias $F_{2:3}$ derivadas de un cruzamiento entre un material del tipo oleaginoso y uno confitero. Se construyó un mapa de ligamiento consistente en 165 marcadores de polimorfismo de amplificación de regiones objetivo (TRAP) y 44 marcadores de repetición de secuencias simples (SSR) a partir de 120 plantas F_2. Este mapa contiene 17 grupos de ligamiento y explora una distancia genética total de 1784.3 cM. Se detectó un total de 51 QTLs, 32 de los cuales de identificaron en ambas generaciones. Cada QTL explicó un 5.1-29.3% de la variación fenotípica, lo que sugiere que estos atributos están controlados por múltiples genes. La mayoría de los QTLs están agrupados en seis regiones cromosómicas. Dos de los tres QTLs identificados para diámetro del disco están localizados en dos de las seis regiones cromosómicas. Además, alelos de la línea confitera en dichos QTLs presentaron efectos positivos sobre estos atributos. Tanto la congruencia de QTLs como los análisis de correlación revelaron que bases genéticas diferentes son responsables de la expresión de los atributos forma de semilla, estriado y otros caracteres de los confiteros. La información generada por este estudio facilitará el mejoramiento del girasol confitero.

ÉTABLISSEMENT D'UNE CARTE DE LOCI DES CARACTÉRISTIQUES QUANTITATIVES (QTL) CONTRÔLANT LA MORPHOLOGIE DE LA GRAINE ET LE DIAMÈTRE DU CAPITULE DU TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

La morphologie de la graine et le diamètre du capitule sont les caractères agronomiques les plus importants pour le tournesol de bouche. Cet article rapporte les résultats d'une analyse de LCQ associés à 10 caractères morphologiques parmi lesquels la taille du grain, sa forme et ses stries et le diamètre du capitule, à partir d'une population F_2 et de sa descendance F_2:F_3 issue d'un croisement entre une lignée de type "huile" et d'une lignée de type "de bouche". Une carte de liaison a été établie à partir de 165 marqueurs TRAP (target region amplification polymorphism) et de 44 marqueurs microsatellites (SSR), et sur 120 individus F_2. Cette carte comporte 17 groupes de liaison et couvre une distance génétique totale de 1784.3 cM. Au total, 51 LCQ ont été mis en évidence et 32 d'entre eux l'ont été pour les deux générations. Chaque LCQ explique 5.1 à 29.3% de la variance phénotypique totale, ce qui suggère un contrôle multigénique. La plupart des LCQ se concentre sur six régions chromosomiques. Deux des trois QTL identifiés pour le diamètre du capitule co-localisent avec deux de ces six régions chromosomiques. De plus, le parent de type "de bouche" apporte les allèles favorables à ces caractères. La cohérence des résultats obtenus sur les LCQ et les analyses de corrélations mettent en évidence l'existence de bases génétiques distinctes pour la forme de la graine, ses stries, et les autres caractères d'intérêt pour le tournesol de bouche. L'information produite par cette étude facilitera l'amélioration génétique des tournesols de bouche.