Genetic Analysis of Seed-Oil Concentration across Generations and Environments in Sunflower

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ABSTRACT

Seed-oil concentration is a major consideration in sunflower (*Helianthus annuus* L.) breeding because it is an important component of oil yield. Seed-oil concentration is a complex trait determined by the genotype and the environmental conditions. The objectives of this study were (i) to locate quantitative trait loci (QTL) for seed-oil concentration across generations and environments, (ii) to compare QTL detected among individual F_2 plants and their F_3–generation progeny, and (iii) to assess the genetic relationship between seed-oil concentration and days to flowering in an elite sunflower population. Two hundred thirty-five F_2 plants and F_3 lines of a single-cross population of two divergent inbred lines were evaluated in four environments. Detection of QTL was facilitated with a genetic map of 205 loci defined by restriction fragment length polymorphism (RFLP) and composite interval mapping. Eight QTL on seven linkage groups accounted for 88% of the genetic variation for seed-oil concentration across environments. Gene action was additive for four QTL and dominant or overdominant at the others. In all environments, the QTL on linkage group G_{20cM} had the most influence on seed-oil concentration. Four of the eight QTL were detected in two or more environments and the parental effects were the same across generations and environments. The phenotypic correlation between seed-oil concentration and days to flower (DTF) ranged from −0.05 to –0.29. QTL on two linkage groups (B and L) affected seed-oil concentration and DTF. The highest LOD score for these two QTL associated with seed-oil concentration was observed at the environment with the highest rate of decline of temperature and radiation during the grain-filling period. Additive effects for higher values of DTF and lower values of seed-oil concentration in linkage groups B and L were derived from the same parent.

Seed-oil concentration (achene-oil concentration) is a major consideration in sunflower breeding because it is one of the two components of oil yield. The genetic basis of seed-oil concentration has been described to a limited degree. Seed-oil concentration has relatively high broad sense heritability (0.6–0.7) and high narrow sense heritability (0.5–0.6) on a single plant basis (Martinez et al., 1979; Fick, 1975). Some research has been done to study gene action involved in the expression of the traits. Highly significant additive, dominant, and epistatic effects were determined for seed-oil concentration in two F_2 populations and their reciprocal backcrosses (Gupta and Khanna, 1982). Estimates of general (GCA) and specific combining ability (SCA) (Bedov, 1985; Areco et al., 1985) indicated additive effects were more important for seed-oil concentration. Significant additive effects were also reported for seed oil and seed percentages, although significant dominant effects for seed percentage were detected in irrigated conditions (Refoyo et al., 1988). Additive gene action has also been reported for hull percentage (Vranceanu and Stoenescu, 1969. In general, these results indicate that additive effects predominantly influence oil concentration in the whole seed and its components. However, some non-additive effects also seem to affect these traits. The high heritability and predominately additive gene action of this trait facilitate selection in early generations of inbreeding and cultivar development (Miller and Fick, 1997). Improvement of seed-oil concentration in hybrid progeny has been achieved by increasing seed-oil concentration in the inbred progeny (Miller et al., 1982). This improvement has been accomplished by a reduction of the hull percentage (or increase of seed percentage), and to a lesser extent, by an increase of the seed oil concentration (Gundaev, 1971).

The use of molecular markers provided additional information about the genetic basis of seed-oil concentration. Leon et al. (1995) used a genetic map of 201 RFLP loci and oil data collected from individual plants in the F_2 generation to locate six QTL associated with 57% of the genetic variation. Two of the QTL were related to seed oil concentration (linkage groups C and I), two to seed percentage (linkage groups G and J), and the other two to both traits (linkage groups B and N). Additive gene action was predominant for seed-oil concentration and its components. In a later study, Leon et al. (1996) reported a dominant factor (Hyp) determining the presence of white pigments in the hypodermis that was located in linkage group 'G'. Seeds with white hypodermis had lower oil concentration than those with unpigmented hypodermis. The Hyp factor was located in the same map interval as one QTL with major effects on seed-oil concentration. Mestries et al. (1998) also mapped QTL affecting seed oil content and found two to three QTL for this trait, depending on the environment and generation. These QTLs were associated with 19 to 54% of the phenotypic variance across generations.

Seed-oil concentration of sunflower is sensitive to environmental conditions during the grain-filling period (Connor and Hall, 1997). For example, reductions in oil yield and seed-oil concentration may occur when low temperatures and radiation prevail during the seed-filling period (Andrade and Ferreiro, 1996; Connor and Hall, 1997; de la Vega et al., 2001; de la Vega and Hall, 2002). At high latitudes, such conditions are frequently encountered by late-flowering genotypes and could be a source of genotype × environment interaction for

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seed-oil concentration. Therefore, estimates of QTL positions and effects for seed-oil concentration in sunflower may be enhanced through analyses of phenotypic data collected from replicated progeny evaluated in suitable target environments, and through the coincident evaluation of traits that could alter our assessment of allelic effects on the primary trait (Cowen, 1988). To our knowledge, such investigations have not been reported for seed-oil concentration of sunflower. The objectives of this research were (i) to map genetically and assess QTL for seed-oil concentration by means of replicated progeny evaluated in several environments located within the target environment, (ii) to compare QTL detected among individual F_2 plants and their F_3-generation progeny, and (iii) to assess the genetic linkage between QTL for seed-oil concentration and days to flowering (DTF).

MATERIALS AND METHODS

Germplasm and Trait Characterization

The nonrestorer (B) lines ZENB8 (female) and HA89 (male) were crossed to produce the progeny used in this study. The F_2 seed was produced by self-pollinating a single F_1 plant. ZENB8, a proprietary inbred line, has a seed-oil concentration of approximately 330 g kg^{-1} and reaches anthesis (flowers) approximately 75 d after planting at the photoperiod (15–16 h) and temperatures typical of the growing seasons at locations used in our study (Fargo, ND, in the USA and Venado Tuerto, Daireaux, and Balcarce in Argentina). HA89, an inbred line released by the USDA, has a seed-oil concentration of 490 g kg^{-1} and flowers approximately 65 d after planting under the same conditions at those locations.

The F_2-generation seed was hand planted in rows at Fargo on 14 May 1992, at a rate of two seeds per hill. Seedlings were removed to leave one plant per hill. Hills were 0.30 m apart within a row. The rows were 6 m long and 0.75 m apart. Five rows of each parent and the F_1 were planted at different periods (−10, −5, 0, +5, +12 d relative to the planting date of the F_2 seed) to estimate the within-row error variance (Leon et al., 1995). Before anthesis, individual heads of 235 F_2 plants were covered with pollination bags to ensure self-pollination and production of F_3-generation seed. Single-row plots of the 235 F_3 families were hand planted at Daireaux, Venado Tuerto, and Balcarce on 17, 18, and 20 Nov. 1992, respectively. Fifteen plots of each parent and the F_1 generation were included at each Argentine environment to provide an estimate of the error variance within and across locations. Plots were 3 m long and contained 10 hills. The space between plots was 0.70 m. Three seeds per hill were planted and seedlings were removed to leave one plant per hill. The families, parents, and F_1 genotype were randomly assigned to plots at each location.

Seed-oil concentration (defined as oil weight/seed weight) was measured with a Nuclear Magnetic Resonance (NMR) analyzer. In the sunflower research community, the word *seed* is used synonymously with *achene* and will be used so in this paper. Measurements were made on a dried sample of 10 g of F_3 seed of each F_2 plant as described in Leon et al. (1995). In the F_3 families, oil data were collected in the same way but from a balanced bulk of F_4 seed harvested from the F_3 plants in each row. The balanced bulk was created by taking equal volumes of seed from each plant in the plot.

Sunflower growth stages are defined according to Schneiter and Miller (1981). Days from emergence (VE) to flowering, or anthesis (R5.5; when 50% of the flowers of a capitula are open) were recorded for F_2 plants and their corresponding F_3 families at each environment, as described in Leon et al. (2000). The day of flowering (DTF) of an F_3 family was considered as the day on which 50% of the plants reached the R5.5 stage. The DTF was used to quantify a portion of the life cycle of the parents, F_1 and F_2 generations, and F_3 families in these environments. The mean daily temperature during the period between flowering and physiological maturity was obtained from the nearest meteorological station. The period began when the first progeny reached anthesis and ended 45 d after the date of the anthesis of the latest progeny.

The genetic map and segregation data used have been described previously (Berry et al., 1995; Leon et al., 1995, 1996). The 205 RFLP loci covered 1380 centimorgans (cM) and were arranged in 17 linkage groups, the haploid number of chromosomes in this species. The average interval size was 5.9 cM. The genetic map was constructed using MAPMAKER version 3.0 (Lander et al., 1987). Genotypic classes at 23 loci deviated significantly from the expected ratios. Those loci exhibited a deficiency in the ZENB8 homozygous class. The majority of the loci with deviant ratios (18/23) were located to four regions, representing linkage groups G, L, and P (Berry et al., 1995).

Statistical Analysis

Simple-Pearson phenotypic correlations between seed-oil concentration and DTF were calculated for each location and for the average values for all locations (i.e., the mean environment). Broad-sense heritability was estimated according to Allard (1966) for individual plants in the F_2 generation (Leon et al., 1995). The within-row variance in the F_2 generation was estimated by pooling within-row variances of the parents and F_1 genotype. The error variance among rows was estimated in the F_2 generation. Genetic variation was then estimated by subtracting the within- and among-row variances from the phenotypic variance (Leon et al., 1995). For the F_3 families, broad-sense heritabilities were estimated using variance components according to Fehr (1987); for the heritability on plot basis (for each location) $h^2 = \frac{\sigma_g^2}{\sigma_e^2 + \sigma_g^2}$ and heritability on entry- mean basis (across locations) $h^2 = \frac{\sigma_g^2}{\sigma_e^2/rt + \sigma_{g\times e}^2/t + \sigma_g^2}$, where t and r are the number of environments and replications within environments, σ_e^2 the experimental error variance, σ_g^2 the genotypic variance, and $\sigma_{g\times e}^2$ the genotype × environment interaction variance. Estimates of σ_e^2 within and across locations were obtained from the parents and F_1, according to Hallauer and Miranda (1988). The significance of the genotype × environment (G×E) interaction was tested according to Hallauer and Miranda (1988) by means of the σ_e^2 estimated from the parents and F_1 genotype across locations (Leon et al., 2000).

Composite interval mapping (CIM) was used for mapping QTL. Phenotypic data consisted of the seed-oil concentration for each F_2 plant or F_3 family evaluated at each location and the average value of each F_3 family across locations (herein, the mean environment). The use of single replicates of each family in multiple environments has been described previously for QTL mapping maize for grain yield (Stuber et al., 1992; Beavis et al., 1994) and plant height (Beavis et al., 1991) and in sunflower for days to flowering (Leon et al., 2000) and photoperiod response (Leon et al., 2001). Computations were facilitated by PLABQTL Version 1.1 (Utz and Melchinger, 1996) as described in detail by Bohn et al. (1996) and Austin and Lee (1998). Initially, an analysis was made with the first statement to check the database for errors and outliers. A

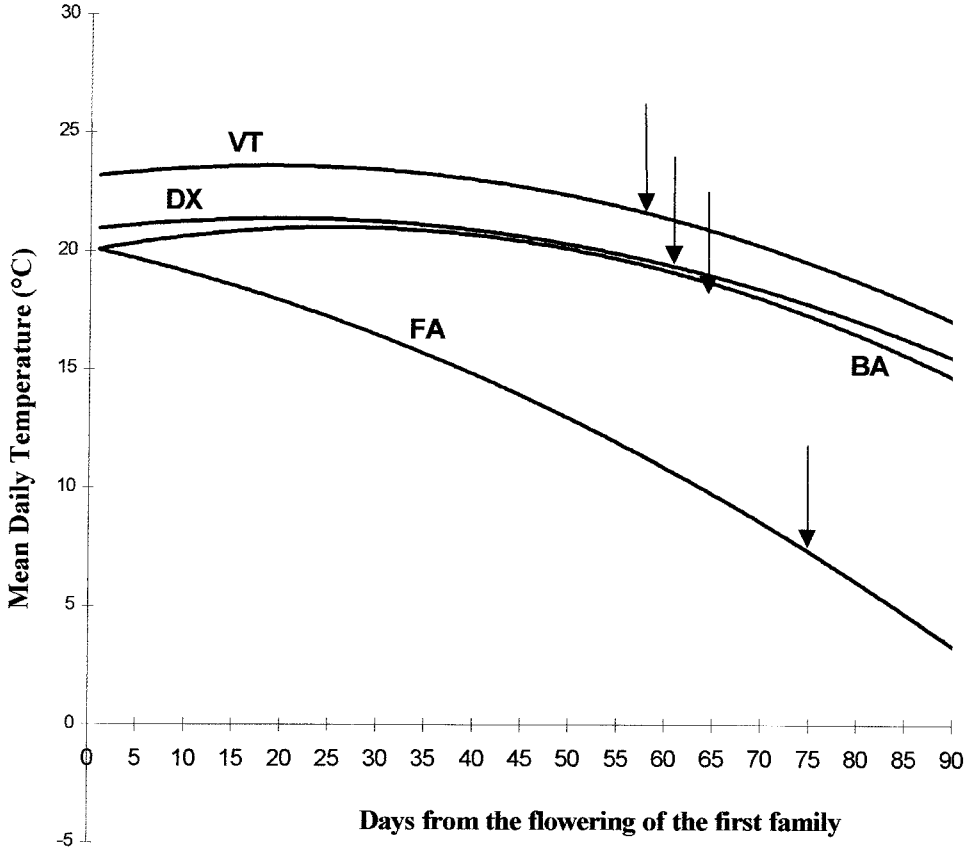

Fig. 1. Mean daily temperature during the phenological period from the flowering of the first sunflower progeny (20 Jan., 24 Jan., 24 Jan. 1993, and 1 Aug. 1992 for Venado Tuerto., Daireaux, Balcarce, and Fargo, respectively). Curves represent results from individual locations Venado Tuerto (VT), Daireaux (DX), Balcarce (BA), Fargo (FA). The arrows represents 45 d after the flowering of the last family (as an estimation of the physiological maturity).

second analysis with the model D and scan statements with a LOD threshold value of 2.5 was conducted to select cofactors. A third analysis was done adding the preselected cofactors in the cov statement and the smodel statement for detection of digenic epistatic interactions between QTL with significant main effects. The coefficient of determination (R^2) from the model for the mean environment was compared with the estimated broad-sense heritability to estimate the amount of genetic variation associated with RFLP loci in multiple regression. Epistatic effects among all pairs of loci were assessed with two-way Analyses of Variance using the program EPISTACY (Holland, 1998). A total of 20 910 pairwise tests were performed and the interlocus interaction variance was partitioned into additive × additive, additive × dominance, dominance × additive, and dominance × dominance interactions. Because of the high number of comparisons, an epistatic interaction was declared when the *F*-test was significant at the $P < 0.0001$ level. Interactions were then added to the multiple regression model in the seq statement of the PLABQTL program to estimate the amount of genetic variation associated with the complete model.

Estimates of the additive (a) and dominant (d) effects were obtained by fitting a model including all QTL as described in Bohn et al. (1996). The d/a (dominant/additive) ratio scale described by Edwards et al. (1987) was used to classify gene action [A = additive or partial dominance ($0 < |d/a| < 0.55$); D = partial dominance or dominance ($0.55 < |d/a| < 1.20$), OD = overdominance ($|d/a| > 1.20$)].

RESULTS AND DISCUSSION

All genotypes reached physiological maturity in each environment. The changes in mean daily temperature were similar among the Argentine environments (Fig. 1). At Fargo, temperatures were lower throughout the period and they decreased at a faster rate (Fig. 1). HA89 had a higher seed-oil concentration than ZENB8 in all environments (Table 1). Some degree of dominant gene

Table 1. Means, variance components, and broad-sense heritabilities for seed-oil concentration for the ZENB8 × HA89 sunflower population.

Environments	Venado Tuerto	Daireaux	Balcarce	Fargo	Mean environment
Oil means			g kg^{-1}		
ZENB8	300 ± 10†	310 ± 10	330 ± 11	330 ± 16	320 ± 8
HA89	430 ± 10	480 ± 10	470 ± 11	520 ± 16	480 ± 8
F_1	410 ± 10	450 ± 10	420 ± 11	460 ± 16	430 ± 8
F_2				440 ± 16	
F_3	380 ± 10	410 ± 10	410 ± 12		
Variance components‡					
σ^2_e	228	206	306	965	572
σ^2_g	334	682	272	866	342
$\sigma^2_{g\times e}$					039
σ^2_{ph}	562	888	578	1831	
H	0.59	0.77	0.47	0.47	0.69

† Mean ± 2 standard errors of mean.
‡ σ^2_e = experimental error variance, σ^2_g = genotypic variance, $\sigma^2_{g\times e}$ = genotype × environment interaction variance, σ^2_{ph} = phenotypic variance, H = Broad-sense heritability.

Table 2. Analysis of Variance for seed-oil concentration for 235 F2 and F3 families of the ZENB8 × HA89 sunflower population evaluated at four environments.

Source of variation	MSE†	F test
Environment (location)	15 230	24.9***
Family (genotype)	1 980	3.2**
Environment × Family	610	1.1
Error ‡	570	

** Significant at the 0.01 probability levels.
*** Significant at the 0.001 probability levels.
† Mean square error.
‡ Variance error was estimated from the parents and F_1 genotype replicated 15 times in each environment.

action was evident because mean oil values of the F_1, F_2, and F_3 generations were closer to the value of HA89 in each environment. Broad-sense heritabilities ranged from 0.47 in Fargo and Balcarce to 0.77 at Daireaux. The heritability estimated on an entry basis in the mean environment was 0.69 (Table 1). These values are similar to those obtained with other populations in other environments for seed-oil concentration (Miller and Fick, 1997).

QTL Mapping

Since the G×E interaction was not significant (Table 2), only the QTL detected in the mean environment are discussed in detail (Table 3). Eight QTL on seven linkage groups affected with seed-oil concentration. Those eight QTL accounted for 59 and 86% of the phenotypic and genotypic variation, respectively. The QTL in linkage groups B, G_{20cM} (interval C0290–C0887), and N has the largest effects, as indicated by their LOD scores and R^2 values. Collectively, they accounted for 70% of the genetic variation. By contrast, the six QTL detected in a previous study for seed-oil concentration accounted for 57% of the genetic variation (Leon et al., 1995). The increase could be attributed to enhanced estimation of trait values with replicated progeny, better sampling of the target environment, and the more refined approach of composite interval mapping.

Alleles for increased seed-oil concentration were all derived from HA89, the parent with the higher values for that trait in each environment. Gene action was additive at four QTL and dominant or overdominant at the other four. The sum of the additive effects for higher values of seed-oil concentration (72 g kg^{-1}) accounted for most of the difference between the values of the parents (160 g kg^{-1}). Most reports have emphasized the importance and prevalence of additive gene action (Bedov, 1985; Miller and Fick, 1997). However, dominant gene action has been detected in studies without DNA markers (Gupta and Khanna, 1982; Refoyo et al., 1988; Russell, 1953). The fact that the mean values of the F_1, F_2, and F_3 generations were closer to HA89 than to ZENB8 in all environments, suggested the presence of dominant gene action. Evidence of additive × dominance digenic epistasis was found between QTL in linkage groups C and M; the interaction accounted for 2% of the total genetic variation. No significant epistatic effects were found among all pair of loci.

Of the eight QTL associated with seed-oil concentration (Table 3), five (linkage groups B, C, G_{20cM}, I, and N) were reported in a previous study based on F_2 plants and a single environment (Leon et al., 1995). In agreement with the previous report, QTL on linkage groups B, G_{20cM}, and N had the greatest effect on seed-oil concentration (Table 3). The QTL in linkage group G_{20cM} was related to seed percentage, whereas those on linkage groups B and N were related to seed oil concentration and seed percentage (Leon et al., 1995). In all environments, the QTL on linkage group G_{20cM} had the most influence on the seed-oil concentration (Table 4). This

Table 4. LOD score for QTL associated with seed-oil concentration at different locations for the HA89 × ZENB8 sunflower population.

Linkage group†	Position	Locations			
		Venado T.	Daireaux	Balcarce	Fargo
B	66	0.9	**4.0‡**	1.9	6.1
C	12	3.1	2.4	0.9	1.1
G	20	3.6	7.3	13.5	7.4
	70	1.8	1.8	4,3	1.0
I	10	1.1	5.1	2.8	0.9
L	62	1.0	0.5	0.9	3.1
M	66	1.8	1.4	2.1	1.4
N	20	2.0	3.3	4.7	2.6

† Letters represents linkage groups.
‡ LOD scores in bold are higher than the threshold value of 2.5.

Table 3. Parameters of QTL for seed-oil concentration detected in the mean environment for the sunflower population HA89 × ZENB8.

Linkage group	Position (cM)†	Left-right‡ mark	LOD	R^2§	a¶	d#	|d/a|††	Gene‡‡ action
B	66	C1735-C0741	5.59	10.4	−10.0	−2.0	0.20	A
C	12	C0838-C1302	3.21	6.1	−7.0	3.4	0.48	A
G	20	C0290-C0887	14.41	24.7	−18.3	−14.6	0.80	D
	70	C1470-C1002	3.47	6.6	−7.2	6.6	0.92	D
I	10	C0649-C1407	4.28	8.1	−7.9	−4.0	0.51	A
L	62	C0628-C0589	2.85	5.5	−5.3	11.4	2.15	OD
M	66	C1004-H2178	2.94	5.6	−7.1	0.2	0.02	A
N	20	C1965-C1562	5.91	11.0	−8.6	−9.8	1.14	D
Total§§				61.0				

† Position of likelihood peak (highest LOD score).
‡ Markers flanking the likelihood peak for a putative QTL.
§ Coefficient of determination: Percentage of phenotypic variance explained by the QTL.
¶ Additive (a) value. Negative sign (−) indicates an increase of the mean value of the trait due to HA89 alleles. A positive sign (+) indicates an increase of the mean value of the trait due to ZENB8 alleles.
# Dominant (d) values. A positive sign means dominance for higher value of the trait. A negative value means dominance for lower value of the trait.
†† Absolute ratio of the average dominant and additive effects at a QTL.
‡‡ A = additive or partial dominance ($0 < |d/a| < 0.55$); D = partial dominance or dominance ($0.55 < |d/a| < 1.20$), OD = overdominance ($|d/a| > 1.20$). Based on the scale of the ratio d/a.
§§ Estimate of total variance (including epistasis) obtained from the simultaneous fit of all QTL detected for seed-oil concentration.

region was also associated with seed hypodermis color (Leon et al., 1996). Such regions are excellent candidates for marker-assisted selection. Four of the eight QTL listed in Table 3 (linkage groups B, G_{20cM}, I, and N) were detected in two or more environments. Whereas the QTL on linkage group *M* was significant in the mean environment but not in individual environments (Table 4). QTL × E interactions were highly significant ($P < 0.01$). Four of the eight QTL for seed oil concentration (linkage groups B, G_{20cM}, L, and N) had significant QTL × E interactions.

Association between Seed-Oil Concentration and Days to Flowering

The phenotypic correlation coefficients between DTF and seed-oil concentration were highly significant ($P < 0.001$) at Fargo (−0.29), and in the mean environment (−0.27). At the other environments, the correlation coefficients were smaller (−0.13 to −0.05) and insignificant except at Venado Tuerto (−0.13; $P < 0.05$). The coincidence between the QTL associated with both traits is consistent with these data. Five QTL in linkage groups A, B, H, I, and L were associated with DTF when these families were evaluated in the same environments (Leon et al., 2000). Those QTL accounted for 73% and 89% of the phenotypic and genotypic variation of DTF in the mean environment. The QTL on linkage groups A and B had the highest LOD scores in each environment and in the mean environment (LOD 38.4 and 10.8, respectively). The two QTL on linkage groups B and L were associated with both seed-oil concentration and DTF. LOD score peaks were at 64 cM for DTF, and 66 cM for seed-oil concentration for QTL in linkage groups B, and 54 cM for DTF, and 62 cM for seed-oil concentration for QTL in linkage group L. There were QTL in linkage group I for seed-oil concentration and for DTF, but they had quite different LOD score peaks (10 and 56 cM, respectively). Consistent with the phenotypic correlations, additive effects for higher values of DTF and lower values of seed-oil concentration in linkage groups B and L were derived from ZENB8. The highest LOD score for these two QTL associated with seed-oil concentration were observed at Fargo (Table 4), the environment with the highest rate of decline in temperature and radiation during the seed-filling period (Fig. 1). This is consistent with the QTL×E interaction reported above.

In areas with short growing seasons, such as Fargo, late flowering genotypes were exposed to poor environmental conditions during seed filling (Fig. 1) and produced less seed-oil than early flowering genotypes. Decreases in temperature and in incident radiation have negative effects on seed growth rate and seed oil concentration (Andrade and Ferreiro, 1996; Connor and Hall, 1997; Hall, 2000; Dosio et al., 2000). Moreover, in areas with short seasons, the probability of sudden interruption of the seed filling period is high. Thus, the environmental conditions during seed filling at Fargo explain the coincidence between the QTL for seed-oil concentration and DTF in linkage groups B and L. These QTL were associated with factors controlling DTF (Leon et al., 2001) and not oil concentration directly. This knowledge helps marker assisted selection programs to choose regions of the genome associated with seed-oil concentration that are not confused by phenology and environment.

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