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Comparative genetic analysis of quantitative traits in sunflower (Helianthus annuus L.). 2. Characterisation of QTL involved in developmental and agronomic traits

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Abstract Seed weight and oil content are important properties of cultivated sunflower under complex genetic and environmental control, and associated with morphological and developmental characteristics such as plant height or flowering dates. Using a genetic map with 290 markers for a cross between two inbred sunflower lines and 2 years of observations on F3 families, QTL controlling seed weight, oil content, plant height, plant lodging, flowering dates, maturity dates and delay from flowering to maturity were detected. QTL detected were compared between the F2 and F3 generations and between the 2 years of testing for the F3 families in 1997 and 1999. Some of the QTL controlling seed weight overlapped with those controlling oil content. Several other co-localisations of QTL controlling developmental or morphological characteristics were observed and the relationships between the traits were also shown by correlation analyses. The relationships between all these traits and with resistance to *Sclerotinia sclerotiorum* and *Diaporthe helianthi* are discussed.

Keywords Cultivated sunflower · Morphological and developmental characteristics · Genetic map · QTL · F_2 and F_3 generations

Introduction

Breeding programmes in sunflower (*Helianthus annuus* L., 2n = 2*x* = 34) began relatively recently compared with other crop species, and have focused mainly on yield and oil content. Identification of genetic factors that affect agronomic and economically important traits in sunflower could help to improve breeding methods. A polygenic pattern of inheritance has been indicated for sunflower oil content with heritabilities estimated at 65 to 70% (Fick 1975). In contrast, seed yield is known to be dependent on both additive and non-additive gene actions, and genotype × environment interactions are an important component of variance giving quite low heritabilities (Fick 1978).

Sunflower can be grown under a wide variety of climatic conditions so a wide range of total crop durations are required around the world. In addition, knowledge of the relative lengths of the period from sowing to flowering, when potential seed number is determined, and of the period from flowering to maturity, when seed filling occurs, can be of importance in breeding for yield. Present-day sunflower varieties show wide variation for these characters. Although plant height is not an essential character in breeding for yield or earliness, susceptibility to lodging may be related to height. Genotypes with a long pre-flowering period tend to be tall, as stem growth continues until flowering, but modern varieties show a wide range of heights and, for a given yield, a shorter plant is preferred (Miller and Fick 1997).

The rapid and recent development of molecular markers has largely contributed to the establishment of saturated molecular maps which have enabled the mapping of qualitative traits and the dissection and localisation of factors underlying quantitative traits, with the goal of using QTL-marker associations in marker-assisted selection. Many studies have been conducted in crops to identify QTL for important characteristics in agronomic and developmental traits such as days to flowering and photoperiod response (Leon et al. 2001) or somatic embryogenesis (Flores Berrios et al. 2000) in sunflower. Mestries et al. (1998) reported QTL for seed weight per capitulum, seed oil content and flowering date employing F3 families from a cross used primarily to study resistance to *Sclerotinia sclerotiorum*.

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The present paper made a similar study on F3 progenies of different genetic origins, but with additional observations of height and of maturity date to obtain some information about the general relations between all these characters, with the main objective of determining the QTL concerned and whether they are general, or specific to different crosses, and their possible use in breeding.

Materials and methods

Sunflower genotypes

The two parental inbred lines of the cross were bred by INRA (Institut National de la Recherche Agronomique), France. XRQ was a selection for downy mildew resistance from a cross between HA89 and the Russian population Progress (Vear et al. 1998). PSC8 was selected from a population made from a wide range of sunflower lines and subjected to recurrent selection for capitulum resistance to *S. sclerotiorum* (Vear et al. 1992). XRQ is an unbranched PET-1 CMS maintainer whereas PSC8 is a male-fertility restorer line with the apical branching gene (*b1*) phenotype. These lines were chosen for their different reactions to downy mildew, *S. sclerotiorum* and *Diaporthe helianthi*, but for many agronomic characters they are quite similar: over several years, compared with control inbreds, PSC8 flowers 1 day later than XRQ, is 10% taller (15 cm) but has two points less oil (46% compared with 48% under open pollination). Since PSC8 is branched, although with a main head, its seed production per capitulum is less than that of XRQ.

The male-fertile forms of the two lines were crossed in both directions. The F1 plants were selfed by covering the capitula with grease-proof paper bags a few days before flowering to obtain the 220 F2 generation, which was in turn selfed to obtain the 220 F3 families.

Field observations

Flowering and maturity dates and the number of days from flowering to maturity were observed in 1997 and 1999 on the two replicates of 25 plants of each F3 or F3 family used to test the *S. sclerotiorum* head rot resistance (Bert et al. 2002). In 1997, 220 families were tested, and in 1999, 180 families, excluding 20 branched and 20 heterozygous families, were tested at random. Sowing dates were April 17 1997 and April 13 1999. Flowering date for each plant was the date (day number in years) when the first tubular florets opened. Maturity date, when the capitulum was brown and dry, was noted for plants which did not show *S. sclerotiorum* symptoms (40% of plants in 1997, 60% of plants in 1999). Observations of maturity were made from mid August to the end of September, by which time most sunflower crops sown in April have been harvested. Plants not dry at the end of September were excluded from calculations of maturity date.

Some lodging occurred in these trials in 1997 and lodged plants were counted. Plant height, seed weight per capitulum and seed oil content were observed in 1996 on the F2 plants that were selfed to obtain the F3 generation and, in 1997 for each F3 family, on one replicate of five selfed plants that were not tested for disease resistance. Seed weight was obtained after drying at 40 °C, and oil content was measured on 2 g samples from each capitulum by nuclear magnetic resonance (Bruker Mini-Spec 10).

Molecular analysis and map construction

A genetic map of the cross XRQ × PSC8 based on 220 F2 individuals was constructed (Bert et al. 2002) using RFLP (available upon request) and AFLP markers, using the software package JOINMAP 2.0 (Stam 1993) and the software program MAPMAKER 3.0b (Lander et al. 1987). Analyses were performed with a LOD score threshold of 4.0 and a maximum recombination value of 40% (θ = 0.40) for grouping and ordering markers. Haldane's mapping function was applied for map-distance calculation.

Statistical analyses and QTL detection

Normality of the different traits were assessed according to the Shapiro and Wilk test (PROC UNIVARIATE of SAS). The data were transformed when necessary with a function to fit a normal distribution. Normality of the residuals and homogeneity of variances using Bartlett's test were also checked (SAS institute Inc).

Data sets were analysed by generalised linear modelling (GLM) using the SAS program (SAS institute Inc.). One way ANOVA were performed with the marker locus as the factor, and the three (for codominant markers) and two (for dominant markers) genotypes as levels. Significant *F*-values were interpreted to indicate segregation of a QTL linked to the marker locus. Due to the large number of ANOVA performed, we have chosen to present only the results from interval mapping. Using the framework map orders, results were confirmed using the method of interval mapping of MAPMAKER/QTL software with a LOD score threshold of 3.0. This value was chosen from the theoretical consideration of Lander and Botstein (1989). The R^2 global value for each trait was calculated using MAPMAKER/QTL when examining multiple QTL simultaneously.

Phenotypic correlations were analysed using STATGRAPHICS/Plus to detect associations between characters.

Results

Phenotypic variation and correlations between traits

The frequency distribution of phenotypes of the 220 F3 families for each trait are shown in Figs. 1 and 2. All traits showed approximately normal distributions. Statistical analyses (mean phenotypic values, *F*-values and broad-sense heritabilities) of the observations of the quantitative traits are presented in Table 1. In all cases there were significant differences between F3 families.

Mean flowering date was 5 days later in 1997 than in 1999, whereas mean maturity date was 4 days earlier. This earlier maturity date in 1997 was confirmed by the fact that only 25/12,000 (0.2%) plants observed were not dry on 29/09/1997, whereas on 28/09/1999 the figure was 261/10,000 (2.6%). Different weather conditions in the 2 years were probably the main cause of these differences, but the absence of some branched families and the lower frequency of disease in 1999 may also have had some effects on maturity date. Flowering dates within families were grouped in 1997, leading to an F genotype of 17.2, compared with 9.8 in 1999. Maturity dates within and between families were always spread wider than flowering dates: for example, in 1997 mean F3 flowering dates were spread over only 10 days, whereas maturity dates ranged over 33 days. The genotypic effect for maturity date was greater in 1999 than in 1997, probably related to the larger number of disease-free plants available for observation. Oil-content and plant-height values between F2 and F3 generations were quite similar whereas mean

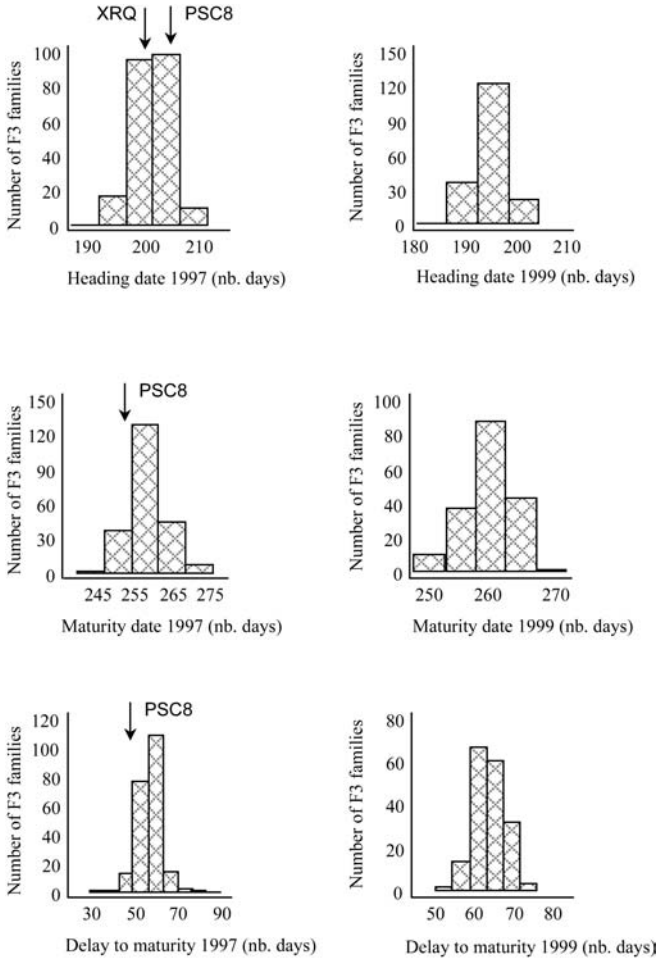

Fig. 1 Frequency distribution of phenotypes for each developmental trait in the 220 F3 families. Phenotypes of parental inbred lines XRQ and PSC8 are indicated by *arrows* when data were available

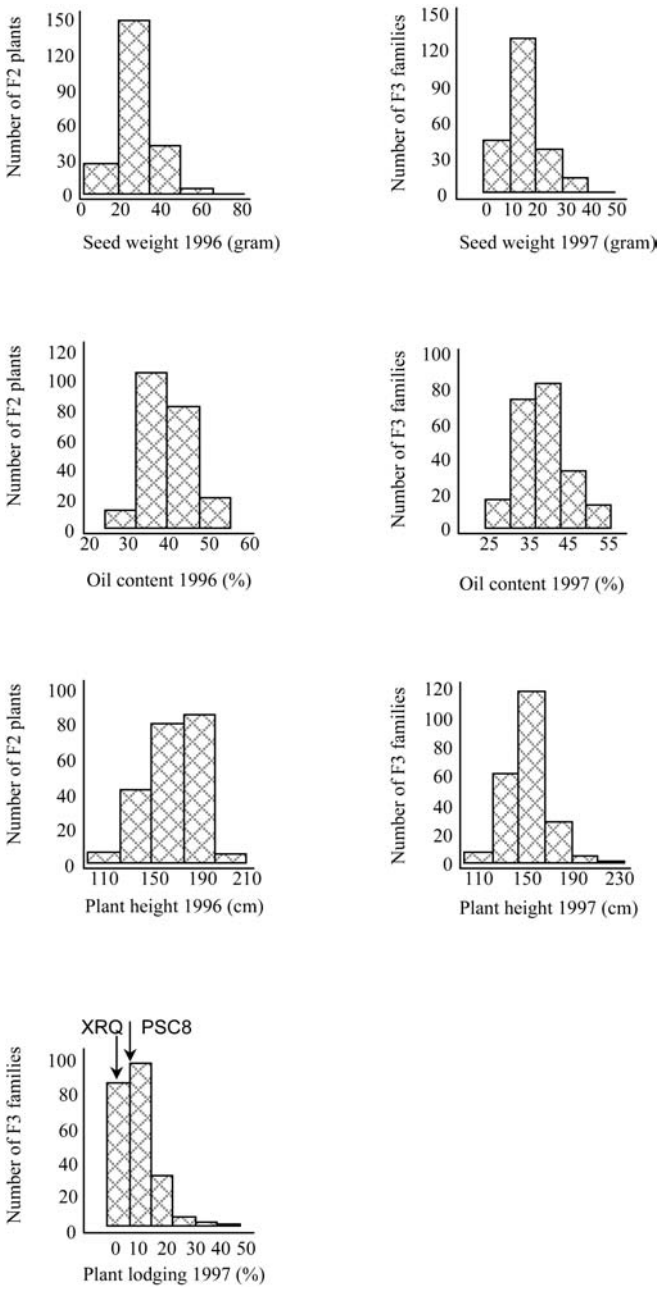

Fig. 2 Frequency distribution of phenotypes for each agronomic trait in the 220 F3 families. Phenotypes of parental inbred lines XRQ and PSC8 are indicated by *arrows* when data were available

seed weight/capitulum was greater for the F2 than for the F3 generation, probably due to year effects.

Correlations (Table 2) between data for different years or generations were always highly significant. They were closest for flowering, $r = 0.79$ compared with 0.42 and 0.39 for maturity dates and days between flowering and maturity, respectively. Correlations between years or generations for seed weight, oil content and height were surprisingly close ($r = 0.33$, 0.66 and 0.60 respectively) considering that F2 observations were on single plants.

Maturity is the final stage of the developmental cycle and thus is partly dependent on flowering date, as confirmed by the highly significant correlation observed between the two traits. The number of days from flowering to maturity was more closely correlated with maturity date than with flowering date, perhaps because of the wider range of values for the former character. As expected, height was significantly correlated with flowering date, but not with the number of days from flowering to maturity. Seed production and oil content showed very little correlation with lengths of the vegetative stages. Lodging was not significantly correlated with height, but showed a negative relation with oil content. There was a slight negative correlation between oil content of the F3, and seed weight in the F2 and F3.

Characterisation of QTL

The results of molecular analyses and map construction were described in detail by Bert et al. (2002). The map

Table 1 Summary of the statistical results from developmental and agronomic traits for the XRQ × PSC8 F2 individuals and F3 families

Character	Number of F3 families	Mean	F genotype	C.V. (%)	Heritabilities
Flowering date 1997	220	201.50 (day)	17.17***	1.07	0.89
Flowering date 1999	177	196.00 (day)	9.84***	1.55	0.82
Maturity date 1997	220	257.50 (day)	2.64***	1.99	0.45
Maturity date 1999	175	259.50 (day)	4.06***	1.26	0.60
Delay to maturity 1997	220	54.00 (day)	2.67***	9.45	0.45
Delay to maturity 1999	175	64.30 (day)	3.42***	3.59	–[a]
Plant height F2	220	167.50 (cm)	–[a]	9.95	–[a]
Plant height F3	220	171.00 (cm)	5.55***	9.19	0.69
Lodging	220	22.32 (%)	1.66***	87.96	–[a]
Seed weight F2	220	35.50 (g)	–[a]	36.63	–[a]
Seed weight F3	220	18.96 (g)	2.62***	49.77	0.45
Oil content F2	220	40.65 (%)	–[a]	13.92	–[a]
Oil content F3	216	40.88 (%)	7.91***	11.56	0.78

***$P > 0.0001$ **$P > 0.001$
[a] – = data not available

Table 2 Pearson correlation coefficients among developmental and agronomic traits in XRQ × PSC8 F2 individuals and F3 families. $r = 0.132$ at $P = 0.05$. fd: flowering date, md: maturity date, dtm: delay to maturity, oc: oil content, sw: seed weight, ph: plant height

Trait	fd97	fd99	md97	md99	dtm97	dtm99	ocF2	ocF3	swF2	swF3	phF2	phF3
Flowering date 1997												
Flowering date 1999	0.797											
Maturity date 1997	0.165	0.233										
Maturity date 1999	0.399	0.517	0.419									
Delay to maturity 1997	–0.239	ns[a]	0.886	0.265								
Delay to maturity 1999	–0.226	–0.290	0.292	0.624	0.387							
Oil content F2	ns	ns	ns	ns	ns	ns						
Oil content F3	ns	ns	ns	ns	ns	ns	0.616					
Seed weight F2	ns	ns	ns	ns	ns	ns	ns	–0.234				
Seed weight F3	–0.147	ns	ns	ns	ns	ns	ns	–0.170	0.332			
Plant height F2	0.633	0.637	0.169	0.387	ns	ns	ns	ns	ns	ns		
Plant height F3	0.612	0.571	0.248	ns	ns	ns	ns	ns	ns	ns	0.597	
% Lodging	ns	ns	ns	0.326	ns	–0.194	–0.148	–0.257	ns	ns	ns	ns

[a] ns: non-significant

covered by 290 markers and based on 220 F2 individuals was 2,318 cM in length. This map is presented in Fig. 3, with 17 linkage groups (1 to 17) identified using concordance of RFLP loci on the CARTISOL map and two anonymous linkage groups (18 and 19). Most of the significant QTLs were located on the same linkage groups for the two years of observations. They are presented in Tables 3 and 4.

Flowering date

Three significant genomic regions on linkage groups 1, 4 and 5 were associated with flowering date for both 1997 and 1999, each explaining from 14 to 25% of the phenotypic variance. Another putative region was identified on group 11 for both 1997 and 1999. Three other QTLs specific to the year were mapped on groups 6 and 18 (1999) and 7 (1997), and each explained about 8–11% of the phenotypic variation.

Maturity date

Two genomic regions were identified for this trait in both years on linkage groups 8 and 11, accounting for 7 to 35% of the phenotypic variation. Two additional QTLs were identified, on linkage group 12 for 1997, and on group 17 for 1999.

Delay from flowering to maturity

One region on group 11 was found for both 1997 and 1999. Two QTLs were specific to 1997 (on groups 10 and 12) and two others for 1999 (groups 8 and 17).

Fig. 3 Genetic linkage map of the sunflower genome based on an intraspecific F2 population of a cross between inbred lines XRQ and PSC8 lines at a LOD score of 4. Genetic distances are in Haldane cM. On the left of each linkage group, QTLs detected for developmental and agronomic traits are indicated in *italic type*. *Ellipses and rectangles* indicate respectively the approximate position of some QTLs detected for resistance to *S. sclerotiorum* and *D. helianthi* (Bert et al. 2002)

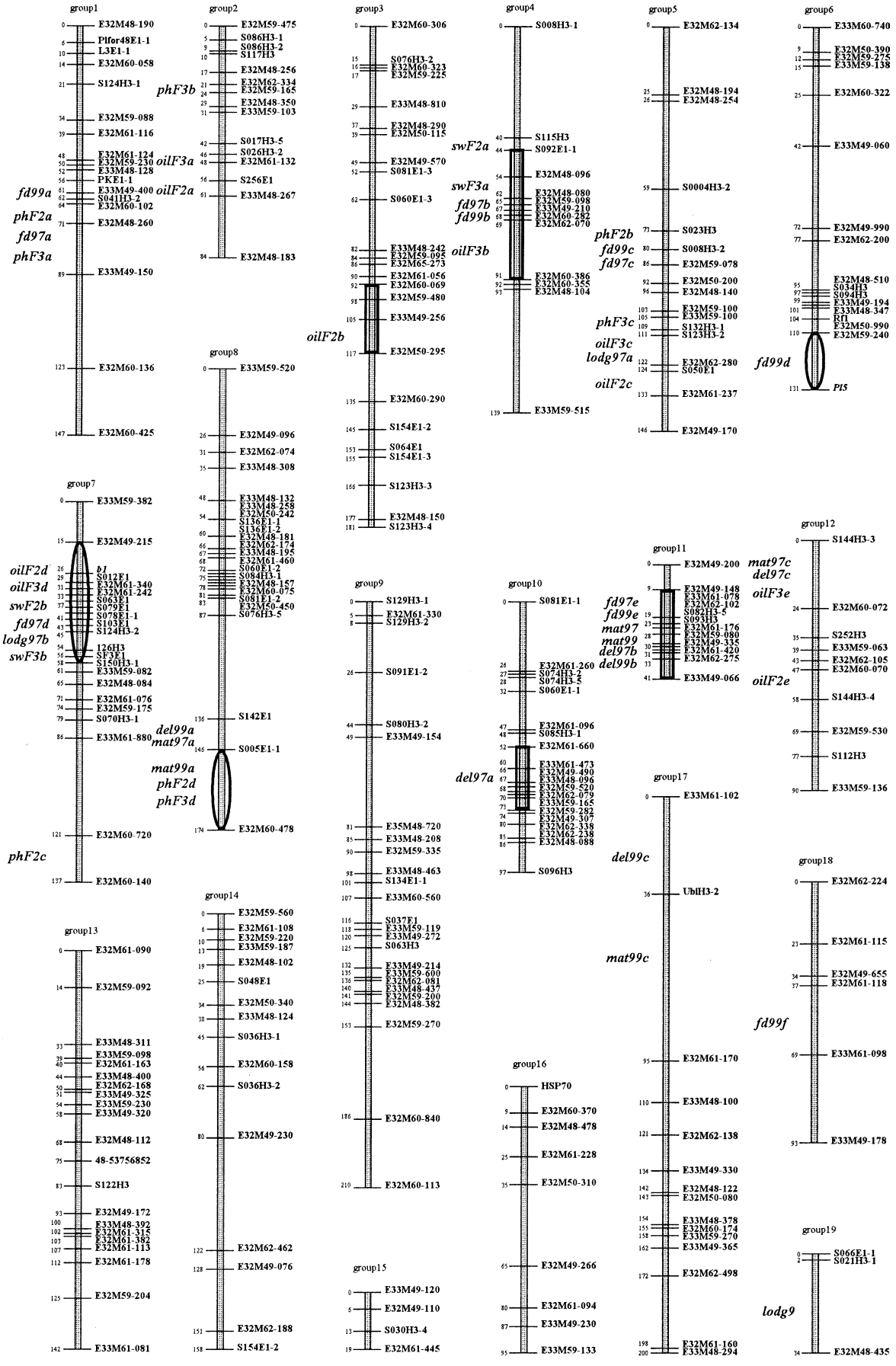
group1
E32M48-190
Plfor48E1-1
L3E1-1
E32M60-058
S124H3-1
E32M59-088
E32M61-116
E32M61-124
E32M59-230
E33M48-128
PKE1-1
E33M49-400
S041H3-2
E32M60-102
E32M48-260
E33M49-150
E32M60-136
E32M60-425
fd99a
phF2a
fd97a
phF3a
group2
E32M59-475
S086H3-1
S086H3-2
S117H3
E32M48-256
E32M62-334
E32M59-165
E32M48-350
E33M59-103
S017H3-5
S026H3-2
E32M61-132
S256E1
E33M48-267
E32M48-183
phF3b
oilF3a
oilF2a
group3
E32M60-306
S076H3-2
E32M60-323
E32M59-225
E33M48-810
E32M48-290
E32M50-115
E32M49-570
S081E1-3
S060E1-3
E33M48-242
E32M59-095
E32M65-273
E32M61-056
E32M60-069
E32M59-480
E33M49-256
E32M50-295
E32M60-290
S154E1-2
S064E1
S154E1-3
S123H3-3
E32M48-150
S123H3-4
oilF2b
group4
S008H3-1
S115H3
S092E1-1
E32M48-096
E32M48-080
E32M59-098
E33M49-210
E32M60-282
E32M62-070
E32M60-386
E32M60-355
E32M48-104
E33M59-515
swF2a
swF3a
fd97b
fd99b
oilF3b
group5
E32M62-134
E32M48-194
E32M48-254
S0004H3-2
S023H3
S008H3-2
E32M59-078
E32M50-200
E32M48-140
E32M59-100
E33M59-100
S132H3-1
S123H3-2
E32M62-280
S050E1
E32M61-237
E32M49-170
phF2b
fd99c
fd97c
phF3c
oilF3c
lodg97a
oilF2c
group6
E33M60-740
E32M50-390
E32M59-275
E33M59-138
E32M60-322
E33M49-060
E32M49-990
E32M62-200
E32M48-510
S034H3
S094H3
E33M49-194
E33M48-347
Rf1
E32M50-990
E32M59-240
PI5
fd99d
group7
E33M59-382
E32M49-215
bl
S012E1
E32M61-340
E32M61-242
S063E1
S079E1
S078E1-1
S103E1
S124H3-2
126H3
SF3E1
S150H3-1
E33M59-082
E32M48-084
E32M61-076
E32M59-175
S070H3-1
E33M61-880
E32M60-720
E32M60-140
oilF2d
oilF3d
swF2b
fd97d
lodg97b
swF3b
phF2c
group8
E33M59-520
E32M49-096
E32M62-074
E33M48-308
E33M48-132
E33M48-258
E32M50-242
S136E1-1
S136E1-2
E32M48-181
E32M62-174
E33M48-195
E32M61-460
S060E1-2
S084H3-1
E32M48-157
E32M60-075
S081E1-2
E32M50-450
S076H3-5
S142E1
S005E1-1
E32M60-478
del99a
mat97a
mat99a
phF2d
phF3d
group9
S129H3-1
E32M61-330
S129H3-2
S091E1-2
S080H3-2
E33M49-154
E35M48-720
E33M48-208
E32M59-335
E33M48-463
S134E1-1
E33M60-560
S037E1
E33M59-119
E33M49-272
S063H3
E33M49-214
E33M59-600
E32M62-081
E33M48-437
E32M59-200
E32M48-382
E32M59-270
E32M60-840
E32M60-113
group10
S081E1-1
E32M61-260
S074H3-2
S074H3-5
S060E1-1
E32M61-096
S085H3-1
E32M61-660
E33M61-473
E32M49-490
E33M48-096
E32M59-520
E32M62-079
E33M59-165
E32M59-282
E32M49-307
E32M62-338
E32M62-238
E32M48-088
S096H3
del97a
group11
E32M49-200
E32M49-148
E33M61-078
E32M62-102
S082H3-5
S093H3
E32M61-176
E32M59-080
E32M49-335
E32M61-420
E32M62-275
E33M49-066
fd97e
fd99e
mat97
mat99
del97b
del99b
group12
S144H3-3
E32M60-072
S252H3
E33M59-063
E32M62-105
E32M60-070
S144H3-4
E32M59-530
S112H3
E33M59-136
mat97c
del97c
oilF3e
oilF2e
group13
E32M61-090
E32M59-092
E33M48-311
E33M59-098
E32M61-163
E33M48-400
E32M62-168
E33M49-325
E33M59-230
E33M49-320
E32M48-112
48-53756852
S122H3
E32M49-172
E33M48-392
E32M61-315
E32M61-382
E32M61-113
E32M61-178
E32M59-204
E33M61-081
group14
E32M59-560
E32M61-108
E32M59-220
E33M59-187
E32M48-102
S048E1
E32M50-340
E33M48-124
S036H3-1
E32M60-158
S036H3-2
E32M49-230
E32M62-462
E32M49-076
E32M62-188
S154E1-2
group15
E33M49-120
E32M49-110
S030H3-4
E32M61-445
group16
HSP70
E32M60-370
E32M48-478
E32M61-228
E32M50-310
E32M49-266
E32M61-094
E33M49-230
E33M59-133
group17
E33M61-102
UblH3-2
E32M61-170
E33M48-100
E32M62-138
E33M49-330
E32M48-122
E32M50-080
E33M48-378
E32M60-174
E33M59-270
E33M49-365
E32M62-498
E32M61-160
E33M48-294
del99c
mat99c
group18
E32M62-224
E32M61-115
E32M49-655
E32M61-118
E33M61-098
E33M49-178
fd99f
group19
S066E1-1
S021H3-1
E32M48-435
lodg9

Table 3 Characteristics of QTL affecting developmental traits in a XRQ × PSC8 F3 population. * = putative QTL with a 2 < LOD < 3. R^2 = phenotypic variation explained by each QTL for non-transformed data. Direction = the parent improving agronomic trait

Trait	QTL	Marker interval	Group	LOD	R^2 (%)	Direction
Flowering date 1997	*fd97a*	E32M48-260_E33M49-150	1	6.99	20.6	PSC8
	fd97b	E32M59-098_E33M49-210	4	4.11	21.1	PSC8
	fd97c	S008H3-2_E32M59-078	5	6.53	16.9	XRQ
	fd97d	S103E1_S124H3-2	7	3.46	8.2	PSC8
	*fd97e**	E32M49-148_E33M61-078	11	2.29	5.5	XRQ
			R^2 total (%)		64.1	
Flowering date 1999	*fd99a*	PKE1-1_E33M49-400	1	4.98	13.7	PSC8
	fd99b	E32M59-098_E33M49-210	4	4.52	25.0	PSC8
	fd99c	S023H3_S008H3-2	5	5.14	16.3	XRQ
	fd99d	E32M59-240_Pl5	6	3.17	10.8	XRQ
	*fd99e**	E32M49-148_E33M61-078	11	2.13	7.1	XRQ
	fd99f	E32M61-118_E33M61-098	18	3.10	32.9	PSC8
			R^2 total (%)		74.9	
Maturity date 1997	*mat97a**	S142E1_S005E1-1	8	2.82	6.8	XRQ
	mat97b	S093H3_E32M61-176	11	6.01	12.9	XRQ
	mat97c	S144H3-3_E32M60-072	12	3.13	19.7	XRQ
			R^2 total (%)		23.6	
Maturity date 1999	*mat99a*	S005E1-1_E32M60-478	8	11.2	35.5	XRQ
	mat99b	E32M59-080_E32M49-335	11	4.79	12.7	XRQ
	mat99c	*Ubi*H3-2_E32M61-170	17	4.79	43.1	PSC8
			R^2 total (%)		58.3	
Delay to maturity 1997	*del97a*	E33M61-473_E32M49-490	10	3.35	9.9	PSC8
	del97b	S093H3_E32M61-176	11	3.50	7.6	PSC8
	del97c	S144H3-3_E32M60-072	12	3.10	18.6	PSC8
			R^2 total (%)		26.6	
Delay to maturity 1999	*del99a*	S142E1_S005E1-1	8	8.97	23.2	XRQ
	*del99b**	E32M61-176_E32M59-080	11	2.11	5.8	XRQ
	del99c	E33M61-102_UbiH3-2	17	5.11	24.4	PSC8
			R^2 total (%)		36.0	

Plant height

Three significant QTLs were found for both F2 and F3, on linkage groups 1, 5 and 8. An additional QTL was also identified on group 7 for F2 plants.

Lodging

Three QTL were identified for lodging, on linkage groups 5, 7 and 19. They each explained only 7 to 9% of the phenotypic variation.

Seed weight

Both F2 plants and F3 families showed QTL on two identical genomic regions, on linkage groups 4 and 7.

Oil content

Four QTL were identified in both F2 plants and F3 families, on groups 2, 5, 7 and 12. Two others were specific to F2 plants (group 3) and F3 families (group 4).

Discussion

A complex trait such as yield is regulated by a number of elementary factors and must be dissected into a number of component traits in order to dissect the underlying Mendelian factors. Comparing phenotypic correlations and their QTL locations should make it possible to improve understanding of the relationships between these components.

The present results show a slight negative correlation between the number of days from flowering to maturity and flowering date, which may be considered as a measure of the length of the pre-flowering stage, since all F3 families were sown on the same day. Thus, it appears that the two periods of sunflower development are not independent. This may be truly the case, but it should be noted that irrigation was carried out if it did not rain, such that the plants received at least 20 mm of water per week from flowering until mid September. This may have retarded the complete maturity of early flowering genotypes so that they appeared to have a long period of maturation. Evidence for the quasi-independence of the lengths of the two periods comes from their different QTL, with only one, on linkage group 11, with a small effect, common to the three traits analysed. Since maturity date and the number of days from flowering to maturity were only observed on plants with good *S. sclerotiorum* resistance, no QTL for these characters would be expected in the regions controlling the percent-

Table 4 Characteristics of QTL affecting agronomic traits in a XRQ × PSC8 F3 population. * = putative QTL with a 2 < LOD < 3. R^2 = phenotypic variation explained by each QTL for non-transformed data. Direction = the parent improving the trait

Trait	QTL	Marker interval	Group	LOD	R^2 (%)	Direction
Seed weight F2 1996	*swF2a**	S115H3_S092E1-1	4	2.10	6.4	PSC8
	swF2b	S063E1_S079E1	7	7.62	16.0	PSC8
			R^2 total (%)		16.0	
Seed weight F3 1997	*swF3a*	E32M48-080_E32M59-098	4	7.1	20.6	PSC8
	swF3b	SF3E1_S150H3-1	7	4.68	9.9	XRQ
			R^2 total (%)		25.2	
Oil content F2 1996	*oilF2a**	S256E1_E33M48-267	2	2.23	7.9	PSC8
	oilF2b	E33M49-256_E32M50-295	3	2.14	13.9	PSC8
	*oilF2c**	S050E1_E32M61-237	5	2.42	6.1	XRQ
	oilF2d	b1_S012E1	7	25.59	43.5	XRQ
	*oilF2e**	E32M60-070_S144H3-4	12	2.24	10.3	XRQ
			R^2 total (%)		68.1	
Oil content F3 1997	*oilF3a*	S026H3-2_E32M61-132	2	5.24	11.7	PSC8
	oilF3b	E32M62-070_E32M60-386	4	3.20	30.1	XRQ
	oilF3c	S123H3-2_E32M62-280	5	3.25	7.9	XRQ
	oilF3d	b1_S012E1	7	27.88	47.6	XRQ
	*oilF3e**	S144H3-3_E32M60-072	12	2.37	17.6	XRQ
			R^2 total (%)		70.1	
Plant height F2 1996	*phF2a**	E32M60-102_E32M48-260	1	2.66	7.5	PSC8
	phF2b	S023H3_S008H3-2	5	5.71	15.5	XRQ
	phF2c	E32M60-720_E32M60-140	7	5.56	48.5	XRQ
	phF2d	S005E1-1_E32M60-478	8	4.92	39.3	XRQ
			R^2 total (%)		67.5	
Plant height F3 1997	*phF3a*	E32M48-260_E33M49-150	1	4.45	12.4	PSC8
	phF3b	E32M62-334_E32M59-165	2	3.27	8.3	XRQ
	phF3c	E33M59-100_S132H3-1	5	3.53	8.6	XRQ
	phF3d	S005E1-1_E32M60-478	8	3.53	17.9	XRQ
			R^2 total (%)		36.4	
Plant lodging 1997	*lodg97a*	S122H3-2_E32M62-280	5	3.18	7.0	PSC8
	lodg97b	S103E1_S132H3-1	7	4.21	9.1	PSC8
	lodg97c	S021H3-1_E32M48-435	19	3.24	6.9	PSC8
			R^2 total (%)		20.6	

age head rot attack (5, 6, 7 and 13 in 1997, 3, 6, 10 and 18 in 1999, Bert et al. 2002).

The QTL for flowering date on linkage groups 1 and 5 are probably the same as those for height, since confidence intervals of the markers are the same. Although there was no phenotypic correlation, it may be noted that the other main QTL for height is situated in the same region as one for the maturity date on linkage group 8. It is possible that a tall genotype has a large mass of vegetation which takes longer to dry than a smaller genotype.

Although flowering date was the character with least variation (small CV and high *F*-genotype values) and the QTL were the same in the 2 years (with the exceptions of those on groups 7 in 1997 and 6 in 1999), they are not on the same linkage groups as those reported by Mestries (1996) and Mestries et al. (1998) for the cross between sunflower inbred lines GH and PAC2 (groups 6, 7, 10 and 12). The QTL reported by these authors accounted for 20 to 40% of the phenotypic variation, whereas for the present cross the QTL accounted for 64% of the variation in 1997 and 75% in 1999. This was in the same range value of 73% for the variation explained by the five QTL identified by Leon et al. (2001). Leon et al. (2001) dissected the flowering date into growing degree days and photoperiod components in multiple environments. They reported two QTL for photoperiod that co-located with two of the six QTL associated with growing-degree days. Since flowering date is an important agronomic trait for adaptation, several QTL analyses have been carried out on crop species and particularly in rice. Based on their chromosomal positions, it has been suggested that some QTL for photoperiod sensitivity and basic vegetative growth (the main determinants of this trait) are the same loci as major genes (Yano et al. 1997; Lin et al. 2000; Yamamoto et al. 2000; Zhou et al. 2001).

Lodging was not correlated with height in this cross and the only common QTL were those on group 5 for the F3 in 1997. Since susceptibility to lodging can be catastrophic for yield, but occurs irregularly at normal cropping densities, it would be extremely useful to find markers that would help to eliminate the most susceptible plants in breeding. In the present study, in 1997 90% of the F3 families showed some lodging, but only 30% had more than 10% of the plants lodged, so that genotype effects were small.

The seed-weight character measured is not "yield" in the agricultural sense of seed production per hectare of hybrid genotypes. It was seed weight per capitulum on selfed plants, with little involvement of heterosis. The QTL on the same linkage group 7 as the branching gene *b1*, also reported by Mestries et al. (1998) for GH × PAC2

linked to the same markers, is almost certainly linked to capitulum size. The other QTL for seed weight, on linkage group 4, is close to the one for flowering date. However, the only significant correlation between F3 seed weight and 1997 flowering was negative, so the relation may simply be due to linkage, the parent XRQ, which has greater seed production, being slightly earlier flowering than PSC8. The other QTL reported by Mestries et al. (1998) were not common to those found in the present cross.

For oil content, the branching gene again appears important. However, the present results contrast with those from Mestries et al. (1998), who suggested that the effect of this gene on oil content could be pleiotropic, since branched plants have smaller capitula with smaller seeds often containing more oil. In the cross XRQ × PSC8, the effects are in the opposite direction, the unbranched allele from XRQ being linked to higher oil contents. The relation could therefore be due to genetic linkage. The other QTL found by Mestries et al. (1998) was on a small supplementary linkage group, so it is not possible to compare this with the other QTL in the present cross. Oil content might have been expected to be related to the number of days between flowering and maturity, when seed-filling occurs, but no correlation was found here. It may be that there is a link with the strict period of seed filling, but not of capitulum drying.

The QTL for developmental and agronomic traits presented here were compared with those for resistance to *S. sclerotiorum* and *D. helianthi* reported by Bert et al. (2002) and presented in Fig. 3. Co-localisations were observed on several linkage groups. A QTL for the percentage of plants attacked by *S. sclerotiorum* in F3 families was detected on 1999 on linkage group 6 close to one for flowering date, with the allele of the parental line XRQ that reduced the number of days to flowering being linked with increased resistance to *S. sclerotiorum*. However, this may also have been due to environmental effects, giving more favourable conditions for *Sclerotinia* attack than for later flowering dates. Another co-localisation was observed on linkage group 8 with QTL for development (plant height, maturity dates, flowering to maturation period) and for the percentage of plants attacked by *S. sclerotiorum*, and for *S. sclerotiorum* mycelium extension on the capitulum. Linkage group 7 carried, in the same confidence interval, QTL for resistance to *S. sclerotiorum* (latency index and percentage of plants attacked) and QTL for seed weight and oil content, as already reported by Mestries et al. (1998). Studies of unbranched plants only are in progress only to determine the effect of the branching gene *b1* on both *S. sclerotiorum* resistance and agronomic characters.

Resistance to *D. helianthi* was also correlated with components of plant morphology. Linkage groups 3, 4, 10 and 11 carried QTL for resistance to *D. helianthi* measured by mycelium extension on leaves and semi-natural attacks in the same regions, where QTL for oil content, seed weight, plant height and flowering and maturity dates were detected. Particular associations between traits such as plant height and flowering date with resistance to pathogens have previously been reported in the literature. Dirlewanger et al. (1994) observed a negative correlation between the number of nodes measured and *Ascochyta* blight resistance and a possible common QTL. Li et al. (1995a) observed a negative correlation between plant height and sheath blight response-ratings in rice. They also co-located three QTL for resistance with loci associated with heading date. In that case, it was expected that increased resistance would be associated with earlier flowering because there would be less time for disease development, so that there may have been a pleiotropic effect on heading date and plant height genes (Li et al. 1995b). However, resistance to *D. helianthi* in sunflowers was measured by lesion length on young fully grown leaves, so there is no particular physiological explanation for the correlation which would appear to be due to genetic linkage.

These co-locations will need further studies as they may affect the efficiency of breeding programmes. The small seed-weight of branched genotypes linked to *Sclerotinia* resistance should not be important if it is a pleiotropic effect of the branching gene, since hybrid varieties are unbranched. In contrast, it will be necessary to determine, by fine mapping, whether it is possible to break the unfavourable linkages between lateness and *Sclerotinia* resistance on linkage group 6, and lateness and Phomopsis resistance on group 4, if these QTL are to be used in breeding.

In conclusion, this study has demonstrated several QTL for developmental and agronomic characters that are stable over 2 different growing seasons, but which show few co-locations with those reported by other authors on other sunflower genotypes, when comparisons are possible. For traits such as flowering dates, which are frequently observed at the same time as other characters, it will be important to be able to compare maps to determine on a large number of crosses whether QTL are common or genotype specific. Future work should probably include observations on the appearance of the reproductive system (star bud stage) and of physiological maturity, when seed weight and oil content are at a maximum, to divide up vegetative stages and determine QTL for shorter periods. QTL for oil content and plant height on inbred lines should be usable to predict values of hybrid genotypes also, but studies of the relation and common QTL (if any) between seed production in inbreds and yield of hybrids are necessary. In addition, studies need to be made of seed number and 1,000 seed weight and other characters to advance in the understanding of the genetic determination of yield.

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