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AFLP mapping of QTLs for in vitro organogenesis traits using recombinant inbred lines in sunflower (*Helianthus annuus* L.)

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Abstract Genetic control for two in vitro organogenesis traits, the number of shoots per explant plated (S/E) and the number of shoots per regenerating explant (S/RE), was investigated in 75 recombinant inbred lines (RILs) of sunflower and their two parents (PAC-2 and RHA-266). Genetic variability was observed among the 75 RILs for the organogenesis traits studied. Some RILs presented significant differences when compared with the best parental line (RHA-266). Genetic gain, in terms of the percentage of the best parent, for 32% of the selected RILs was significant. A set of 99RILs from the same cross including the 75 mentioned above was screened with 333 AFLP markers and a linkage map was constructed based on 264 linked loci. Six putative QTLs for the S/RE (tentatively named *osr*) and seven QTLs for the S/E (*ose*) trait were detected using composite interval mapping. For each trait, the QTLs explained 52% (*ose*) and 67% (*osr*) of the total phenotypic variance. These results suggested that additive gene effects predominate in explaining a large proportion of the observed genetic variation associated with regeneration ability. The coincidental location of QTLs for S/E and S/RE is discussed.

Keywords Sunflower · *Helianthus annuus* L. · AFLP · Genetic mapping · in vitro regeneration · Recombinant inbred lines

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Introduction

Sunflower is the second largest annual oil scrop in the world, after soybean. Therefore the ability to regenerate a large number of plants is important for the development of biotechnology in this species. Plant regeneration by organogenesis can offer the possibility of obtaining a high number of regenerated shoots and fertile plants. Organogenesis is influenced by the nature and developmental stage of the explant (Power 1987; Witrzens et al. 1988; Espinasse and Lay 1989; Chraîbi et al. 1992), and the culture medium composition (Paterson and Everet 1985; Pugliesi et al. 1991).

Furthermore, regeneration frequency by organogenesis depends on genotype and its interaction with culture conditions. There are very few reports about the genetic control of organogenesis in sunflower. It has been shown that organogenesis parameters are under additive genetic control (Sarafi et al. 1996a, b) and evidence of a cytoplasmic effect and nucleo-cytoplasm interaction was also demonstrated by Deglene et al. (1997). Regeneration by organogenesis may either be direct or indirect, i.e. having an intermediate callus phase, and shoots are produced mainly in superficial cell layers of the cotyledons (Bronner et al. 1993). The precise and limited zone of induction observed in organogenesis raise questions about the special features of the cells implicated in the process. The very early events leading to shoot or to somatic embryo formation in sunflower do not seem to be similar. As early as the 2nd day of culture, clear differences occurred between two process; cells giving rise to somatic embryos started to accumulate lipids and proteins while cells involved in shoot formation did not (Bronner et al. 1993). Molecular characterization of regeneration potential has been approached by the identification of organogenesis-specific proteins in *Cucumis melo* (Leshem and Sussex 1990) and *Populus deltoides* (Coleman and Ernst 1991), and only few proteins seem to be correlated with the regeneration process.

Identification of chromosome regions with effects on regeneration traits would increase our understanding of

the genetic control of these characters. The development of molecular-marker techniques has provided an additional tool to determine quantitative trait loci (QTLs) contributing to shoot regeneration from immature barley embryos calli (Mano et al. 1996), rice seed callus (Taguchi-Shiobara et al. 1997) and anther culture in maize (Beaumont et al. 1995). As far as we know, QTLs involving organogenesis in sunflower have not been reported in the literature. However morphological markers and a small number of QTLs controlling important traits such a disease resistance (Mestries et al. 1998) or major genes such as fertility restoration, branching, fatty acid composition, plant height and maturity have been determined in sunflower (Berry et al. 1995; Gentzbittel et al. 1995; Mouzeyar et al. 1995; Miller and Fick 1997; Gentzbittel et al. 1999).

Linkage map construction is the first step in a systematic QTL analysis. The AFLP (amplified fragment length polymorphism) assay is a powerful technique for genetic fingerprinting in sunflower (Hongtrakul et al. 1997) as well as genome mapping and genetic variability studies (Vos et al. 1995; Ridout and Donini 1999). AFLP markers are typically dominant, but codominant markers that vary in size and intensity can also be identified. Most of the AFLP fragments correspond to a unique position on the genome and, hence, can be exploited as landmarks in genetic and physical mapping (Meksem et al. 1995; Thomas et al. 1995).

Some studies have shown the advantages of recombinant inbred lines (RILs) for detecting QTLs (Austin and Lee 1996). RILs are homozygous and can be propagated without further segregation. The RILs undergo multiple cycles of meiosis before homozygosity is reached; in consequence, linked genes have a great probability of recombination and the pleiotropic effect can be detected (Burr and Burr 1991). This effect increases the power of testing differences between genotypic classes.

The objective of the investigation presented here was to evaluate the variability and genetic gain for organogenesis traits in recombinant inbred lines (F_8) of sunflower. We also constructed a genetic map based on AFLP markers and carried out a QTL analysis to characterize the chromosome regions involved in organogenesis traits.

Materials and methods

Regeneration by organogenesis

Recombinant inbred lines (RILs) of *Helianthus annuus* L. were used in this experiment. The RILs population consisted of 75 F_8-derived lines developed through single seed descent (SSD) from a cross between 'PAC-2' and 'RHA-266'. The material was kindly provided by INRA (France).

After sterilization, the seed of 75 RILs (F_8) and their parents were germinated on solidified hormone-free half-strength MS medium (Murashige and Skoog 1962) containing 10 g/l of sucrose and 7 g/l of agar-agar. Cultures were maintained at 25±2°C under a 16 h light/8 h dark cycle. Two days after culturing, cotyledons were excised and divided into two pieces. The explants were cultured in 55 mm-diameter Petri dishes containing 10 ml of regenerating medium (Chraîbi et al. 1991) and each Petri dish received four explants. The culture conditions were the same as for germination. The experiment was designed in randomized complete blocks with three replications and 77 genotypes (75 RILs and the two parents). Each replication consisted of ten Petri dishes with four explants. Regeneration parameters were scored 4 weeks after the initial explant culture by recording the average number of shoots per explant plated (S/E) and the average number of shoots per regenerating explants (S/RE).

DNA extraction

Ninety nine recombinant inbred lines of the cross 'PAC-2×RHA-266', including the 75 RILs used in the organogenesis experiment, and their parents were grown in pots in the greenhouse and used for AFLP analysis. Leaf tissue from 20 day-old plants of each line was collected for the extraction of total DNA. Genomic DNA was isolated according to the Nucleon Phytopure Kit extraction and purification protocol (Amersham Life Science). Leaf samples (0.1 g) were placed in 1.5-ml tubes and frozen at –20°C. After breaking the cell wall, the cells were lysed in a reagent containing potassium SDS which is known to form complexes with proteins and polysaccharides. Chloroform was then added along with a modified resin. After incubation and centrifugation DNA pellets were washed once with 70% ethanol and re-suspended in 100 µl of TE.

AFLP procedure

Amplified fragment length polymorphism was resolved according to the AFLP Analysis System-I and AFLP Starter Primer Kit from Life Technologies, following the manufacturer's instructions, using *Taq* DNA Polymerase (Life Technologies) and γ^{33}P [ATP] from Amersham Pharmacia Biotech. Two hundred and fifteen nanograms of total DNA for each genotype sample were used. All reactions were made in the Gene Amp PCR System 9700 Thermocycler (Perkin Elmer Applied Biosystems). The bands were first amplified with primers each having one selective nucleotide. Then, the diluted PCR products were used as a template for the second amplification with primers containing three selective nucleotides (see Table 3). The procedure was performed as described in the kit.

Following the amplification, reactions products were mixed with an equal volume of formamide dye (98% formamide, 10 mM EDTA, bromophenol blue and xylene cyanol). After denaturation for 3 min at 90°C, 4 µl of each sample was located on a 6% denaturing polyacrylamide gel in a 38×50 cm Sequi-Gen GT Nucleic Acid Electrophoresis Cell (BIO-RAD). The gel was pre-equilibrated by passing an electric current through it (at a constant 95 W) for 30 min. The reactions products were then separated, under these conditions, for 2 h 30 min. Following the separation, the gel was vacuum dried and exposed to Hyperfilm MP (Amersham Pharmacia Biotech) for 2 days. Mapping data were obtained by visual scoring of autoradiograms. AFLP markers were named using the two last letters of the selective nucleotides in the *Eco*RI primer/*Mse*I primer, followed by numbers in descending molecular-weight order.

Linkage analysis

AFLP polymorphic bands were scored as present (1) or absent (0) on autorads and unreliable ambiguous bands were scored as missing (–). A molecular linkage map based on a data set consisting of 333 AFLP marker loci was constructed using MAPMAKER 3.0 (Lander et al. 1987). Linkage groups were obtained with a recombination value of less than 0.30 and a LOD score of 3.5. The Kosambi mapping function was used to transform the recombination frequency to genetic distances (Kosambi 1944).

QTL analysis

Analyses of variance were performed for the organogenesis traits. Narrow-sense heritabilities were calculated according to Kearsey and Pooni (1996), using least square estimates of the genetic parameters. Data were normalized by using a $\sqrt{x+3/8}$ transformation. The chromosomal location of QTLs for organogenesis traits were resolved by composite interval mapping (CIM) using QTL Cartographer version 1.13 (Basten et al. 1999). The QTL carte model 6 integrated two parameters for CIM: the number of markers to control for the genetic background (n_p=15) and a window size (w_s=10) that will block out a region of the genome on either side of the markers flanking the test site. The inclusion of background markers makes the analysis more sensitive to the presence of a QTL in the target interval. At each interval, the significance of the association was tested by a likelihood ratio statistic (Haley and Knott 1992).

Results

Genetic analysis of in vitro organogenesis

Analysis of variance of 75 RILs and their parental genotypes 'PAC-2' and 'RHA-266' showed a highly significant genotypic effect for both organogenesis traits (Table 1). Estimates of additive variance (V_A) and environmental variance (V_E) were determined for the average number of shoots per explant plated (V_A=255.4, V_E=97.99) and the average number of shoots per regenerating explant (V_A=328.54, V_E=136.73) from the mean squares of the ANOVA tables. Parental genotype RHA-266 showed higher values when compared with 'PAC-2' for the average number of shoots per explant plated (S/E) and the average number of shoots per regenerating explant (S/RE). The difference between all recombinant F_8 lines ($\overline{X}_{F8}$) and their parents ($\overline{X}_P$) was not significant (Table 2), indicating that the 75 RILs in this experiment are representative of the total possible recombinant lines from the cross 'PAC-2×RHA-266.' The best parent (RHA-266) compared with the best RIL and with the mean of the 24 out 75 RILs (32%) which have higher values for both traits when compared with the best parent, presented significant differences for the two traits studied. This phenomenon considered as genetic gain might be due to the polygenic nature of organogenesis and the accumulation of favourable alleles for organogenesis ability in the RILs. By contrast, 20% of the RILs presented lower values for both traits compared with the parent line PAC-2 which has low values. Finally, the rest of RILs (48%) have intermediate values compared with their parents. Some of the RILs have high values for one trait and low values for the other, which shows that the two studied characters (S/E and S/RE) are not totally dependant. Narrow-sense heritabilities were 0.72 and 0.70 for S/E and S/RE respectively.

Molecular-marker linkage map

A total number of 19 primer combinations (Table 3), chosen among primer pairs selected for sunflower (AFLP Analysis System protocol), were used to determine the linkage map of our material. Out of a total number of 333 markers analysed, 264 were placed in 18 groups using a minimum LOD score of 3.5 and a maximum recombination value of 0.30 (Fig. 1). The groups ranged from 32 to 224 cM in length and each carried 4–37 markers (Table 4). The total length of the map is 2558 cM, with is at least one marker for every 10 cM on average. Sixty nine markers remain unlinked to any group.

QTLs for in vitro organogenesis

Thirteen putative loci associated with in vitro organogenesis traits were identified (Table 5), located on seven different linkage groups (Fig. 2). The QTLs were designated as follows: *ose* (organogenic shooting explant) for shoots per explant plantes (S/E) and *osr* (organogenic shooting regenerant) for the number of shoots per regen-

Table 1 Mean squares for organogenesis parameters of sunflower recombinant inbred lines (RILs) and their two parents

Source of variation	*df*	S/E[a]	S/RE[b]
Total	230	603.88	788.40
Genotype	76	1630.39***	2107.96***
Block	2	43.91 ns	142.28 ns
Residual	152	97.99	136.73

[a] S/E=Average number of shoots per explant plated
[b] S/RE=Average number of shoots per regenerant explant
***=Significant at P=0.001, ns=not significant, *df*=degrees of freedom

Table 2 Genetic gain and heritability for two organogenesis traits in recombinant inbred lines (RILs) of sunflower

Item	S/E[a]	S/RE[b]
RHA266 (P1)	30.64	39.16
PAC2 (P2)	7.84	10.67
P1–P2	22.80*	28.49*
$\overline{X}_P$=(P1+P2)/2	19.24	24.91
$\overline{X}_{RILs}$[d]	26.95	35.27
$\overline{X}_{RILs}-\overline{X}_P$	7.71 ns	10.36 ns
Best RIL (BRIL)	105.24	125.85
GG[e]=BRIL-BP[c]	74.6*	86.69*
32% SF_8L	54.53	64.79
GG[f]=32%SF_8[g]L-BP	23.89*	25.63
h^2_n	72.27	70.61
LSD (Least significant difference)	15.84	18.71

* Significant at P=0.05, ns=not significant at P=0.05
[a] S/E, average number of shoots per explant plated
[b] S/RE, average number of shoots per regenerating explant
[c] BP, best parent ('RHA-266')
[d] XRIL, mean of all recombinant inbreds lines
[e, f] GG, genetic gain when the best RIL or 32% of the selected RILs are compared with the best parent ('RHA-266')
[g] 32%SF_8, 32% of the best recombinant F_8 lines

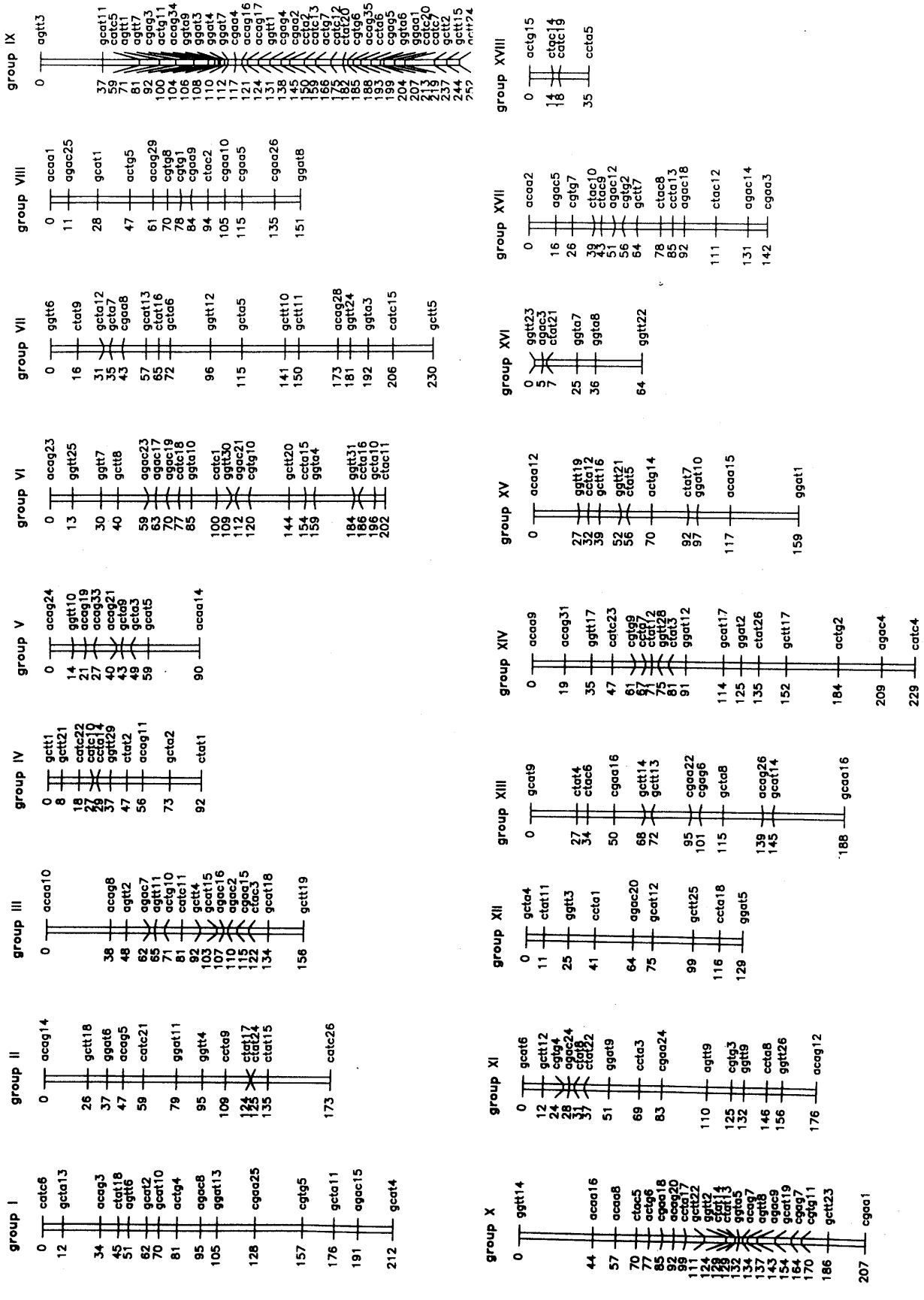

Fig. 1 AFLP-linkage map of *H. annuus* L. based on a RILs population of a cross between PAC-2 and RHA-266. The names of the markers are shown on the right of the group and their map position (Kosambi cM) at the left

Table 3 AFLP primer combinations tested and number of mappable markers in each primer pair used for map construction

Primer combination	Number of markers	Primer combination	Number of markers
E-AAC/M-CAA	13	E-AGG/M-CTA	11
E-AAC/M-CAG	29	E-AGG/M-CTT	27
E-AAC/M-CTG	15	E-AGG/M-CAT	13
E-AAG/M-CTT	10	E-AGC/M-CTA	12
E-AAG/M-CAC	24	E-AGC/M-CAA	21
E-ACC(M-CTA	16	E-AGC/M-CTT	24
E-ACA/M-CTC	24	E-AGC/M-CAT	20
E-ACG/M-CAA	19	E-ACT/M-CAT	24
E-ACG/M-CAG	6	E-ACT/M-CAC	14
E-ACG/M-CTG	11		

erating explants (S/RE), followed by the corresponding number of linkage group and the corresponding number of QTLs on the linkage group. The percentage of phenotypic variation explained by each QTL ranged from 6% to 17%. The percentage of phenotypic variance explained sum to 52% (*ose*) and 67% (*osr*) of the total phenotypic variance, respectively.

Table 4 Marker distribution among the linkage groups

Linkage group	Marker number	Length (cM)	Average distance (cM)
I	15	187	12.5
II	11	114	10.4
III	15	137	9.1
IV	10	82	8.2
V	9	82	9.1
VI	20	185	9.2
VII	16	186	11.6
VIII	14	153	10.9
IX	37	224	6.1
X	20	175	8.7
XI	18	173	9.6
XII	9	116	12.9
XIII	13	117	9.0
XIV	18	217	12.1
XV	14	180	12.8
XVI	6	57	9.5
XVII	15	141	9.4
XVIII	4	32	8.0
Unlinked	69	–	–
Total	333	2558	9.95

Discussion

Significant genetic variability for two organogenesis parameters was observed in this study, which is in agreement with results previously reported (Espinasse and Lay 1989; Sarrafi et al. 1996a, b; Deglene et al. 1997). Parental genotype ‘RHA-266’ performed better than ‘PAC-2’ for the two traits (S/E) and S/RE) studied. The best parent compared with the best RIL or with 32% of RILs presenting higher values than the best parent, showed significant differences for both organogenesis parameters. This phenomenon, considered as genetic gain, might result from the accumulation of favourable alleles in RILs. Sarrafi et al. 1996a, b and Deglene et al. (1997) reported the importance of additive genetic control for organogenesis traits in sunflower.

We have assessed the potential of the AFLP technique for detecting genetic variation among sunflower recombinant inbred lines from the cross ‘PAC-2×RHA266.’ The 99 genotypes, including the 75 RILs used in the organogenesis experiment, were screened with 333 markers in order to construct the AFLP linkage map; 264 loci were mapped onto 18 linkage groups covering 2558 cM

Table 5 Map positions and effect of QTLs detected in recombinant inbred lines (RILs) for in vitro organogenesis traits in sunflower

Trait	QTL	Linkage			Proportion of variance explained[a]	Additive effect (*a*)
		Group	Position[d]	LOD		
S/E[b]	*ose2.1*	II	70	5.1	0.09	–0.83
	ose2.2	II	101	3.1	0.08	0.45
	ose6.1	VI	71	4.5	0.10	–0.32
	ose7.1	VII	101	4.1	0.07	–0.15
	ose9.1	IX	132	3.7	0.07	–0.06
	ose17.1	XVII	90	5.5	0.11	0.45
S/RE[c]	*osr2.1*	II	71	6.4	0.10	–0.83
	osr2.2	II	96	6.5	0.12	1.09
	osr6.1	VI	71	8.7	0.17	–0.95
	osr7.1	VII	110	4.2	0.06	–0.11
	osr8.1	VIII	52	4.7	0.07	–0.14
	osr15.1	XV	137	4.1	0.07	–0.13
	osr17.1	XVII	90	3.9	0.08	0.19

[a] Value determined by QTL Cartographer, Version 1.13 (Basten et al. 1999)
[b] S/E, average number of shoots per explant plated
[c] S/RE, average number of shoots per regenerating explant
[d] Position, expressed in cumulative Kosambi cM, from north of the linkage group

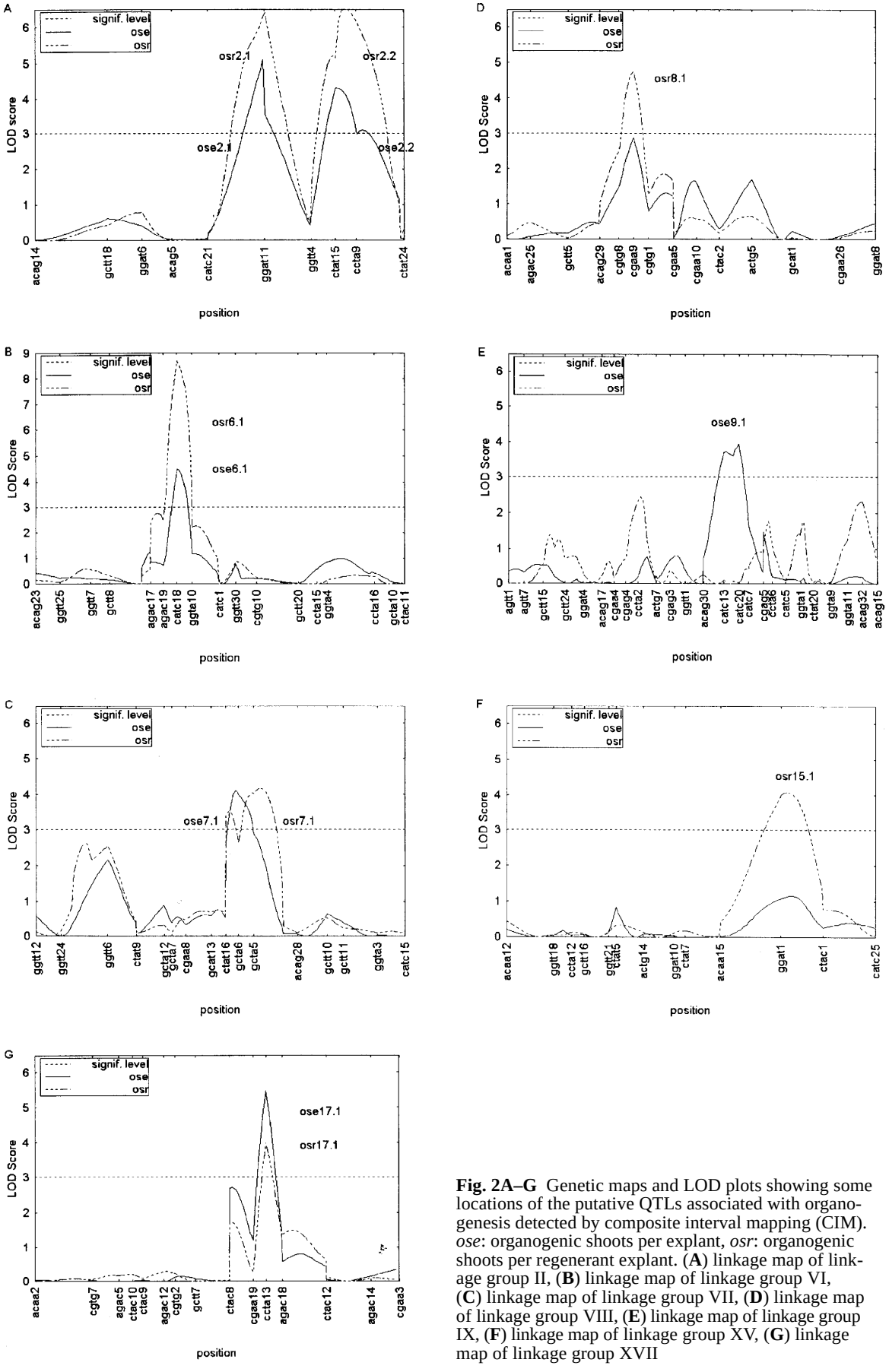

Fig. 2A–G Genetic maps and LOD plots showing some locations of the putative QTLs associated with organogenesis detected by composite interval mapping (CIM). *ose*: organogenic shoots per explant, *osr*: organogenic shoots per regenerant explant. (**A**) linkage map of linkage group II, (**B**) linkage map of linkage group VI, (**C**) linkage map of linkage group VII, (**D**) linkage map of linkage group VIII, (**E**) linkage map of linkage group IX, (**F**) linkage map of linkage group XV, (**G**) linkage map of linkage group XVII

of the sunflower genome. This demonstrates that high-resolution mapping of regions around genes of interest can be readily accomplished. The length of the map is significantly greater than those previously described (Berry et al. 1995; Gentzbittel et al. 1995, 1999) and corresponds to an expected two-fold expansion of the distances when using RILs created through selfing. The markers mapped are sparse in some regions of the genome and dense in others, as previously described. Specific biological differences in the levels or location of DNA polymorphism, the rates of recombination, the variation in copy number of specific genomic sequences or the sampling error are factors that could affect the distribution of markers (Van Eck et al. 1995). Cultivated sunflower (*H. annuus* L.) possess 17 haploid chromosomes. Our results (18 linkage groups and unlinked markers) indicate that further mapping is still needed to obtain a saturated map. It has been suggested that increasing population size, and not the number of markers, would most likely reduce the number of linkage groups (Keim et al. 1997). Furthermore, the AFLP markers can be converted to either PCR primers or RFLP probes to facilitate integration with other linkage maps (Baudaracco-Arnas and Pitrat 1996).

Using the AFLP technique a considerable amount of polymorphism has been revealed. Thirteen QTLs related to the two in vitro organogenesis traits (S/E and S/RE) were identified. RILs showed clearly their predicted potential in resolving linked QTLs, as demonstrated with *ose2.1* and *ose2.2*, two QTLs founded to be linked on linkage group II and showing opposite additive signs. Five QTLs are common for both traits and a QTL colocated with *osr8.1* could be suspected for S/E (Fig. 2D). This result could be expected as the two parameters studied are related to each other. However, QTLs specific to each organogenesis trait are also found like *ose9.1* and *osr15.1*. This result is in agreement with the description of some of the RILs which have high values for one trait and low values for the other, which shows that the two studied characters are not totally dependant. The question is raised to what extent the same 'genes' control the two process. A large number of phenotypic and physiological changes are induced during organogenesis in response to in vitro culture, and it should be expected that the number of QTLs involved in the expression of this process can be large. The number of QTLs detected in the present study (six *osr* and seven *ose* QTLs) is thus consistent with this hypothesis. This result is not in complete agreement with previous results. Komatsuda et al. (1995) determined a locus which explained 65% of the total genetic variance for shoot differentiation in barley. Armstrong et al. (1992) also detected three loci which explained 82% of the total phenotypic variance of regenerable calli in maize.

The induction of cells toward organogenesis involves a re-programming of gene expression and at least some of the QTLs could be hypothesized to represent transcription factors acting as positive or negative regulators of gene expression. However, it has been shown that genotypes having positive alleles for all of the detected QTLs did not always have the highest regeneration ability (Taguchi-Siobara 1997). The morphogenetic event is a function also of the auxin/cytokinin ratio and of the developmental stage of the cotyledon. It has been suggested that explants with many meristematic and undifferentiated cells, as in the immature stomatal complex present in young cotyledons (like those used in this study), are generally predisposed to form shoots (Yeung et al. 1981). These shoots are formed near the wound sites because the cells of these regions had the characteristics of meristematic cells.

The results presented here revealed four regions related to the in vitro regeneration by organogenesis in recombinant inbred lines. Although these regions need to be more precisely mapped, the information obtained should help in the selection of organogenesis-responsive genotypes and the transfer of regeneration ability to genotypes that respond poorly. Crosses between RILs contrasted for their organogenesis parameters and exhibiting molecular polymorphism in detected QTLs will be made, in order to focus more precisely on the genomic region of interest. Sunflower which was previously classified as a rather recalcitrant plant concerning in vitro organogenesis is slowly being integrated with other regenerable species.

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References

Armstrong CL, Romero-Severson J, Hodges TK (1992) Improved tissue culture response of an elite maize inbred through backcross breeding, and identification of chromosomal regions important for regeneration by RFLP analysis. Theor Appl Genet 84:755–762

Austin DF, Lee M (1996) Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in maize. Theor App Gener 92:817–826

Basten CJ, Weir BS, Zeng Z-B (1999) QTL cartographer version 1.13: program in statistical genetics. Department of statistics, North Carolina State University

Baudracco-Arnas S, Pitrat M (1996) A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor Appl Genet 93:57–64

Beaumont V, Rocheford T, Widholm J (1995) Mapping the anther culture response genes in maize (*Zea mays* L.). Genome 38: 968–975

Berry ST, Leon AJ, Hanfrey CC, Challis P, Burkolz A, Barnes SR, Rufener GK, Lee M, Caligari PDS (1995) Molecular-marker analysis of *Helianthus annuus* L. 2. Construction of an RFLP map for cultivated sunflower. Theor Appl Genet 91:195–199

Bronner R, Jeannin G, Hahne G. (1993) Early cellular events during organogenesis and somatic embryogenesis induced on immature zygotic embryos of sunflower (*Helianthus annuus* L). Can J Bot 72:239–248

Burr B, Burr F (1991) Recombinant inbred lines for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Chraîbi B, Latche A, Roustan JP, Fallot J (1991) Stimulation of shoot regeneration from cotyledons of *Helianthus annuus* L. by the ethylene inhibitors, silver and cobalt. Plant Cell Rep. 10:204–207

Chraîbi M B, Castelle JC, Latche A, Roustan JP, Fallot J (1992) A genotype-independent system of regeneration from cotyledons of sunflower (*Helianthus annuus* L.). The role of ethylene. Plant Sci 89:215–221

Coleman GD, Ernst SG (1991) Protein differences among *Populus deltoides* internodal stem explants determined for shoot regeneration or callus growth. Plant Sci. 75:83–92

Deglene L, Lesingnes P, Alibert G, Sarrafi A (1997) Genetic control of organogenesis in cotyledons of sunflower (*Helianthus annuus* L.). Plant Cell Tissue and Org Cult 48:127–130

Espinasse A, Lay C (1989) Shoot regeneration of callus derived from globular to torpedo embryos from 59 sunflower genotypes. Crop Sci 29:201–205

Gentzbittel L, Vear F, Zhang X-Y, Bervillé A, Nicolas P (1995) Development of a consensus linkage RFLP map of cultivated sunflower (*Helianthus annuus* L.). Theor Appl Genet 90: 1079–1086

Gentzbittel L, Mestries E, Mouzeyar S, Mazeyrat F, Badaoui S, Vear F, Tourvieille de Labrouhe D, Nicolas P (1999) A composite map of expressed sequences and phenotypic traits of the sunflower (*Helianthus annuus* L.) genome. Theor Appl Genet 99:218–234

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324

Hongtrakul V, Huestis GM, Knapp S (1997) Amplified fragment length polymorphism as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. Theor Appl Genet 95:400–407

Kearsey MJ, Pooni HS (1996) The genetical analysis of quantitative traits. Chapman and Hall, London, UK

Keim P, Schupp JM, Travis SE, Clayton k, Webb DM (1997) A high-density soybean genetic map based upon AFLP markers. Crop Sci 37:537–543

Komatsuda T, Taguchi-Shiobara F, Oka S, Takaiwa F, Annaka T, Jacobsen HJ (1995) Transfer and mapping of the shoot-differentiation locus Shd_1 in barley chromosome 2. Genome 38: 1009–1014

Kosambi DD (1944) The estimation of map distances from recombinational values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Leshem B, Sussex IM (1990) Polypetides of culture melon cotyledons serve as markers of root and shoot organogenesis. J Plant Physiol 137:155–159

Mano Y, Takahashi H, Sato K, Takeda K (1996) Mapping genes for callus growth and shoot regeneration in barley. Breed Sci 46:137–142

Meksem K, Leister D, Peleman J, Zabeau M, Salamini, Gebhardt C (1995) A high-resolution map of the vicinity of the *R1* locus on chromosome V of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81

Mestriest E, Gentzbittel L, Tourvieille de Labrouhe D, Nicolas P, Vear F (1998) Analysis of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.) using molecular markers. Mol Breed 4: 215–226

Miller JF, Fick GN (1997) The genetics of sunflower. In: Shneither A (ed) Sunflower technology and reproduction. ASA monograph, Madison, Wisconsin, pp. 441–495

Mouzeyar S, Roeckel-Drevet P, Gentzbittel L, Philippon J, Tourvieille De Labrouhe D, Vear F, Nicolas P (1995) RFLP and RAPD mapping of the sunflower Pl_1 locus for resistance to *Plasmopara halstedii* case 1. Theor Appl Genet 91: 733–737

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15: 473–497

Murigneux A, Bentolila S, Hardy T, Baud S, Guitton C, Juillen H, Ben Tahar S, Freyssinet G, Beckert M (1994) Genotypic variation of quantitative trait loci controlling in vitro androgenesis in maize. Genome 37:970–976

Paterson KE, Everett NP (1985) Regeneration of *Helianthus annuus* L. inbred plants from callus. Plant Sci 42:125–132

Power CJ (1987) Organogenesis from *Helianthus annuus* L. inbreds and hybrids from the cotyledons of zygotic embryos. Am J Bot 74:497–503

Pugliesi C, Cecconi F, Mandolfo A, Baroncelli S (1991) Plant regeneration and genetic variability from tissue cultures of sunflower (*Helianthus annuus* L.). Plant Breed 106:114–121

Ridout CJ, Donini P (1999) Use of AFLP in cereals research. Trends Plant Sci 4:76–79

Sarrafi A, Bolandi AR, Serieys H, Bervillé A, Alibert G (1996a) Analysis of cotyledon culture to measure genetic variability for organogenesis parameters in sunflower (*Helianthus annuus* L.) Plant Science 121:213–219

Sarrafi A, Roustan JP, Fallot J. Alibert G (1996b) Genetic analysis of organogenesis in the cotyledons of zygotic embryos of sunflower (*Helianthus annuus* L) Theor Appl Genet 92:225–229

Taguchi-Siobara F, Lin SY, Tanno K, Komatsuda T, Yano M, Sasaki T, Oka S (1997) Mapping quantitative trait loci associated with regeneration ability of seed callus in rice (*Oryza sativa* L.). Theor Appl Genet 95:828–833

Thomas CM, Vos P, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8:785–794

Van Eck HJ, Vandervoort JR, Draaistra J, Vanzandvoort P, Vanenckevort E, Segers B, Peleman J, Jacobsen E, Helder J Bakker J (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Mol Breed 1:397–410

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Witrzens B, Scowcroft WR, Larkin PJ (1988) Tissue culture and plant regeneration from sunflower (*Helianthus annuus* L.) and interspecific hybrids (*H. tuberosus*×*H. annuus*). Plant Cell Tissue Org Cult 13:61–76

Yeung EC, Aitken J, Biondi S, Thorpe TA (1981) Shoot histogenesis in cotyledon explants of radiata pine. Bot Gaz 142:494–501

Yu K, Pauls K (1993) Identification of a RAPD marker associated with somatic embryogenesis in alfalfa. Plant Mol Biol 22:269–277

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application 92402629.7; Publication. Number EP0534858A1