USE OF RFLP MARKERS FOR GENETIC LINKAGE ANALYSIS OF DAYS TO FLOWERING IN SUNFLOWER (*Helianthus annuus L.*)

Alberto Leon, Advanta Semillas S.A.I.C., Balcarce Research Station, Ruta 226 km 60.3, Casilla de Correo 30, 7620 Balcarce, Argentina
Fax: +54 2266 430002; e-mail: alberto.leon@advantaseeds.com

Fernando Hector Andrade, Mar del Plata University, (7620) Balcarce, Pcia. de Bs. As., Argentina
Fax: +54 2266 421756; e-mail: fandrade@inta.gov.ar

Michael Lee, Department of Agronomy, Iowa State University, Ames, IA 50011-1010, USA.
Fax: +1 515 2943163; e-mail: mlee@iastate.edu

Summary

The number of days from seedling emergence to flowering (DTF) is a major consideration in sunflower breeding because the maximum yield of the crop can only be achieved if the cultivars are phenologically adapted to the production environment. DTF is a complex trait determined by the genotype, environmental conditions and interactions. Identification of genetic factors which affect flowering could create opportunities for improved breeding methods and for more fundamental investigations of this important trait. The objectives of this study were to locate quantitative trait loci (QTL) for DTF in an elite sunflower population evaluated in several environments.

Two hundred thirty-five F_2-generation plants and their F_3 progeny of a single-cross population of two divergent inbred lines were evaluated in four environments. QTL detection was facilitated with a genetic linkage map of 205 loci defined by restriction fragment length polymorphism (RFLP) and composite interval mapping.

Five QTL of five linkage groups accounted for 89% of the genetic variation for DTF. Gene action was additive at four QTL and dominant or overdominant at the other locus. Three QTL were detected in all environments and generations. The parental effects and the relative magnitudes of the genetic effects of those QTL were consistent across generations and environments.

Introduction

The genetic and environmental controls of flowering in sunflower are certainly complex and mostly undefined. Our abilities to investigate and manipulate the phenotype in selection programs could be enhanced with improved resolution of genetic factors that influence flowering and the rate at which genotypes proceed from seedlings to anthesis. Most genetic studies of flowering in sunflower have assessed the phenotype as the number of days from seedling emergence to anthesis (DTF). Polygenic inheritance patterns have been reported in most studies (Stoenescu, 1974; Machacek, 1979) although there is some evidence of genetic factors with major, qualitative effects (Jan, 1986). Additive gene action has the greatest influence on flowering (Miller et. al., 1980; Roath, 1982) but dominant effects have been noted (Jan, 1986). Estimates of broad-sense heritability have ranged from 0.62 to 0.95 (Shabana, 1974; Miller and Fick, 1997).

The genetic components of flowering in sunflower have not been described within the context of contemporary genetic analysis and molecular linkage maps. Thus, there is very limited information on genetic factors affecting flowering or DTF, their locations in the genome, and their linkage and interaction with other genes, traits and environmental cues. Therefore, our understanding of this complex trait would be advanced through genetic mapping of quantitative trait loci (QTL) with DNA markers. Ultimately, such information could facilitate marker-assisted selection in breeding programs and other more fundamental inquiry. The objectives of this study were: 1) to locate QTL for DTF using replicated progeny evaluated in several environments and 2) to compare detection of QTL for DTF using individual plants in the F_2 generation and their F_3 families.

Materials and Methods

Germplasm and Field Design

A cross between non-restorer inbred lines (B lines) ZENB8 (female) and HA89 (male) was made to create the F_2 population and their respective F_3 progenies. The seed of the F_2 generation was created by self-pollinating a single plant of the F_1 generation. ZENB8, a proprietary inbred line, flowers approximately 75 days after planting at photoperiods (15-16 hours) and temperatures of the growing seasons typical of the locations used in this study (Fargo, ND in the USA and Venado Tuerto, Daireaux and Balcarce in Argentina). HA89, released by the USDA, flowers approximately 65 days after planting under the same conditions at those locations.

The F_2 generation was planted at Fargo on May 14, 1992. Two seeds per hill were sown with a hand planter and thinned to one plant per hill. The space between rows was 75 cm and the distance between hills within a row was 30 cm. Five rows of each parent and the F_1 were planted at different periods (-10, -5, 0, +5, +12 days relative to the F_2 planting date) to estimate the within-row error variance (Leon et al., 1995). Before anthesis, individual heads were covered with pollination bags to ensure self-pollination and production of F_3 generation seed. Two hundred thirty-five $F_{2:3}$ families were planted with a hand planter at Daireaux, Venado Tuerto and Balcarce on November 17, 18 and 20, 1992. One row per family was planted at each location. Fifteen replicates of each parent and the F_1 hybrid were included to provide an estimate of the error variance within and across locations. Rows were three meters long and contained ten hills. The space between rows was 70 cm. Three seeds per hill were planted and seedlings were thinned to leave one plant per hill. The families, parents and F_1 were randomly assigned to plots at each location.

The number of days from emergence (VE) to 50% flowering (R5.5) was recorded for individual F_2 plants and their corresponding progenies. The day of flowering of an F_3 progeny was the day when 50% of the plants reached the R5.5 stage. Herein, sunflower

growth stages are defined according to Schneiter et al. (1981).

The RFLP map and segregation data used herein have been described (Berry et al., 1995; Leon et al., 1995; Leon et al., 1996). The 205 RFLP loci covered 1380 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 (see Berry et al., 1995, for further details).

Statistical Analysis

To estimate the total phenotypic variability due to genetic effects, the broad-sense heritability was estimated according to Allard (1966) for individual plants in the F2 generation (Leon et al. 1995). The within-row variance in the F_2 generation was estimated by pooling within-row variances of the parent and F_1 rows. 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). The significance of the genotype by environment (GxE) interaction was tested according to Hallauer and Miranda (1988).

Composite interval mapping (CIM) (Zeng, 1994) was used for mapping QTL. Phenotypic data consisted of trait values for each F_2 plant or F_3 family evaluated at each location and the average value of the F_3 families across locations (herein, the mean environment). The use of single replicates of each family in each environment has been described previously for QTL mapping in maize for grain yield (Stuber et al., 1992; Beavis et al., 1994) and plant height (Beavis et al., 1991). Computations were carried out using PLABQTL Version 1.1 (Utz and Melchinger, 1996).

The initial analysis was made with the 'first' statement to check the database for errors and outliers. A second analysis was conducted to select cofactors using the 'model D' and 'scan' statement with a LOD threshold value of 2.5. The third analysis was done adding the preselected cofactors in the 'cov' statement and the 'smodel' statement for detection of digenic epistatic interactions between QTL that had significant main effects. The coefficient of determination (R^2) of the model for the mean environment (the average of the other environments) was compared with the broad-sense heritability to calculate the amount of genetic variation associated with the RFLP loci.

The QTL and their positions were used in simultaneous multiple regression to estimate the additive (*a*) and dominance (*d*) effects for the F_2 and F_3 generations. 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

ZENB8 flowered later than HA89 at each location by five to twelve days. The average difference was eight days. Directional dominance for earliness was indicated as the F_1 had similar values to HA89 and the means of the F_2 and F_3 generations were between the mid-parent value and HA89 (Table 1). Coefficients of Skewness were positive: 0.61, 0.85, 0.73, 2.14, and 1.17 for Venado, Daireaux, Balcarce, Fargo, and the mean environment, respectively. Broad-sense heritabilities ranged from 0.60 in the F_2 generation to 0.92 for the F_3 families at Venado. The heritability estimated on an entry basis in the mean environment

was 0.82 (Table 1). These values are similar to those obtained with other populations in other environments (Shabana, 1974; Miller and Fick, 1997). The genotype by environment interaction was not significant (Table 2).

Table 1. Means, variance components and broad-sense heritabilities for days to flowering (DTF) for the ZENB8 x HA89 sunflower population.

Environments	Venado T	Daireaux	Balcarce	Fargo	Mean environment
			%		
DTF Means					
ZENB8	69 ± 2.0†	72 ± 1.2	74 ± 1.0	86 ± 2.0	76 ± 0.6
HA89	59 ± 2.0	67 ± 1.2	62 ± 1.0	80 ± 2.0	68 ± 0.6
F_1	60 ± 2.0	66 ± 1.2	64 ± 1.0	78 ± 2.0	67 ± 0.6
F_2				81 ± 2.0	
F_3	63 ± 2.0	68 ± 1.2	67 ± 1.0		
Variance Components‡					
σ^2_e	1.01	4.12	1.57	9.16	4.21
σ^2_g	10.89	10.04	13.80	13.98	5.60
σ^2_{gxe}					0.71
σ^2_{ph}	11.90	14.16	15.37	23.14	
H	0.92	0.71	0.90	0.60	0.82

† Mean ± 2 standard errors of mean.

‡ σ^2_e= experimental error variance, σ^2_g= genotypic variance, σ^2_{gxe}= genotype x environment interaction variance, σ^2_{ph}= phenotypic variance, *H*= Broad sense heritability.

Table 2. Analysis of variance for days to flowering (DTF) for 235 F3 families of the ZENB8 x HA89 sunflower population evaluated at four environments.

SOURCE OF VARIATION	MSE†	F-test
Environment (location)	14503.7	2959.9***
Family (genotype)	49.7	10.1***
Family x environment	4.9	1.2
Error ‡	4.2	

*** Significant at the 0.001 probability level.

† Mean square error.

‡ Variance error was estimated from the parents and F_1 that were replicated 15 times in each environment.

Five QTL were associated with DTF in linkage groups A, B, H, I, and L (Table 3). Those QTL accounted for 73% and 89% of the phenotypic and genotypic variation in the mean environment. QTL in 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) and they accounted for 84% of the genetic variation associated with RFLP loci. Evidence of additive x dominance digenic epistasis was found between QTL in Linkage Groups A and H. That interaction accounted for 2% of the genetic variation attributable to marker loci. The genetic

positions and parental effects of the QTL were very consistent across environments and generations: with exception of QTL of linkage groups H and I, all QTL were detected in every environment (Figure 1).

Table 3. Summary of QTL associated with days to flowering (DTF) in the mean environment for the ZENB8 x HA89 sunflower population.

Linkage Group	Position (cM) †	RFLP loci ‡	LOD	R^2§	a¶	d#	| d/a | ††	Gene‡‡ action
A	38	C0266-C0341	10.8	19.1	-2.22	0.04	0.02	A
B	64	C1735-C0741	38.4	52.9	3.19	-2.69	0.84	D
H	70	C0523-C0515	2.7	5.2	1.04	0.26	0.25	A
I	56	C1891-C0851	4.8	9.1	1.11	-0.58	0.52	A
L	54	C0230-C0628	7.8	14.2	1.54	-0.42	0.27	A
Total§§				72.9				

† Position of likelihood peak (highest LOD score).
‡ RFLP loci flanking the likelihood peak of the QTL according to the linkage map of Berry et al. (1995).
§ 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 values of the trait. A negative value means dominance for lower values of the trait.
†† Absolute ratio of the average dominance 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 reported by Edwards et al. (1987).
§§ Estimate of total variance obtained from the simultaneous fit of all QTL detected for DTF.

The genetic effects for higher values of DTF at four QTL were derived from the late-flowering parent, ZENB8. The only exception was the QTL on linkage group A. With the exception of the dominant effects at the QTL of linkage group B, gene action was additive and in accordance with previous reports (Miller et al., 1980; Roath et al., 1982).

In sunflower, DTF is controlled primarily by the genotype, photoperiod and temperature (Goyne et al., 1977; Marc et al., 1981; Goyne and Hammer, 1982). The lack of G x E interaction could be explained by the similarity of photoperiod among environments. With the exception of the cool conditions towards the end of the growing season at Fargo, temperatures among the locations were also very similar throughout the growing season.

The QTL identified in this study could be used for marker assisted selection for these and related environments. Since it is known that the inbred lines ZENB8 and HA89 are photoperiod sensitive (A. Leon, unpublished), further research is being conducted to genetically resolve that component of flowering. Further understanding of the components of DTF and the interaction with the environment will refine the use of marker assisted selection for modifying DTF for a wider range of environmental conditions and for understanding the influence of DTF on the expression and perception of other traits such as grain quality.

ACKNOWLEDGEMENTS

The authors thank Dr. Florin Stoenescu, Nora Costa, and Abelardo de la Vega for supervising the experiments at their research stations, Dr. Simon Berry for reviewing the manuscript, Dr. Martin Grondona for statistical support and Dr. Ian Bridges and Zeneca Semillas (ADVANTA group) for making this research possible.

REFERENCES

Allard, R.W. 1966. Principles of plant breeding. John Wiley and Sons, New York.

Beavis, W.D., D. Grant, M. Albertsen and R. Fincher. 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor. Appl. Genet. 83:141-145.

Beavis, W.D., O.S. Smith, D. Grant and R. Fincher. 1994. Identification of quantitative trait loci using a small sample of topcrossed and F4 progeny from maize. Crop Sci. 34:882-896.

Berry, S.T., A.J. Leon, C.C. Hanfrey, P. Challis, A. Burkholz, S.R. Barnes, G.K. Rufener, M. Lee, and P.D.S. Caligari. 1995. Molecular markers analysis of *Helianthus annuus* L. 2. Construction of an RFLP map for cultivated sunflower. Theor. Appl. Genet. 91:195-199.

Edwards, M.D., Stuber C.W., Wendel J.F. 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in Maize. I. Numbers, genomic distribution and types of gene action. Genetics 116: 113-125.

Fehr, W. 1987. Principles of Cultivar Development. Vol. 1. McGraw-Hill, New York.

Falconer, D.S. 1981. Introduction to Quantitative Genetics. 2nd ed. Longman, New York, NY.

Goyne, P.J. and G.J. Hammer. 1982. Phenology of sunflower cultivars. II. Controlled environments studies of temperature and photoperiod effects. Aust. J. Agric. Res. 33:251-261

Goyne, P.J., D.R. Woodruff and J.D. Churchett. 1977. Prediction of flowering in sunflowers. Aust. J. Exp. Agric. Anim. Husb. 17:475-481.

Haley, C.S., and S.A. Knott, 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity: 69:315-324.

Hallauer, A.R., and J.B. Miranda. 1988. Quantitative Genetics in Maize Breeding. 2nd ed. Iowa State Univ. Press, Ames, IA.

Jan, C.C. 1986. The inheritance of early maturity and short-stature of *H. annuus* line. *In* Proc. of the 9th Sunflower Research Workshop, Fargo, ND. National Sunflower Assoc., Bismarck, ND.

Lander, E.S., P. Green, J. Abrahamson, A. Batlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Leon, A.J., M. Lee, G.K. Rufener, S.T. Berry, and R.P. Mowers 1996. Genetic mapping of a locus (*Hyp*) affecting seed hypodermis color in sunflower. Crop Sci. 36:1666-1668.

Leon, A.J., M. Lee, G.K. Rufener, S.T. Berry, and R.P. Mowers. 1995. Use of RFLP markers for genetic linkage analysis of oil percentage in sunflower seed (*Helianthus annuus*). Crop Sci. 35:558-564.

Machacek, C. 1979. Study of the inheritance of earliness in sunflower (*Helianthus annuus* L.). Genet. a Slecht. 15(3):225-232.

Marc, J., and J.H. Palmer. 1981. Photoperiodic sensitivity of inflorescence initiation and development in sunflower. Field Crop Res. 4:155-164.

Miller, J.F. and G.N. Fick, 1997. The genetics of sunflower. p441-495. *In* A.A. Schneiter (Ed). Sunflower technology and production. ASA, CSSA, and SSSA, Madison, WI.

Miller, J.F., J.J. Hammond, and W.W. Roath. 1980. Comparison of inbred vs. single-cross testers and estimation of genetic effects in sunflower. Crop Sci. 20: 703-706.

Roath, W.W., J.J. Hammond, and J.F. Miller. 1982. Genetic effects of days to flowering in sunflower (*Helianthus annuus* L.) under short day regime. p.247-249. *In* Proc. of the 10th Int. Sunflower Conf., Surfers Paradise, Australia. 14-18 March 1982. Int. Sunflower Assoc., Paris, France.

Schneiter, A.A. and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

Shabana, R. 1974. Genetic variability of sunflower varieties and inbred lines. p. 263-269. *In* Proc. of the 6th Int. Sunflower Conf., Bucharest, Romania. 22-24 July 1974. Int. Sunflower Assoc., Paris, France.

Stoenescu, F. 1974. Genetics. p. 92-125. *In* Vranceanu, A.V. (ed.) Floarea-soarelui. Editura Academiei Republicii Socialiste, Romania, Bucaresti.

Stuber, C. W., S. E. Lincoln, D. W. Wolff, T. Helentjaris, and E .S. Lander. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823-839.

Utz, H.F., and A.E. Melchinger. 1996. PLABQTL: A program for composite interval mapping of QTL. J.Quant.Trait Loci 2:1 (http://probe.nalusda.gov:8000/ otherdocs/jqtl/jqtl1996-01/utz.html).

Zeng, Z.B. 1994. Precision mapping of quatitative trait loci. Genetics. 136:1457-1468.