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RFLP PROFILES IN LOW OLEIC SUNFLOWER USING *SDI-*, A STEAROYL-ACP, AND AN OLEOYL-PC DESATURASES cDNAs

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SUMMARY

Polymorphisms revealed by cDNA corresponding to genes involved in different agronomic traits were compared between a set of public lines and commercial hybrids. We expected that only few probes should reveal polymorphisms and that these polymorphisms should be more frequent in hybrids than in lines. In contrast, we found that all these probes revealed polymorphisms among lines and that the distribution of these polymorphisms were about the same between lines and hybrids. However for two of the probes we observed much more profiles in lines than in hybrids. Three hypotheses are discussed to explain these facts.

Moreover, two possible applications appeared: (1) lines identification and (2) to determine whether the environment, the genetic background or the genes involved in the fatty acid metabolism are responsible for variations in oleic and linoleic content.

Key words: **Sunflower, stress drought induced genes, desaturases, polymorphism**

INTRODUCTION

Randomly chosen cDNAs are usually poorly polymorphic in a cultivated species. The range of frequency varies from 50% to a few per cent depending upon the species and the genetic basis of the cultivated material. Consequently cDNAs have to be screened before used in RFLP. For sunflower the genetic basis of elite lines has led to expect about 10% of randomly chosen cDNA usable for diversity and mapping (Berry *et al.*, 1994; Gentzbittel *et al.*, 1994, 1995). This means that these cDNA are expected to reveal polymorphism between a pair of lines randomly chosen. When cDNA have been isolated by physiologists, it is logical to use them to look for polymorphisms between lines which represent the genetic basis of the cultivated

species. Now, for sunflower, there are many cDNA sequences corresponding to genes involved in a known function related to traits of agronomic interest. Such cDNAs corresponding to genes directly involved in a known function are those, *i.e.*, corresponding to desaturases genes and stress-induced genes. The desaturase genes control the desaturation of fatty acid (TG) before introduction into membranes (phospholipids) and direct oil composition in the seed. Other genes isolated for responding to drought stress (Ouvrard *et al.*, 1996) have already revealed polymorphisms in cultivated sunflower (Lacombe *et al.*, 1998).

For sunflower, the genetic basis of hybrids is narrow, likely due to the fact that maintainer lines (B lines) for Pet 1 cytoplasm and restorer lines (R lines) are selected to generate elite hybrids. The distribution of RFLP in commercial hybrids in comparison with elite lines was therefore performed to check whether this is verified.

Here we compared the RFLPs revealed by these cDNA for a set of lines and of hybrids with these probes. We found that each cDNA revealed between 3 to more than 40 RFLP on 43 lines. Most of the RFLP are fairly distributed in lines and commercial hybrids. However, RFLP revealed by two of these probes ($\Delta 9$ and *sdi-1*) are reduced in hybrids in comparison with lines. This striking fact leads to speculate on the causes of such a bottleneck for the polymorphisms and to propose experiments to solve this dilemma.

Table 1: List of the plant material used in RFLP experiments: **Part A,** Commercial hybrids

CODE	STRUCTURE
Florine	hybrid
Coril	hybrid
Viki	hybrid
Trisun 860	hybrid
Santiago	hybrid
Albéna	hybrid
Athis	hybrid
Platon	hybrid
Aril	3 way hybrid
Sélect	hybrid
Santafé	hybrid
Pistol	hybrid
Flamme	hybrid
Jaguar	hybrid
Agrisol	hybrid
Sarah	hybrid
NSH 45	hybrid

MATERIAL AND METHODS

DNAs from twenty-four public lines (Table 1A), and seventeen commercial hybrids (Table 1B) were studied using the RFLP technique with cDNAs corresponding to genes involved in fatty acid desaturation (sunflower Δ9 and Δ12) and responding to drought stress (*sdi1*, *sdi6*, *sdi8*,... , *sdi10*) (Ouvrard *et al.*, 1996) (Table 2). DNAs were restricted either by *Eco*RI or by *Hind*III, and after agarose slab gel electrophoresis were transferred according to Southern to Nylon membranes.

Table 1: List of the plant material used in RFLP experiments: **Part B.** Lines, their origin and lane number

Code	Structure	Pedigree derived from	Δ9 desaturase *Eco*RI or *Hind*III RFLP	
S1	line	ignored	E1	H1
R1	line	ignored	E2	H2
LR 1	no fixed	85 B6 x *H. debilis* 215	E3	H3
LR 2	line	FS 20.6.2 x *H. argophyllus*	E4	H4
LR 4	line	NSH 45	E5	H5
RHA 271	line	*H. petiolaris*	E6	H6
RHA 274	line	*H. petiolariss*	E7	H7
RHA 373	line	*H. petiolaris*	E8	H8
89 B 1	line	*H. praecox runyonii*	E9	H9
89 B 2	line	*H. niveus canescens*	E10	H10
85 B 3	line	Vniimk 6540, Cernianka	E11	H11
HA 74	line	*H. argophyllus* x Arm 9345	E12	H12
HA 74	line	idem	E12	H12
HA 89	line	Vniimk 8931	E13	H13
H 52	line	ignored	E14	H14
125	line	Moroccan population(CIRO)	E15	H15
83 HR 4	line	Vniimk 6540 & (G509)	E16	H16
XK	line	Peredovik x *H. tuberosus*	E17	H17
2603	line	Moroccan population (CIRO)	E18	H18
AA 7-2-4	line	selection in *H. argophyllus*	E19	H19
CANP 3	line	Armavirsky 9345 Russia	E20	H20
PNRM 6.5.1	no fixed	Vniimk 6540 x *H. anomalus*	E21	H21
FS 20 6 2	line	Cernianka x (Sunrise)	E19	H19

Hybridization was performed with ^{32}P labelled cDNAs. Different length of exposure was performed according to the high or faint signals of the radioactive spots on the membrane. The Δ9 and Δ12 polymorphisms were studied further on nineteen other lines (Table 1C) because they displayed the highest and the lowest polymorphisms, respectively.

Table 1: List of the plant material used in RFLP experiments: **Part C.** Lines studied with the two desaturases only

Code	Pedigree derived from	Δ9 desaturase *Eco*RI or *Hind*III RFLP	
BD70032		-	-
Olea7-3		-	H22
PB3		-	H22
HA124		-	H23
Z2736-2		-	H23
HA821		-	H24
RT1B11	INRA	E22	H24
HA300		-	H24
H52		-	
H55		-	H25
RHA266		-	H26
XRQ		E22	H27
CANP3	INRA	-	-
HA335		-	H27
UX		-	-
RHA274		-	-
LC1004A	Romania	E22	H1
LC1103C	Romania	-	H1
V94		-	H28
SD		-	-
HA99		-	-

Table 2: List of the cDNA used as a probe, reference number in genebank and literature references

cDNA	Accession number	reference
sdi-1	X92646	Ouvrard *et al.* (1996)
sdi-5	X92645	Ouvrard *et al.* (1996)
sdi-6	X92649	Ouvrard *et al.* (1996)
sdi-8	X92650	Ouvrard *et al.* (1996)
sdi-9	X92648	Ouvrard *et al.* (1996)
sdi-10	X92651	Ouvrard *et al.* (1996)
Δ9 desaturase	none	Kabbaj *et al.* (1996)
Δ12 desaturase	none	unpublished

RESULTS

On the first set of twenty-four lines (Table 1A, lanes 1 to 23) all probes revealed polymorphism that is surprising in comparison with the screening which has been required to handle enough cDNA leading to RFLP between elite lines (Gentzbittel *et al.*, 1994, 1995; Berry *et al.*, 1994). We noted these polymorphisms as 'simple' (less than four fragments, Figure 1) or 'complex' (more than five fragments) and RFLP profiles were qualified as 'low polymorphic' when they displayed less than four patterns or 'highly polymorphic' when they displayed more than five patterns (Table 3; Figure 2).

Table 3: Quantitation of polymorphisms revealed with cDNA used as a probe on lines and hybrids

		Lines average number of		Hybrids average number of	
cDNA used as a probe	Profile Type	profiles	fragments per profile	profiles	fragments per profile
sdi-1	complex polymorphic	15	9.3	4	10
sdi-5	simple, low polymorphic	2	1.5	2	1.5
sdi-6	simple polymorphic	8	1.8	6	2.8
sdi-8	simple polymorphic	7	2.8	5	3
sdi-9	complex low polymorphic	4	6.2	2	11
sdi-10	simple polymorphic	8	1.4	9	2.3
Δ 9 *desaturase*	complex polymorphic	21.5	4	9	4.6
Δ*12 desaturase*	complex, No polymorphism	1	5	1	5.5

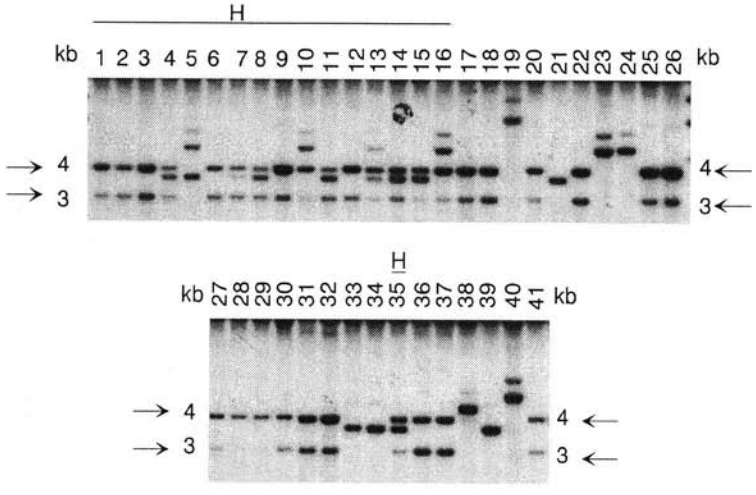

Figure 1 : Autoradiogram of DNAs restricted with EcoRI probed with sdi-6 cDNA from hybrids (H: lanes 1 to 16 and 34) and lines (all the others 17 to 33 and 35 to 40). The number for lanes refers to those in Table 1. Other genotypes are not shown.

These probes revealed on average in the lines between one (Δ12 desaturase), two (*sdi-5*) to up twenty-two profiles (Δ9 desaturase). In the commercial hybrids the polymorphism ranged between one (Δ12) and nine (*sdi-1* and Δ9 desaturase).

With the cDNA corresponding to a Δ9 gene as a probe, we observed 21 RFLP profiles in sunflower lines although the Δ9 profile contains only four fragments (Table 1A, lanes 1 to 23, Figure 2). When we extended the research to more lines (19 more) and we found more profiles both with *Eco*RI and *Hind*III which corresponded to several tens of new profiles in all the lines checked since the *Eco*RI and the *Hind*III polymorphisms are independent (Table 1A, lanes 24 to 44). The *sdi-1* cDNA also displayed many profiles (15) but here the profiles are complex (9.3 fragments on average). With the probe corresponding to the Δ12 gene we revealed three patterns. Furthermore, based on both the Δ9 and the Δ12 desaturases with *sdi-1*, for example, we discriminated and characterized most of the sunflower lines (Table 1A).

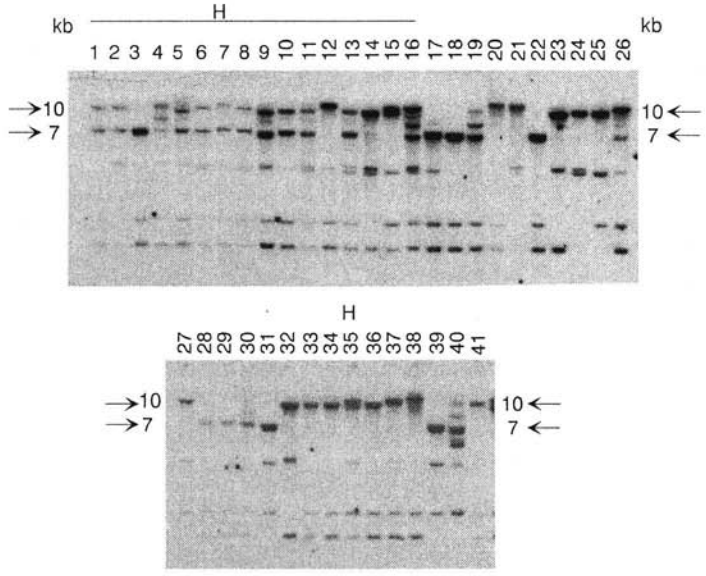

Figure 2 : Autoradiogram of DNAs restricted with EcoRI probed with a cDNA corresponding to a Δ9 desaturase gene from hybrids (lanes 1 to 16 and 34) and lines (all the others). (-) not determined.

In commercial hybrids, with most of the probes we found that these polymorphisms were in average present and, i.e., *sdi-10* displayed as much as polymorphisms in hybrids (9) than in lines (8). On average these probes revealed five profiles for lines and 4.5 for hybrids. However, two probes: *sdi-1* and Δ9, displayed much more polymorphism in hybrids than in lines. These two probes together revealed thirty six profiles in lines but only thirteen in commercial hybrids.

The observation of autoradiograms of DNAs restricted by *Eco*RI and hybridized with Δ9 desaturase cDNA used as probe revealed that the Δ9 profiles for lines displayed either about a 10 kb or a 7 kb fragment (Figure 2) plus shorter fragments. These RFLPs map at two independent loci in our F_2 segregating population resulted from FS 20.6.2 x *H. argophyllus* 92 (Lambert *et al.*, 1998). Moreover, the mapping of these RFLPs revealed that the two higher fragments are allele (Data not shown). Furthermore, for commercial hybrids we observed that most of them (12 out of 17) displayed one 7 kb and one 10 kb fragment whereas one hybrid (lane 3) displayed only two 7 kb fragments and four hybrids (lanes 12, 14, 15, and 35) displayed two 10 kb (Figure 2).

DISCUSSION

All the probes that we studied revealed polymorphism, although the frequency for cDNAs has been found to be about 15-20% (Gentzbittel *et al.*, 1995; Berry *et al.*, 1994). Moreover, most of the cDNA we used, correspond to genes involves in common function (ELIP, nsLPT...), we therefore did not expect many polymorphisms.

The sample of lines was chosen partly at random and partly because we used these lines in our breeding programs. Thus, we believe that the genetic basis of this material is quite wide. The genetic polymorphisms of sunflower lines revealed with our cDNA set is wide. We revealed in average seven different profiles for twenty four lines, this appears unexpected. Moreover, it is of the same extend in lines and hybrids, on average the hybrids did not display more fragments (4.5) than the lines (4.0). Apparently there is no relation between the number of fragments in the profiles and the number of profiles observed.

When we compared the number of profiles in lines and hybrids for the Δ9 and the *sdi-1* probes - the two more polymorphic probes - we found a clear discrepancy since for lines we revealed 36 profiles but only thirteen profiles are recovered in sunflower F_1 hybrids. For the remaining probes, out of thirty profiles observed in lines twenty-five were recovered in commercial hybrids.

Since we found with two probes a severe reduction in the molecular polymorphisms, the questions addressed here are whether or not this reduction has a meaning. Several explanations can be brought to explain this situation, all can be experimented.

1. This could be due to statistical variation by sampling lines and hybrids.
2. This could be due to complement the function of two different alleles of the Δ9 desaturase gene. We effectively observed that broadly these hybrids displays both of the short and long RFLP fragments present either in B or in R lines (Figure 1). A similar proposition is valuable for *sdi1* (ELIP) gene too, however the function is not as clear than for a desaturase gene.

3. The third possibility is that the loci revealed by these cDNAs belong to linkage groups very important to obtained efficient combining effect in the F_1 hybrid.

Hypothesis 1: We did not believe that since some lines were chosen because there are widely used in our breeding programs and the other lines were chosen at random. Moreover, the hybrid set is from five different companies, this warrants that it is unlikely that their genetic basis is narrow.

Hypotheses 2 and 3 are interesting since the combination of lines with such polymorphism could be preferentially produced to check combining ability efficiency. This reduction of polymorphisms from lines to hybrids is, according to most breeders, attributed to the two restorer and maintainer complementation groups based on PET1 cytoplasm. Furthermore, this reduction in polymorphism is expected to be limited around the Rf loci but not for any particular probes. Lacombe *et al.* (1998) have shown that commercial hybrids can result from crosses between two different maintainer groups with R lines. Moreover one of these probes, *sdi-9*, revealed polymorphisms which have been shown to map together with the Rf1 locus. This means consequently that the molecular diversity in the Rf1 region is still maintained. According to our results any of the Δ9 desaturase loci is linked to the Rf loci.

However, if such a reduction exists, it is not possible to use it for prediction of the combining ability of any lines. We therefore wonder here whether the bottleneck in the polymorphism is due either to the two combinations groups including R lines and B lines, to a poor sampling of lines and hybrids or to location of markers in loci important for heterotic effect.

The function of the Δ9 desaturase is to produce the oleic acid (C18:1). This component is highly variable in sunflower oil in a range between 18% to 50% without any knowledge of the genetic and environmental effects. We propose therefore to determine the correlation between the Δ9 desaturase polymorphisms and the level of C18:1 in the seed and the leaf tissues (membranes) of lines and hybrids in a wide range of genetic and molecular variabilities. This correlation will lead to determine those polymorphisms which modify the oleic level and by this way to suggest a variability in enzyme forms. Coupled with a similar study with the Δ12 desaturase polymorphisms we plan to determine the effect of each of the two enzymes in oleic acid accumulation in the low oleic sunflower.

These polymorphisms revealed by a few of these probes (Δ9 and Δ12 desaturases, *sdi-1*, ...) have already applications in our group for lines identification and to check purity of hybrids. They may have, therefore, extended applications to perform seed control and to measure gene flux.

CONCLUSION

The distribution of polymorphisms for breeder is always questionable: here we revealed that two loci (it is likely that the Δ9 and the *sdi-1* loci are independent, the answer will be known soon) are less polymorphic in commercial hybrids than in lines. Around Δ9 desaturase loci there are certainly several causes for such polymorphisms, but this means that on the basis of these polymorphisms we can eliminate some lines from hybrid vigor checks.

The question now is to correlate such polymorphisms with eventual variation in the corresponding genes to determine whether or not slight changes in oil composition are due to genetic or environmental effect. Furthermore we also could look for Δ9 desaturase forms more or less responsive to environment to lower the oleic content when high temperature regimen occurs. For the Δ9 desaturase we believe that some polymorphisms might be associated with slight variations in the function of the enzyme and thus will lead to a correlation between some of them and oleic content. Furthermore we can also check whether some polymorphisms belong to efficient linkats (Demarly, 1979) to predict combining ability of lines. Such a work could be conducted in collaboration between INRA, other public Institutes and companies.

Two possible applications appeared :

1) Lines identification since based on both the Δ9 desaturase, *sdi-1* and the Δ12 probes we characterized most of the sunflower lines.
2) According to the sunflower oil composition in a wide range of lines and hybrids, it is not easy to determine whether the environment, the genetic background or the genes involved in the fatty acid metabolism are responsible for variations in oleic and linoleic content. To estimate whether desaturase polymorphisms could be involved in such variations we suggest to compare RFLP revealed by cDNA desaturases (stearoyl ACP desaturase and an oleoyl PC desaturase) and oil composition in a set of lines and hybrids.

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RFLO PERFILES EN EL GIRASOL CON BAJO CONTENIDO DE ACIDO OLEICO CONSTATADOS POR SDI-, STEAROIL-ACP Y OLEOIL-PC DESATURACION cADN

RESUMEN

Los poliformismos constatados por cADN, que corresponden a los genes responsables para las propiedades agronomas diferentes han sido comparados dentro del grupo consistente de las lineas publicas y de los hibridos comerciales. Hemos expectado que solo un pequeño numero de sondas podria identificar el poliformismo, asi como el poliformismo seria mas frecuente en los hibridos que en las lineas. En contra de expectaciones, fue constatado que todas las sondas indicaban el poliformismo entre las lineas y que el poliformismo era igualmente repartido en las lineas y los hibridos. Entretanto, dos sondas indicaron el numero de perfiles considerablemente mas grande en las lineas que en los hibridos. Los hechos antes observados fueron tratados a base de tres hipotesis.

Los resultados obtenidos pueden ser utilizados de dos maneras: (1) para la identificacion de lineas y (2) para la constatacion de lo que es responsable para la variacion del contenido de acidas oleico y linoleico - medio ambiente, base genetica o los genes que rigen el metabolismo de acidas grasos.

PROFILS RFLP DANS LE TOURNESOL À FAIBLE CONTENU D'ACIDE OLÉIQUE DÉTERMINÉS AU MOYEN DE DÉSATURASES OLEOYL-PC DE ADNc

RÉSUMÉ

Les polymorphismes révélés par l' ADNc correspondant aux gènes responsables de différents traits agronomiques ont été comparés entre des groupes formés de lignes existantes déjà sélectionnées et d'hybrides commerciaux. Nous nous attendions à ce que seulement quelques sondes révèlent des polymorphismes et à ce que ces polymorphismes soient plus fréquents chez les hybrides que sur les lignes. Contrairement à notre attente, nous avons constaté que toutes les sondes révélaient des polymorphismes parmi les lignes et que la répartition de ces polymorphismes étaient à peu près la même entre les lignes et les hybrides. Cependant, dans le cas de deux sondes, nous avons observé un nombre significativement plus important de profils sur les lignes que chez les hybrides. Trois hypothèses pourraient expliquer ces constations.

De plus, deux applications possibles sont apparues: (1) l'identification des lignes et (2) la possibilité de déterminer si c'est l'environnement, la base génétique ou les gènes responsables du métabolisme des acides gras qui sont la cause de la variation du contenu des acides oléique et linoélique.