HELIA, **27**, Nr. 40, p.p. 41-54, (2004) UDC 633.854.78:575.116.4:665.12

THE PERVENETS HIGH OLEIC MUTATION: METHODOLOGICAL STUDIES

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Received: October 08, 2003
Accepted: January 05, 2004

SUMMARY

The Pervenets mutation raises the oleic acid content (OAC) from about 25% (LO linoleic sunflower) to over 75% (HO sunflower) in sunflower seed oil whatever the genetic backgrounds of the genotypes: fixed mutant lines, hybrid between fixed mutant lines and, surprisingly, both direction cross hybrids between high oleic × linoleic and linoleic × high oleic lines. Here we develop the methodology to point out the coincidence between the mutation and the oleate-desaturase locus. The mutation function is modeled to explain the phenotype.

A series of sunflower genotypes developing embryos between 12 and 24 days after pollination without or carrying the Pervenets mutation was used to compare oleate-desaturase transcript accumulation. This revealed the presence and absence of the transcript, in normal LO and Pervenets HO embryos, respectively, whereas stearate-desaturase transcript accumulation was equivalent in both types. The RFLP approach using oleate-desaturase cDNA as a probe has revealed that in comparison with the linoleic sunflower, those carrying Pervenets mutation displayed, besides a common 5.8 kb *Eco*RI fragment, an extra 8 kb *Eco*RI fragment and, with HindIII, the shift from 8 kb to 16 kb of a *Hind*III fragment. The common 5.8 kb *Eco*RI fragment to Pervenets and normal sunflower carries an oleate-desaturase gene. The insertion unique to the sunflower carrying Pervenets mutation has to carry oleate-desaturase sequences. The genetic studies of this mutation have lead to contradictory results in the inheritance pattern of the HO trait due to variable mutation expression and several genetic factors affecting the HO level. Thus, it appeared important to study the inheritance in a set of recombinant inbred lines. Moreover, we pointed out that the presence of the mutation is not sufficient to induce the HO phenotype since a suppressor of the mutation "supole" may mask the HO trait. The supole allele is revealed in half of the HO RI lines, and consequently its accurate mapping is difficult. Thus conjunction of the molecular and genetic methods and of material construction was required to solve this problem and to provide efficient tools to breed HO sunflower.

Key words: DNA diagnostic, *Helianthus*, high oleic oil, Pervenets mutation, marker-assisted selection

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INTRODUCTION

Vegetable oils are required for food and industry. They are mainly composed of triacylglycerol carrying saturated, mono-unsaturated and poly-unsaturated fatty acids. There exists a large variety of oilseed crops but all crops follow the same biochemical pathway for their synthesis. Biosynthesis and desaturation of fatty acids present in seed oil occur exclusively in plastids and on reticulum endoplasmic cytosolic face. The first desaturation of stearic acid (18:0) in oleic acid (18:1) is catalyzed by the stearoyl-ACP desaturase (Δ9-desaturase) in plastids. Other desaturations take place on reticulum endoplasmic cytosolic face. The oleoyl-PC desaturase (Δ12-desaturase) catalyzes the desaturation of oleic acid (18:1) in linoleic acid (18:2). The linoleoyl-PC desaturase (Δ15-desaturase) catalyzes the third desaturation of linoleic acid (18:2) in linolenic acid (18:3) (Ohlrogge and Browse, 1995; Somerville and Browse, 1996). Complementary DNAs corresponding to genes encoding for these enzymes were first isolated in *Arabidopsis thaliana* by genetic methods or heterologous hybridization and mutant complementation (see for review Somerville and Browse, 1996). Such cDNAs were also isolated for most of the oilseed crops like sunflower (*Helianthus annuus* L.) for which stearoyl- and oleoyl-desaturases cDNA were isolated (Hongtrakul *et al.*, 1998a, 1998b; Martinez-Rivas *et al.*, 2001).

The polyunsaturated linoleic (18:2) and linoleic (18:3) fatty acids lower cholesterol atherogenic fraction level but they also lead to decreased cholesterol anti-atherogenic fractions. Diets containing vegetable oil with high oleic acid content have been reported to be most effective for preventing cardiovascular diseases (Delplanque *et al.*, 1997; Broun *et al.*, 1999, Delplanque *et al.*, 2000). Increase of oleic acid content has become one of the major goals to improve vegetable oil quality. In order to reach this purpose, mutagenesis has been used on the major oilseed crops in selecting new varieties with increased oleic acid content in seed oil. Two rapeseed (*Brassica napus*) varieties with high oleic acid content in seed oil were obtained by chemical mutagenesis. The mutagenized locus seems to correspond to a gene encoding for an oleoyl-desaturase located on reticulum endoplasmic (Schierhold *et al.*, 2000). Two different mutations leading to increase oleic acid content in peanut (*Arachis hypogaea*) oil seed were identified. One of them affects the mRNA accumulation of one of the two oleoyl-desaturase genes identified. The second mutation is located in the coding sequence of the other oleoyl-desaturase gene and leads to no enzyme activity (Jung *et al.*, 2000a, 2000b). Transgenic strategies like posttranscriptional gene silencing (PTGS) were also used to increase oleic acid content. High oleic acid content soybean (*Glycine max*) and rapeseed were obtained through antisense-mediated down-regulation of oleoyl-desaturase (Kinney, 1996). Cotton (*Gossypium hirsutum*) transformed with oleoyl-desaturase inverted repeat construct displays a high oleic acid content through hairpin RNA-mediated gene silencing (Liu *et al.*, 2002). As indicated by these examples, the oleoyl-desaturase activity catalyz-

ing oleic acid desaturation seems to be the key step for oleic acid accumulation in seed oil.

The Pervenets sunflower population obtained by chemical mutagenesis displays an oleic acid content in seed oil higher than 65% whereas the wild varieties display an oleic acid content of about 20% (LO varieties for low oleic) (Soldatov, 1976). New varieties with an oleic acid content in seed oil higher than 80% were obtained from Pervenets population by breeding programs (HO varieties for high oleic). This fatty acid composition modification is specifically located in embryo tissues (Garcés *et al.*, 1989). Because of the interest in oleic acid and because of the agronomic performance of HO varieties carrying the Pervenets mutation compared with the LO varieties, these varieties are widely used in the world (about 1.2 million ha, CETIOM 2002). HO genotypes displayed an absence of oleoyl-desaturase activity in their seeds during lipid reserve elaboration steps whereas in LO seeds oleoyl-desaturase activity was detected (Garcés and Mancha, 1989, 1991). Desaturase mRNA accumulation studies performed on embryos from seeds resulting from HO and LO selfed-genotypes by northern (Kabbaj *et al.*, 1996) or by RT-PCR techniques (Hongtrakul *et al.*, 1998) revealed that the Pervenets mutation affects the oleoyl-desaturase mRNA accumulation in immature seeds during lipid reserve elaboration steps. However, since an oleate-desaturase gene is present, the mechanism induced by the mutation that prevents transcript accumulation is still unknown.

In the present study, we explain the methodology that shows that the correlation between the Pervenets mutation and the oleoyl-desaturase mRNA under-accumulation is due to repeated sequences that act *in trans* to induce this mRNA under-accumulation. Furthermore, we developed a molecular study in order to isolate Pervenets mutation based on the candidate gene of the oleate-desaturase (Lacombe and Bervillé, 2000b). For this purpose, we developed a set of recombinant inbred lines to verify the closely genetic linkage between the mutation and a HO specific allele revealed with the oleoyl-desaturase cDNA used as a probe. This enabled to reveal a suppressor for the Pervenets mutation (Lacombe *et al.*, 2001; Lacombe and Bervillé, 2002). According to all these results, we proposed an original mechanism associated to the Pervenets mutation, which has never been described in relation to mutations leading to fatty acid composition modification.

MATERIALS AND METHODS

Plant materials

Segregating recombinant inbred line population: the [LO] line 83HR4 (INRA), male-sterilized by gibberellin, was crossed with the [HOAC] line RHA345 (USA) in our INRA (Mauguio) nursery in 1996. Nine F_1 hybrid seeds were obtained and the F_1 plants were inter-crossed to produce an F_2 generation in a greenhouse during the following winter. From these we obtained 390 F_2 plants, 247 F_3 families, 237

F_4, 196 F_5, and 174 F_6 progenies. These last progenies were used to determine OAC on half-cotyledons of seeds separately for five seeds of each F_6 family obtained in 1999. Five seeds were sown per RI line family. These seeds were sown in Jiffy pots and after keeping for 6 days in a greenhouse they were transferred to the field. For each F_6 family, the RFLP genotype of the second plant in the row was determined with the oleate-desaturase cDNA probe. 83HR4 and RHA345 parental lines were included as controls.

OAC measurement

Measurements of oil composition were performed using Gas Chromatography (GC) (Conte *et al.* 1989).

Molecular methods

Molecular hybridization: DNA preparation, restriction analyses, southern blots, and hybridization were done according to Gentzbittel *et al.* (1994). DNA from [HOAC] or [LO] lines and hybrid genotypes were restricted by *Hind*III and the southern transfers were probed with the oleate-desaturase cDNA. Oleoyl-PC-desaturase probe (oleate-desaturase) was an entire cDNA sequence. The complete sequence has not yet been published (A. G. Abbott, unpublished); it corresponds to accession number HAU1341 (Genbank) by Hongtrakul *et al*. (1998b).

Data management

RFLP profiles revealed on autoradiograms were scored visually. The Qgene package (Nelson, 1997) was used to perform the analysis of variance and to compute linkage, additive and dominance effects.

Map construction

All of the loci were aggregated in a map with MAPMAKER version 3.0b (Lander *et al*., 1987) and the map distance function of Kosambi (Kosambi, 1944). The minimum LOD score value for the threshold was 3.0 and the maximum recombination rate was 0.375 to construct linkage groups.

RESULTS

Functioning of the Pervenets mutation: it causes oleate-desaturase mRNA under-accumulation

We selfed 6 LO and 10 HOAC genotypes and 3 crosses were made between HOAC and LO genotypes. Two of them were reciprocal (Table 1). Seeds from the selfed LO and HO genotypes displayed an OAC between 23 and 38% and between 83 and 91%, respectively. Hybrid seeds displayed an OAC between 61 and 88%. OAC in seeds of reciprocal crosses were in about the same range (78 and 88%) (Table 1).

Table 1: List of the plant material used and level of transcript accumulation determined by northern blot analysis (Lacombe and Bervillé, 2000a)

Genotype	Oleic acid content	Parents phenotype	Source	Embryo production	Desaturase transcript	
					Δ9	Δ12
BE78079		HOAC	Monsanto	lines selfing	+	-
BD40713		LO	Monsanto	lines selfing	+	+
RHA345		LO	USA	lines selfing	+	+
83 HR 4	25%	LO	INRA	lines selfing	+	+
HOC 97	84%	HOAC	Monsanto	lines selfing	+	-
HOC B	86%	HOAC	Monsanto	lines selfing	+	-
HOC 98	85%	HOAC	Monsanto	lines selfing	+	-
HOC 500K	85%	HOAC	Monsanto	lines selfing	+	-
Ha OI 9	86%	HOAC	CSIC	lines selfing	+	-
BD 70080	36%	LO	Monsanto	lines selfing	+	+
BE 78078	85%	HOAC	Monsanto	lines selfing	+	-
BD 70032	35%	LO	Monsanto	lines selfing	+	+
BE 73201.1	84%	HOAC	Monsanto	lines selfing	+	-
BE 73201.2	83%	HOAC	Monsanto	lines selfing	+	-
BE 73201.4	86%	HOAC	Monsanto	lines selfing	+	-
BE 73201.5	85%	HOAC	Monsanto	lines selfing	+	-
90 R 19	23%	LO	INRA	lines selfing	+	+
63 B	29%	LO	INRA	lines selfing	+	+
Santiago	38%	LO	Novartis	hybrid selfing	+	+
Trisun 870	91%	HOAC	Mycogen	hybrid selfing	+	-
Olbaril	84%	HOAC	Pioneer	hybrid selfing	+	-
83 HR 4 × HOC	61%	LO × HOAC	INRA	lines crosses	+	-
Ha 342 × RHA 345	77%	HOAC × HOAC	INRA	lines crosses	+	-
Ha 342 × OPA 2	81%	HOAC × HOAC	INRA	lines crosses	+	-
63 A × HOC B	78%	LO × HOAC	INRA	lines crosses	+	-
HOC A × 63 B	88%	HOAC × LO	INRA	lines crosses	+	-

Stearate and oleate-desaturase mRNA accumulation were examined by northern-blot analysis of RNA isolated from developing embryos during lipid reserve elaboration. A 1.6 kb mRNA was revealed by the stearate desaturase cDNA used as a probe in lanes corresponding to 12- and 16-DAF embryos. No significant intensity difference was shown between all LO and HO embryos resulting either from selfing or crosses (Table 1, Figure 1). The oleate-desaturase cDNA used as a probe revealed a 1.4 kb mRNA in lanes corresponding to 12- and 16-LO embryos. No hybridization signal was revealed in HO embryos resulting either from selfing or crosses. This shows that the Pervenets mutation leading to the HOAC trait is corre-

lated to an absence or a weak oleate-desaturase mRNA accumulation in embryos and acts as dominant.

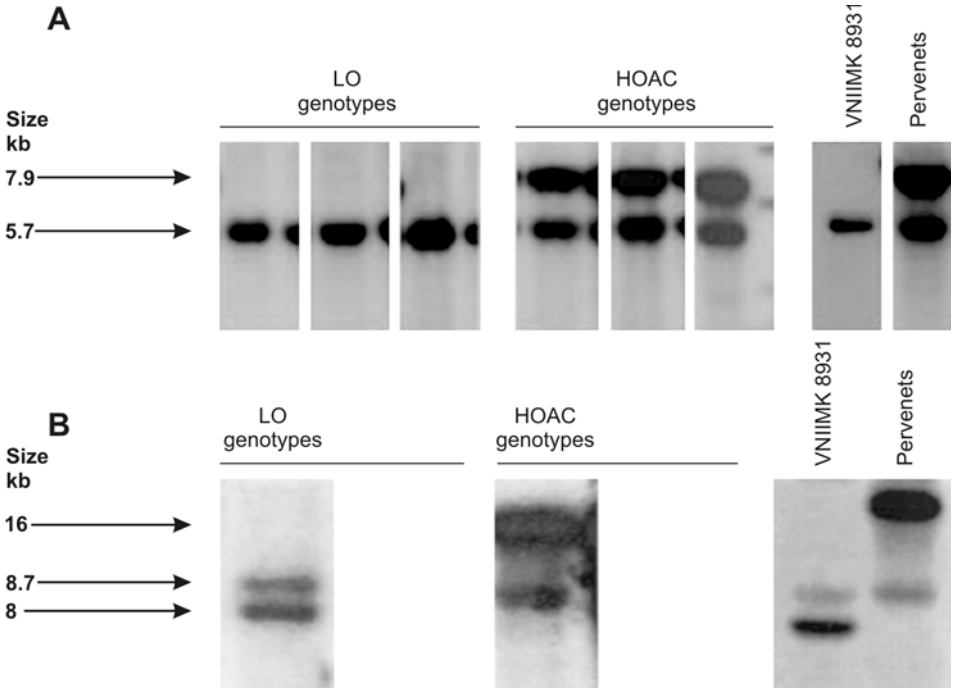

Figure 1: Autoradiogram of southern blot restricted DNA from LO and HO genotypes including VNIIMK 8931 and Pervenets, hybridized with the oleate-desaturase cDNA as a probe
***A:** restriction with EcoRI. Arrows indicate the fragments which differ between profiles in both LO and HO genotypes.*
***B:** restriction with HindIII. Arrows indicate the fragments which differ between profiles in both LO and HO genotypes.*

Characterization of oleate-desaturase genomic regions in HOAC compared with LO genotypes

The LO genotypes carry a HOAC and LO common 5.8 kb *Eco*RI fragment using an oleate-desaturase cDNA as a probe. In addition, the oleHOS allele carries an extra 7.9 kb *Eco*RI fragment revealed with the oleate-desaturase cDNA used as a probe, showing that this extra fragment displays similar oleate-desaturase sequences.

The DNA restricted with *Hind*III displayed a 8 kb fragment in LO genotypes and the hybridized fragment shift from 8 kb to 16 kb in HOAC genotypes. This suggests that the HOAC extra fragment is adjacent to the 5.8 kb *Eco*RI fragment common to both oleHOS and oleLOR alleles. Double *Eco*RI-*Hind*III restriction revealed a common 2.2 kb fragment in both HOAC and LO genotypes and the *Eco*RI 7.9 kb HOAC specific fragment in HOAC genotypes (Figure 1).

Confirmation of genetic linkage between HO trait and Pervenets mutation in a F_2 population

From these data, we constructed physical maps of the oleoyl-desaturase genomic regions in HOAC and LO genotypes with *Eco*RI, *Hind*III and oleoyl-desaturase positions (Figure 2). The oleHOS allele displays 2 adjacent regions carrying oleate-desaturase sequences: the common 5.8 kb *Eco*RI fragment and an extra specific fragment. This extra fragment is exclusively revealed in HOAC genotype carrying the Pervenets mutation. It carries or is in the neighborhood of the mutated allele.

Coincidence of the mutation and the oleate-desaturase locus

Linkage between oleHL and the [HOAC] trait

The [HOAC] and [LO] parents of RI lines displayed oleHOS and oleLOR, respectively. For RI lines, only 6 plants were heterozygous at this locus and eliminated from further analyses. The other 168 RI lines were fixed at the oleHL locus. OleLOR:oleHOS segregated 1:1 (90:78), in agreement with two alleles at the oleHL locus (χ^2 test, $p>0.05$). All RI lines displaying the oleLOR (90) were [LO]. The seventy-eight RI lines displaying the oleHOS allele were distributed equally between [HOAC] (35) and [LO] (43) (1:1, χ^2 test $p>0.3$). Thus, the class of RI lines with oleLOR and [HOAC] was lacking (Figure 2, Table 2).

Table 2: Number of RI lines in each [HOAC] or [LO] class according to the oleLOR or oleHOS alleles at the oleHL locus

	OleLOR	oleHOS
All RILs	96	78
[LO] RILs	96	43
[HOAC] RILs	0	35

One hundred and sixteen RI lines were genotyped with 169 SSR primer pairs out of 805 assayed. Forty-two RI line families were discarded due to amplification troubles. Alleles at 116 loci were not distorted (χ^2 test, $p>0.05$). All of the loci were aggregated in a map of 15 linkage groups covering 508.9 cM with six SSR and the Δ12HL loci remaining unlinked. Using variance analysis with two factors, a non-significant association between SSR polymorphism and OAC variation was detected. However, any line without the Pervenets mutation displayed the HO trait. No interaction was detected between the oleHL alleles (either OleHOS or oleLOR) by computing loci effects two a two.

DISCUSSION

An oleate-desaturase locus maps with Pervenets mutation

One locus carrying the oleHOS allele is characterized by the *Hind*III fragment of more than 15 kb for [HOAC] genotypes and it corresponds to the 8 kb *Hind*III fragment for the [LO] genotypes (Hongtrakul *et al.*, 1998, Lacombe and Bervillé, 2001).

Full dominance of the [HOAC] trait is quite surprising. It has not been reported for rapeseed or peanut, for which high oleic mutants do exist but in which mutations are never dominant (Moore and Knauft, 1989; Schierholt *et al.*, 2000). Dominance occurred when the trait was of transgenic origin (i.e., antisense oleate-desaturase construct) (Lacombe and Bervillé, 2000b).

Perez-Vich *et al.* (2000) have also shown by QTL analyses that the QTL peak for OAC, explaining 84.5% of the variation, coincided with an oleate-desaturase locus. Moreover, several authors have shown under-accumulation for oleate-desaturase transcript in the developing embryos between 10 to 20 days after fertilization (Kabbaj *et al.*, 1996; Hongtrakul *et al.*, 1998; Lacombe and Bervillé, 2000a). All plants in these preceding studies had also been characterized for oleHL locus by Lacombe and Bervillé (2001) and they displayed oleHOS when [HOAC]. The strict correlation in [HOAC] plants between under-accumulation of the oleate-desaturase transcript and oleHOS suggests a functional relationship between these features leading to the absence of oleate-desaturase activity giving rise to the [HOAC] trait (Lacombe and Bervillé, 2000a).

Supole locus

We assumed that since we found only 6 RI lines heterozygous for oleHOS / oleLOR, each RI line family verified to be fixed at oleHL locus could be represented by a single plant. So the genotype of each family was based on the information gained from a single genotyped plant (plant number 2). The mode of inheritance of the [HOAC] trait was directed by one locus in the F_2, so we expected the trait to be directed by one locus in the RI lines also (Lacombe *et al.*, 2001). However, the 3:1 [LO]:[HOAC] ratio was evidence that the [HOAC] trait was directed by two loci in the RI line population.

We hypothesized a suppressor locus because we observed that RI lines carrying oleHOS were split equally into [LO] and [HOAC] classes (1:1, χ^2 test, $p>0.3$), whereas plants carrying oleLOR were all [LO]. The oleHOS region is therefore required for the [HOAC] trait. As we had eliminated the possibility of recombination between the oleHL locus and the locus carrying the Pervenets mutation allele, this distribution indicates another independent locus controlling the [HOAC] phenotype of the RI lines. In the [LO] RI lines carrying oleHOS, the effect of the oleHOS region on the [HOAC] trait could be suppressed by one allele at another locus (supole allele) (Table 2). We therefore explain the 3:1 [LO]:[HOAC] segregation pattern by 2 loci directing the [HOAC] trait.

Organization of the Pervenets mutation

At DNA level, the previously reported Pervenets mutation is carried by or closely linked to a HO-specific allele (oleHOS) revealed with oleoyl-desaturase cDNA used as a probe whereas LO genotypes carried another oleoyl-desaturase allele, oleLOR (Hongtrakul *et al.*, 1998; Lacombe *et al.*, 2001; Lacombe and Bervillé,

2001). Results reported here revealed that the oleHOS carries 2 adjacent regions displaying oleoyl-desaturase sequences. One of these regions is common between oleHOS and oleLOR allele.

Functioning of the Pervenets mutation

A decrease in oleate-desaturase transcript accumulation has been previously reported concerning one HO genotype compared with one LO genotype (Kabbaj *et al.*, 1996) and three partially HO/LO isogenic lines (Hongtrakul *et al.*, 1998). Our experiments, performed on a more important number of unrelated genotypes, lower the probability that the correlation between the HO phenotype and the oleoyl-desaturase mRNA under-accumulation may be due to genetic background effect. They rather emphasized that the Pervenets mutation should affect the oleoyl-desaturase mRNA accumulation in HO embryos leading to the oleoyl-desaturase decreased activity and thus to the oleic acid accumulation.

The 3 hybrid seeds obtained from HO × LO crosses displayed an oleic acid content between 61 and 88%. This agrees with the results concerning the Pervenets mutation dominant effect obtained by genetic approaches (Urie, 1985; Miller *et al.*, 1987; Fernandez-Martinez *et al.*, 1989; Dehmer and Friedt, 1998; Demurin *et al.*, 2000; Velasco *et al.*, 2000; Pérez-Vich *et al.*, 2000; Lacombe *et al.*, 2001). Moreover, we reported the same oleoyl-desaturase mRNA under-accumulation in these hybrids' embryos as observed in HO embryos coming from HO genotype by selfing. This shows that the Pervenets mutation acts *in trans* to induce this mRNA under-accumulation in HO embryos during lipid reserve elaboration steps.

Two different conclusions emerged concerning the [HOAC] trait: the first concerns the Pervenets mutation. We revealed that it was linked to an oleHL locus and led to an OAC of 65%. Here we show that the oleHOS allele is an efficient marker for assisted selection of genotypes carrying the Pervenets mutation. The second conclusion concerns the [HOAC] trait, which was controlled by at least three loci: oleHL, supole, and modifier loci. The complexity of this trait explains the difficulties for breeders when attempting to convert [LO] lines into [HOAC] lines. Moreover, this complex genetic determination of [HOAC] might explain the discordant results based on OAC distribution found in the literature for different crosses. According to the genotypes chosen in studying [HOAC] mode of inheritance, supole and modifier alleles may be present or not leading to discordant conclusions concerning the genetic determinism of the [HOAC] trait.

Acknowledgements

CIFRE Contract and then postdoctoral fellowship between INRA and Monsanto supported Séverine Lacombe. This joint work involved D. Vares (INRA – Montpellier), and P. Jouve, S. Veillet, C. Millet, H. Guillot

and W. Dioh (Monsanto) with the collaboration of A.G. Abbott (Clemson University, SC, USA).

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MUTACIÓN ALTAMENTE OLEICA PERVENETS: INVESTIGACIONES METODOLÓGICAS

RESUMEN

La mutación Pervenets incrementa el contenido del ácido oleico (CAO) en la semilla de girasol de unos 25% (girasol linólico BO) en más de 75% (girasol AO) independientemente de la base genética del genotipo (línea mutante fija, híbrido entre las líneas mutantes fijas, o, lo que es sorprendente, los híbridos formados por cruzamiento en ambas direcciones entre las líneas altamente oleicas y linólicas tanto como linólicas y altamente oleicas). En el presente trabajo desarrollamos la metodología con la cual se indica la coincidencia entre esta mutación y el locus oleato desaturasa. La función de la mutación se modela con el fin de explicar fenotipo.

La acumulación del transcripto de oleato desaturasa fue comparada en la serie de genotipos de girasol en los cuales se presenta la formación de

embrión, 12 a 24 días tras la polinización con o sin presencia de la mutación Pervenets. Los resultados confirmaron la presencia de este transcripto en los embriones normales de BO y la ausencia del mismo en los embriones Pervenets de AO, mientras que la acumulación de transcripto de la desaturasa estearato era igual en ambos tipos de girasol. El método RFLP en el cual se utiliza cADN de oleato desaturasa como sonda, demostró que el girasol con mutación Pervenets, en comparación con el girasol linólico, posee, aparte del fragmento habitual, 5.8kb *Eco*RI, y el fragmento adicional 8kb *Eco*RI y con HindIII paso del 8kb a 16kb del fragmento *Hind*III. El fragmento 5.8kb *Eco*RI, habitual para el girasol normal y para el girasol Pervenets, lleva el gen de oleato desaturasa. El inserto único para el girasol que lleva la mutación Pervenets, tiene que llevar las secuencias de oleato desaturasa. Las investigaciones genéticas de esta mutación dieron resultados contradictorios en la matriz de herencia de las propiedades de AO, gracias a las diferentes expresiones de mutación y al número definido de los factores genéticos que influyen en el alto contenido de ácido oleico. Por tal razón, se consideraba que era importante investigar esa herencia en un conjunto de las líneas consanguínea recombinantes. También hemos senalado que la presencia de esta mutación no es suficiente para inducir la formación del fenotipo de AO, debido a que puede ocurrir que el supresor de la mutación "supole" disfraza la característica de AO. Este alelo supole se encuentra en la mitad de las líneas de AO RI, por lo cual su mapeado preciso resulta difícil. Por ello era necesario combinar los métodos moleculares y genéticos, tanto como las construcciones materiales, para solucionar este problema y para garantizar los instrumentos eficientes para el mejoramiento de girasol de AO.

MUTATION D'ACIDES OLÉIQUES GRAS PERVENETS: RECHERCHES MÉTHODOLOGIQUES

RÉSUMÉ

La mutation Pervenets augmente la teneur en acide oléique chez le tournesol normal (LO) d'environ 25% à plus de 75% (tournesol HO) dans l'huile des graines quel que soit le fond génétique: lignées mutantes fixées, hybrides entre lignées mutantes et dans tous les hybrides quel que soit leur sens entre LO et HO. Ici nous développons la méthodologie pour démontrer la coïncidence entre la mutation et un locus d'oléate-désaturase. La fonction possible de la mutation a été modélisée pour expliquer le phénotype.

Dans une série de génotypes de tournesol des embryons entre 12 et 24 jours après la pollinisation sans et portant la mutation Pervenets ont été utilisés pour comparer avec ceux ne la portant pas, l'accumulation des transcrits de l'oléate-désaturase. Ceci a révélé la présence et l'absence du transcrit respectivement chez le normal et Pervenets alors que pour le transcrit de l'oléate-désaturase l'accumulation est équivalente.

L'étude RFLP a révélé par comparaison au tournesol LO que ceux qui portent la mutation Pervenets montrent à côté du fragment *Eco*RI de 5.8 kb, un RFLP avec un fragment supplémentaire de 8 kb et avec *Hind*III le glissement du fragment de 8 kb à 16 kb, révélé par une sonde d'oléate-désaturase.

Le fragment *Eco*RI commun à Pervenets et au LO de 5.8 kb porte un gène d'oléate-désaturase. L'insertion unique au tournesol portent la mutation Pervenets portent aussi dune séquence d'oléate-désaturase selon l'étude RFLP.

Les études génétiques de cette mutation ont conduit à des résultats contradictoires sur le mode d'hérédité du caractère HO en raison d'un expression variable de la mutation et des facteurs multiples qui contrôlent la teneur oléique. Ainsi, il apparaît important d'utiliser des lignées recombinantes pour étudier le mode d'hérédité. En outre, nous avons ainsi montré que la présence de la mutation n'est pas suffisante pour induire le phénotype HO puisque un suppresseur de la mutation supole peut masquer le caractère HO. Le suppresseur supole n'est révélé que dans la moitié des lignées HO ce qui rend son marquage difficile.

La conjonction de méthodes génétiques et moléculaires ainsi que la construction de matériels sont requis pour résoudre les problèmes posés en amélioration du tournesol HO afin d'acquérir la maîtrise de cette mutation