Genetic, molecular and expression features of the Pervenets mutant leading to high oleic acid content of seed oil in sunflower

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Summary : Pervenets is a sunflower population that displays seed oil with a high oleic acid content [HOAC]. Our aim is to reconcile all the data gathered on this mutant in a unique explanatory mechanism. All Pervenets-derived [HOAC] lines display no accumulation or a very reduced accumulation of the DELTA12-desaturase transcript in the embryos during the stages for oil accumulation. They also carry oleHOS specific RFLP markers revealed by an DELTA12-desaturase cDNA used as a probe. The linoleic or [LO] genotypes do not carry this RFLP marker, but another allele: oleLOR (oleHL locus). Linkage disequilibrium between the oleHOS allele and [HOAC] was verified. We studied the mode of inheritance of [HOAC] in two segregating populations. A F2 progenies revealed one dominant allele for [HOAC] that co-segregated with the oleHOS allele showing that the Pervenets mutation and oleHOS were closely linked. F6 recombinant inbred lines, showed the [HOAC] trait due to two independent loci: the locus carrying the oleHOS allele and another locus sup. One allele, supole, at this second locus may suppress the effect of the oleHOS allele on the [HOAC] trait. Northern analyses performed on [HOAC] lines and F1 ([HOAC] x [LO]) hybrids revealed under-accumulation of DELTA12-desaturase transcript. Thus Pervenets mutation acts in trans. The oleHOS genomic region that may carry the Pervenets mutation was cloned. A genomic library was constructed in lambdafixII with the DNA from the RHA345 [HOAC] line and screened with a DELTA12-desaturase cDNA as a probe. Two overlapping clones were entirely sequenced and revealed carrying a gene for an DELTA12-desaturase probably located in the RE. This corresponds to the invariant part of the oleHL locus. Another clone (11.1) probably carries DELTA12-desaturase repeated sequences that cause instability of the clone. We showed that the 11.1 clone carries most of cDNA sequence, but due to its organization it is not yet sequenced. A mutation mechanism is proposed: duplicated DELTA12-desaturase sequences probably act in trans to silence the normal gene. The existence of gene silencing for the Pervenets mutation and modifier alleles (supole) that probably act on gene silencing mechanism have never been suggested to explain [HOAC] trait. These facts and explanations may explain the discordant results found in literature.

Keywords : cloning, gene silencing, high oleic acid content, mutant, DELTA12-desaturase desaturase, RFLP, sunflower, suppression.

ARTICLE

Pervenets is a sunflower population with a seed oil oleic acid content greater than 65% constituted from one mutant. The mutant has been obtained after mutagenesis treatment on VNIIMK 8931 [1]. About 200 sunflower heads from the population VNIIMK 8931 were treated with dimethyl sulphate. Thirty M_1 seeds from each 200 sectors were sown on one lane and plants were self-pollinated and the operation was repeated until the M_3 generation. To detect eventual fatty acid mutants, Soldatov collected pollen on 10 different M_3 progenies to pollinate one normal plant. Thus, Soldatov repeated this operation 200 times. He sowed 30 seeds per pollinated head and after self-pollination of each plant he used 30 seeds per plant to extract oil. Thus, he analyzed 6,000 oil samples per iodine method or gas chromatography and only one displayed an Oleic Acid Content (OAC) of 50.3% higher than those of other plants. He sowed the remaining seeds from the plants that had such an OAC of 50.3% and self-pollinated each of them. He used again 30 seeds per head to extract oil and mixed the seeds from all the progenies, which displayed an OAC higher than 40%. Thus, he constituted the Pervenets population. After two cycles, by intercrossing plants in Pervenets, he enhanced the average OAC of Pervenets to 65% (range 60 to 80%). This report clearly shows that the Pervenets mutant came from only one pollen grain (Soldatov, personal communication). The Pervenets population is used worldwide as a [HOAC] source in breeding programs to produce [HOAC] lines and commercial hybrids with an OAC over 83%. Thus, these [HOAC] genotypes cumulate Pervenets mutation effect and other independent factors acting on OAC.

On the other hand, several genetic studies led to various conclusions concerning the mode of inheritance, the dominance and maternal effects of different factors affecting the [HOAC] trait [2]. Moreover, since the mutation was not marked, its effects had not been unravelled from other factors.

Our goals were to determine the step altered by the Pervenets mutation and to explain why the Pervenets mutation controls more or less the OAC in different genetic backgrounds. To unravel the effects of the Pervenets mutation from other factors affecting OAC we first marked the Pervenets mutation using RFLP technology in a diversity analysis. This enabled us to follow the effect of mutation region on OAC variation in a F2 and recombinant inbred line populations segregating for [HOAC]/[LO] traits. Northern analyses performed on seeds resulting from [HOAC] genotypes selfing or from crosses in both directions ([HOAC] x [LO] and [LO] x [HOAC] revealed under-accumulation of DELTA12-desaturase transcript in the seed at the stage for oil deposit. Then we constructed the physical map of the oleHOS region. This region was cloned and characterized.

Finally, we reconciled in a model such a rearrangement of DELTA12-desaturase sequences in relation with the mechanism of Pervenets mutation that decreases DELTA12-desaturase transcript level, and the disturbances in the mode of inheritance that affects OAC content.

Materials and methods

Plant materials

** Segregating F2 population*

The [LO] line BD40713 was used as the female parent in a cross with the [HOAC] line BE78079 from Monsanto. One F1 plant was self-fertilized to produce the F2 progeny, composed of 107 plants in a greenhouse in 1998. They were used both to determine OAC on half a cotyledon of each seed, and to detect RFLP using a sunflower DELTA12-desaturase cDNA probe. Comparisons were made with the BD40713 and BE78079 (Monsanto) parent lines and F1 plants of the same cross were treated in the same way.

** Segregating recombinant inbred line population*

The [LO] line 83HR4 (Inra), male-sterilised by gibberellin, was crossed with the [HOAC] line RHA345 (USA) in our Inra nursery during the summer of 1996. Nine F1 hybrid seeds were obtained and the F1 plants were inter-crossed to produce the F2 generation in a greenhouse during the following winter. From these F1 plants we obtained 390 F2 plants and further 174 F6 progenies. These last progenies were used to determine OAC on half a cotyledon separately for five seeds of each F6 family. The corresponding five seeds were sown per RI line family. Three plants were self-pollinated but only one was randomly chosen to constitute the next generation. The seeds were sown in Jiffypots and after 6 days in a greenhouse they were transferred to the field. For each F6 family in the field, the RFLP genotype of plant number 2 on the row was determined with the DELTA12-desaturase cDNA as a probe. 83HR4 and RHA345 parental lines were included as controls.

Measurement of OAC

Measurements of oil composition were performed using gas chromatography (GC) [3]. When used the refractometer method [4] was performed on the oil from 20 seeds of the next generation. The results from both methods are consistent for lines.

Molecular techniques

Plant DNA preparation, restriction, southern transfer and labelling of probes were done according to Lacombe and Bervillé [5]. DNA preparation from phage genomic clones, and subsequent treatment were done according to the protocol given by the provider.

** Southern analyses: molecular hybridisation*

DNA preparation, restriction analyses, Southern transfers, and hybridisation were done according to Gentzbittel *et al.* [6]. DNA samples from [HOAC] or [LO] lines or hybrid genotypes were restricted by *HindIII* and the Southern transfers were probed with the DELTA12-desaturase cDNA.

** Probes: stearoyl PC desaturase has been described by Kabbaj et al. (1996)*

DELTA9-desaturase has been described by Kabbaj *et al.* [7]. DELTA12-desaturase corresponds to an entire cDNA sequence. The complete sequence has not yet been published (A. G. Abbott unpublished); it corresponds to accession number U91341 (Genbank) by Hongtrakul *et al.* [8].

**** Northern analysis***

A series of genotypes either lines or F1 hybrid were used for Northern analyses *(Table 1)*. Total RNAs were extracted from 200 mug of immature embryos using "RNA Instra Pure System" protocol (Eurogentec). An equal amount of RNA (15 mug) was denatured with formaldehyde and formamide. The denatured RNAs were electrophoresed in a 1.2% agarose-15% formaldehyde gel and the gel was photographed after ethidium bromide (EtBr) staining to demonstrate equal loading of RNA. The gel was transferred to a nylon membrane to obtain Northern blots [9].

The DELTA9-desaturase cDNA and the DELTA12-desaturase cDNA were used to produce labelled probes with [^{32}P] alpha-dCTP by random primer reaction, as described by Feinberg and Vogelstein [10]. Different length of exposure for membranes onto films was performed depending on the level of radioactive spots on the membranes.

**** Genomic library construction***

We used the line RHA345 from USA as source of the genomic DNA. Genomic DNA was partially restricted with *Sau*3A and sized for 15 kb fragments on average. The 15 kb fraction was ligated to the arms of lambdafixII phage (Stratagene) restricted with *Xho*I. The final phage library was 2 x 10^6 phages per ml.

Two million clones were spread on recipient bacteria and plates were Southern transferred to nylon membranes for screening with a DELTA12-desaturase cDNA. Finally, 10 clones hybridizing with the DELTA12-desaturase cDNA were retained and further studied.

Data management

RFLP profiles revealed on autoradiograms were scored visually. The Qgene package [11] was used to perform analysis of variance and to compute linkage, additive and dominance effects.

Results

Diversity analysis

It was performed on 114 [LO] and 125 [HOAC] genotypes representing the diversity of cultivated sunflower of different public institutes and companies. This showed that [LO] and [HOAC] genotypes carry specific DELTA12-desaturase RFLP markers, oleLOR and oleHOS respectively, revealed by an DELTA12-desaturase cDNA used as a probe *(Figure 1)*. It corresponds for the [LO] and the [HOAC] genotypes to a 8 kb and15 kb *Hind*III fragment respectively, whereas no polymorphism for the DELTA9-desaturase was correlated to the [HOAC] trait. Linkage disequilibrium between the oleHOS allele and Pervenets mutation was verified and probably due to linkage or coincidence between the two loci [5].

Segregation between alleles at oleHL locus and Pervenets mutation

We studied the inheritance of [HOAC] in two segregating populations.

**** In F2 segregation population***

The first study concerned 107 F2 progenies from the cross *BD40713* [LO] x *BE78079* [HOAC]. [HOAC] and [LO] F2 population parents displayed oleHOS and oleLOR, respectively. OleLOR:oleHOS segregated 1:2:1 (33 oleLOR/ oleLOR:50 oleLOR/oleHOS:24 oleHOS/oleHOS) in the F2 population, in agreement with 2 alleles at one locus: oleHL (khi^2 test, $p > 0.3$). All the F2 plants with OAC higher than 65% displayed oleHOS in the homozygous or heterozygous state, whereas all the F2 plants with OAC lower than 65% displayed homozygous oleLOR *(Figure 2A)*. On the basis of genotype at the oleHL locus (whether homozygous oleLOR, or homozygous oleHOS and heterozygous), the OAC threshold was 65%, whereas the OAC threshold between [LO] and [HOAC] was 70%. All plants were correctly classified as [LO] or [HOAC] with respect to both OAC and the alleles at the oleHL locus, except for 2 plants that were classified in [LO], even though they carried oleHOS. Linkage between the oleHL locus and the locus directing [HOAC] was computed. The oleHOS allele explained most of the variation in OAC ($R^2 = 0.8642$), with strong linkage ($p < 10^{-4}$) between the oleHL locus and the locus directing [HOAC]. Additivity (a=22.07%) and dominance (d = 19.57%) were highly significant for oleHOS on OAC.

**** In F6 recombinant inbred lines***

[LO] and [HOAC] parents of the RI lines displayed an average of 19 and 84% OAC, respectively. For the 174 F6 RI lines, OAC varied from 12 to 92%. The OAC histogram is shown in *Figure 2B*. The OAC threshold between [HOAC] and [LO] RI lines obviously could be placed at 55%. The segregation pattern, 35 [HOAC] RI lines and 139 [LO] RI lines, agreed with a 1:3 [HOAC]:[LO] ratio (khi^2 test, $p > 0.1$). This ratio supports the hypothesis that [HOAC] is directed by two independent loci. 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 for further analyses. The other 168 RI lines were probably fixed at the oleHL locus. OleLOR:oleHOS segregated 1:1 (90:78) in agreement with two alleles at the oleHL locus (khi^2 test, $p > 0.05$). All RI lines displaying the oleLOR (90) were [LO]. The 78 RI lines displaying the oleHOS allele are distributed equally between [HOAC] (35) and [LO] (43) (1:1, khi^2 test $p > 0.3$). Thus, the class of RI lines with oleLOR and [HOAC] is lacking *(Figure 2B, Table 2)*.

Northern analyses

The oleic acid content of the seeds resulting from selfed plants or crosses is shown in *Table 1*. The range for OAC variation in seeds resulting from [LO] and [HOAC] lines was from 23 to 38% and 83 to 91%, respectively. The content in oleic acid for seeds resulting from crosses between [HOAC] and [LO] lines ranges between [HOAC] and [LO] lines ranges between 61 to 88%. The sense of the cross does not seem to influence greatly the fatty acid composition of the resulting seeds.

**** Accumulation of the* DELTA*9-desaturase transcript***

We checked by staining RNA with EtBr that equivalent amounts were loaded in each lane *(Figure 3A)*. The accumulation of DELTA9-desaturase transcript in immature embryos resulting from selfing of [HOAC] or [LO] genotypes was revealed by the hibridization of Northern blots by the DELTA9-desaturase cDNA used as a probe *(Figure 3B, Table 3)*. A 1.6 kb transcript was revealed in each lane corresponding to the 12- and 16-DAP embryos. No significant intensity difference was shown between all [HOAC] embryos and [LO] embryos in all genotypes examined.

The same protocol was performed to reveal the DELTA9-desaturase transcript accumulation in immature embryos resulting from crosses between [HOAC] x [LO] or [LO] x [HOAC] lines. A 1.6 kb transcript was revealed in each lane corresponding to the 12- and 16-dap embryos. The intensity of these bands was comparable to the intensity of the bands revealed in Northern blots of RNA of embryos resulting from [HOAC] or [LO] *(Figure 3B, Table 1)*.

**** Accumulation of* DELTA*12-desaturase transcript***

The accumulation of DELTA12-desaturase transcript in immature embryos resulting from selfing of [HOAC] or [LO] genotypes was revealed by the hybridisation of Northern blots by the DELTA12-desaturase cDNA used as a probe *(Figure 3C, Table 1)*.

A 1.3 kb transcript was revealed in each lane corresponding to the 12- and 16-DAP [LO] embryos. However, no hybridization signals was revealed for [HOAC] embryos.

The same protocol was performed for the embryos resulting from [HOAC]x[LO] or [LO] x [HOAC] crosses. No hybridisation signal was revealed in any of these [HOAC] embryos *(Figure 3C, Table 3)*.

Cloning of a fragment carrying the Pervenets mutation

**** Construction of the* DELTA*12-desaturase region physical map***

We first constructed the physical map of the DELTA12-desaturase region in both [LO] and [HOAC] genotypes knowing that at the oleHL locus in the [LO] there is the oleLOR allele corresponding to a 5.8 *Eco*RI and a 8 *Hind*III fragments *(Figure 1)*. The double digest and with *Eco*RI + *Hind*III led to a 2.2 kb fragment carrying the DELTA12-desaturase sequence. In the map of the oleHOS allele, the 5.8 kb *Eco*RI fragment is still present but another extra fragment of 7.9 kb fragment is also present *(Figure 1)*. With *Hind*III, the 8 kb fragment in the [LO] lengthens to 16 kb in the HO. The double restriction also led in the [HOAC] to the 2.2 kb fragment. Moreover, the 7.9 *Eco*RI fragment is still present *(Figure 1)*. Hybridisation signal intensity of the 7.9 kb *Eco*RI extra fragment and PCR analyses with 8 primer pairs covering with overlapping the DELTA12-desaturase cDNA suggest that the fragment carries at least one copy of the DELTA12-desaturase sequence. These data enabled us to construct the oleHL region reference map with *Eco*RI and *Hind*III sites and DELTA12-desaturase sequence position *(Figure 4)*. The oleHOS allele is made of two adjacent regions that carry DELTA12-desaturase sequences, the 5.8 kb EcoRI fragment common to both alleles plus an extra specific fragment.

**** lambda clones carrying oleate similar sequences***

A genomic library was constructed in lambdafixII with the DNA from the RHA345 [HOAC] line with an average insert size of 15 kb. Two millions clones were screened enabling to isolate clones ranging in four groups. Group I genomic clones from two of the four groups displayed only faint signal with the DELTA12-desaturase cDNA as a probe and were therefore not studied further. The eight clones from the group I displayed RFLP fragments hybridizing the DELTA12-desaturase cDNA as a probe with intense signals. Two clones (15.4 and 44.1) from group I, were entirely sequenced and thus revealed that they carry a functional DELTA12-desaturase gene. It is carried by the 5.8 kb EcoRI fragment and it therefore corresponds to the invariant part of the oleHL locus. Further sequence studies show that the clones of group I apparently carry a functional DELTA12-desaturase gene probably located in the endoplasmic reticulum (ER). It is interrupted by a 1,678 bp intron between nt 87 and 88 of the

transcript sequence, 29nt upstream of the initiation codon ATG (5' UTR). The intron is located at the same position in *Arabidopsis* but it is 1,300 bp [12]. Group II clones also hybridized strongly with the DELTA12-desaturase cDNA as a probe, but they differ from group I by carrying another DELTA12-desaturase similar sequence on another *Eco*RI-*Hind*III fragment differing by size to the 2.2 kb previous fragment. Clones 27.1 and 11.1 overlapped but the 11.1 is characterized by an instability leading to a smear when probed with the DELTA12-desaturase cDNA but not when probed with another fragment of the 11.1 clone as a probe. Consequently, the 11.1 sequence probably carries DELTA12-desaturase repeated sequences that cause instability of the clone. PCR amplification products from clone 11.1 DNA with 8 primer pairs covering with overlapping, most of the DELTA12-desaturase cDNA, when hybridized with the DELTA12-desaturase cDNA as a probe showed that the 11.1 clone carries approximately 1kb of an DELTA12-desaturase cDNA corresponding sequence. However, the instability of this clone does not allow its sequencing.

**** Alignment of groups I and II clones on the physical map***

The clones were tentatively aligned on the genomic physical map and we thus obtained a refined map for this region, locating the *Hind*III site in the intron. The common part of both alleles correspond to the group I clones and it carries a DELTA12-desaturase gene. The specific oleHOS part corresponds to the group II clones and carries repeat motives of DELTA12-desaturase sequences.

Consequently, in the [HOAC] mutant Pervenets the organization of this region appears with a tandem repeat of the DELTA12-desaturase sequences whereas there is a single copy in the [LO] genotypes.

Discussion

One or two loci directed [HOAC] trait depending on the crosses

We characterized strong linkage disequilibrium for oleHOS and Pervenets mutation alleles. We can therefore conclude that it is the oleHOS allele at the oleHL locus that carries, or is very close to, the Pervenets mutation allele.

One locus carrying the oleHOS allele is characterized by the *Hind*III fragment of 16 kb for [HOAC] genotypes and it corresponds to the 8 kb *Hind*III fragment for the [LO] genotypes [5, 8]. In the F2 segregating population, we observed co-segregation between the oleHOS allele and OAC higher than 65% ([HOAC] trait). Thus, we revealed a strong genetic linkage between an oleHL locus carrying oleHOS/oleLOR alleles and the Pervenets mutation allele ($R^2 = 0.8642$, $p < 10^{-4}$). This suggests that the Pervenets mutation allele is either at the oleHL locus or strongly linked to it. Moreover, the dominance/additivity ratio (90%) reveals an almost complete dominance effect of the oleHOS region on OAC. This is in agreement with the observed 3:1 [HOAC]:[LO] segregation ratio in the F2, strong evidence that the [HOAC] trait is directed by the oleHOS region carrying the dominant allele.

Despite the strong genetic linkage, two [LO] plants of our F2 population displayed the oleHOS allele of the [HOAC] parent. These two F2 plants had oleHOS, but [LO] were in the upper extreme range of the [LO] class. In order to explain these F2 plants, we excluded recombination between the mutation and the locus directing [HOAC] since such a recombination event was not detected in the diversity study. This suggests that modifier genes could enhance OAC variation leading plants in the lower extreme of the [HOAC] class to shift to a higher [LO] class and *vice versa*.

The hypothesis involving modifier genes (point 3) has already been put forward in the literature [13, 14]. We consider that these genes lead to a modification in OAC, making extreme [LO] or [HOAC] positions uncertain. They may also explain the two F2 plants with oleHOS in the upper extremity of the [LO] class. They should have a small effect on OAC in comparison with the Pervenets mutation allele, but their effect may be sufficient to explain the shift of the OAC threshold from 70 to 65%.

Supole locus

We assumed that since we only found 6 RI lines heterozygous for oleHOS/oleLOR, each RI line family verified to be fixed at oleHL locus and 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 Nr 2). The mode of inheritance of the [HOAC] trait was directed by one locus in the F2, so we expected the trait to be directed by one locus in the RI lines also. 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 equally split into [LO] and [HOAC] classes (1:1, khi^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 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 3)*. We therefore explain the 3:1 [LO]:[HOAC] segregation pattern by 2 loci directing the [HOAC] trait [15].

Strong decrease of* DELTA*12-desaturase transcript accumulation

The measure of the oleic acid content in the seeds revealed that all seeds resulting from [LO] genotypes selfing display a [LO] content (between 23 and 38%). Moreover, all seeds resulting from [HOAC] genotypes selfing or from crosses have a [HOAC] (between 83 and 91%) *(Table 1)*. So, the study of accumulations of both desaturases transcript in these immature embryos reflects what happened during the constitution of linoleic lipid reserve in the case of [LO] embryos or what happened during the constitution of high oleic lipid reserve in the case of [HOAC] embryos.

The study of the DELTA9-desaturase transcript accumulation in immature embryos revealed a transcript accumulation at 12- and 16-DAP. We revealed no difference in DELTA9-desaturase transcript accumulation between [LO] and [HOAC] embryos resulting from selfing or crosses. These results agree with those of Kabbaj *et al.* [7] on one [LO] and one [HOAC] lines. Hongtrakul *et al.* [8] have shown a DELTA9-desaturase transcript accumulation in 3 [LO] and 3 [HOAC] embryos using RT-PCR but with this method, it is difficult to determine the quantity of transcript accumulation. Thus, according to our results on a relative high number of different genotypes (6 [LO], 14 [HOAC] and 5 hybrid genotypes), the [HOAC] phenotype did not appear to be correlated to an over accumulation of the DELTA9-desaturase transcript in [HOAC] embryos during the stages of lipid reserve biosynthesis.

On the other hand, the study of the DELTA12-desaturase transcript accumulation revealed a specific transcript accumulation at 12- and 16-DAP only in [LO] embryos. This result agreed with those of Kabbaj *et al.* [7]. They revealed a DELTA12-desaturase transcript accumulation at 1.3kb only in [LO] embryos at 12-, 16- and 20-DAP and no significant DELTA12-desaturase transcript accumulation in

[HOAC] embryos. Using RT-PCR, Hongstrakul *et al.* [8] have shown a drastically reduced signal in [HOAC] embryos, but this method is not quantitative. It is thus difficult to tell whether this signal corresponds to a significant DELTA12-desaturase transcript accumulation in [HOAC] embryos. So, our results clearly show that in all genotypes tested, the [HOAC] phenotype is correlated to a strong reduction in the level of DELTA12-desaturase transcript accumulation in [HOAC] embryos during lipid reserve biosynthesis.

Dominance of the [HOAC] and no transcript accumulation

The study of hybrid embryos resulting from crosses between [HOAC] and [LO] lines revealed some elements on the possible mechanisms leading to high oleic acid accumulation. Indeed, the measure of the oleic acid content in mature hybrid seeds showed high oleic acid content, whatever the sense of the cross *(Table 1)*. This high oleic phenotype appears consequently to be dominant without major maternal effect. These results agreed with those of Varés *et al.* [16]. Moreover, the study of both desaturases transcript accumulation in immature hybrid embryos revealed the same results as the study on [HOAC] embryos resulting from selfing: the same DELTA9-desaturase transcript accumulation as in [LO] embryos resulting from selfing and an under-accumulation or no-accumulation of DELTA12-desaturase transcript. The expression of the mutation leading to this strong reduction in the level of DELTA12-desaturase transcript accumulation therefore behaves as dominant. Reciprocal crosses indicate no major maternal effects.

CONCLUSION

We have already reported that the main effect of the Pervenets mutation is to decrease the DELTA12-desaturase transcript accumulation leading to prevent enzyme activity during the key stage for lipid deposit causing oleic acid accumulation [7, 17]. A duplication at the DNA level has already been found causing reduction in transcript accumulation in different plant species and *Arabidopsis* [18], here it is causing silencing of the normal DELTA12-desaturase gene, although it is structurally normal.

As a conclusion the genomic organization in the [HOAC] Pervenets mutant appeared repeated motives of a DELTA12-desaturase region. Consequently, the Pervenets mutation is probably the duplicated region. This region causes silencing of the normal DELTA12-desaturase gene and it behaves at dominant. Moreover, the suppressor locus could correspond either to an alternative pathway with a DELTA12-desaturase restoring the normal DELTA12-desaturase function, or to a gene that could prevent silencing, thus leaving the normal DELTA12-desaturase allele functioning. Such a mechanism has already been described by Vaucheret and Fagard [19]. Further studies are undertaken to verify whether gene silencing acts at transcriptional or posttranscriptional levels.

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Illustrations

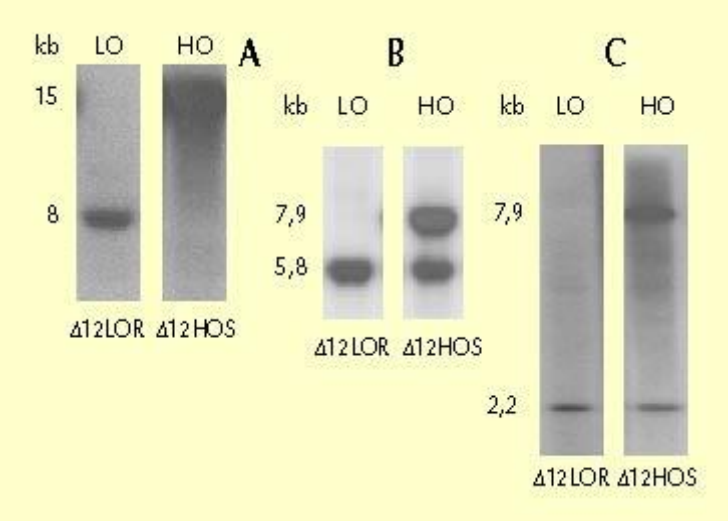
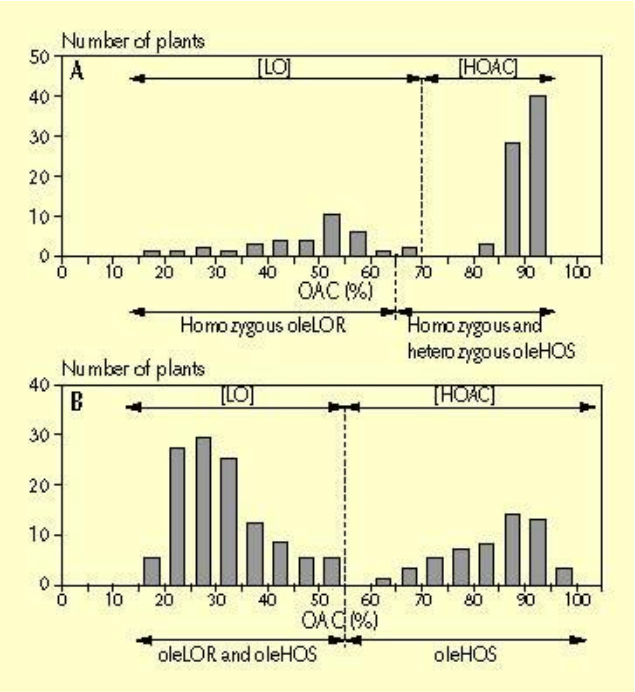


Figure 1. *Autoradiography film of* Hind*III restricted DNA* ***(A)****,* Eco*RI* ***(B)****, and double digest* ***(C)*** *of hybrids, inbred lines and populations transferred into a Southern blot and hybridized with the DELTA12-desaturase cDNA used as a probe.*



Upper dotted vertical lines indicate the OAC threshold between the [LO] and [HOAC] classes and upper horizontal arrows indicate the [LO] and the [HOAC] classes. Lower horizontal arrows indicate the distribution of the oleHOS and oleLOR alleles at the oleHL locus and lower dotted vertical lines indicate the OAC threshold between oleHOS and oleLOR classes.

Figure 2. *Distribution of OAC.* **A)** *for 107 F2 progenies ;* **B)** *distribution of OAC for 174 RILs.*

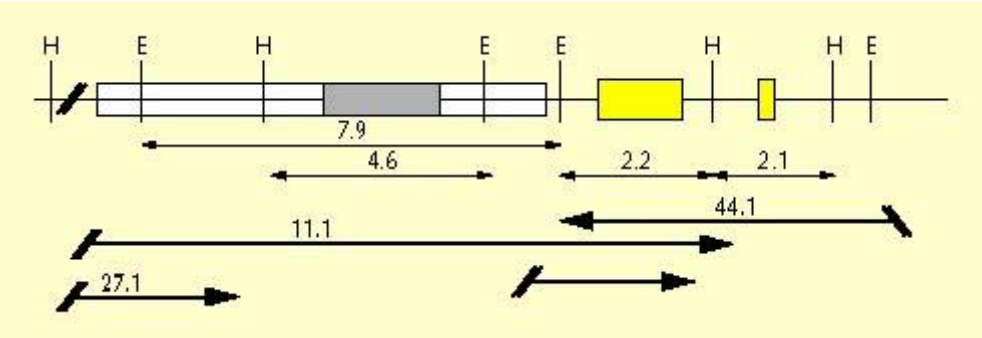


Figure 3. *Northern blots of transcript from embryos harvested 12, 16 or 24 days after flowering (DAF) and* ***A)*** *stained by EtBr, and probed* ***B)*** *with the DELTA9-desaturase, and* ***C)*** *with the DELTA12-desaturase cDNA.*

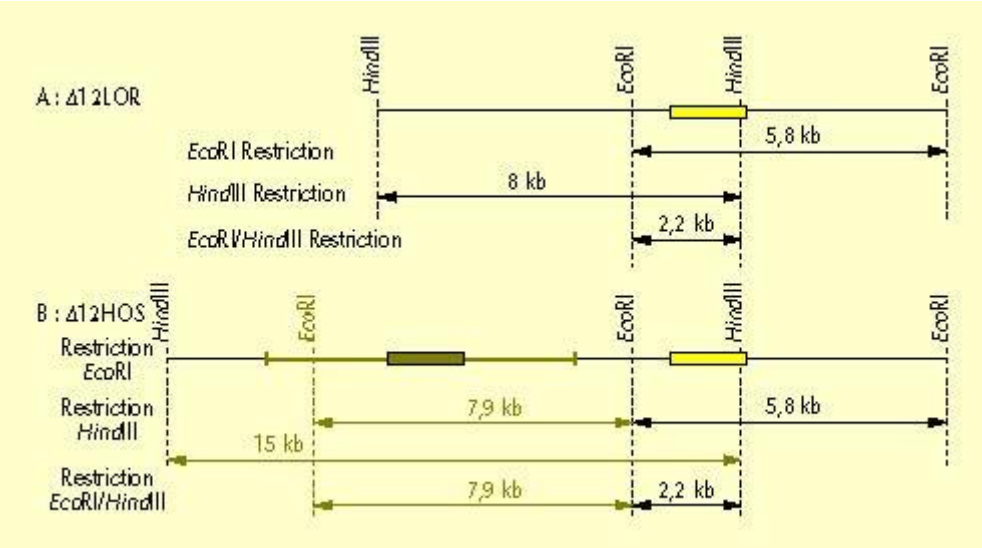


Figure 4. *Physical maps of DELTA12-desaturase regions for **A)** the oleLOR, and **B)** the oleHOS alleles.*

Table 1. *Genotypes used to obtain embryos by selfing or crosses and oleic acid content of the seeds generated (in % of the total seed oil). The presence (+) or absence (–) of desaturase transcript accumulation is noted.*

Genotype DELTA12	Parents	Source	Embryos	Oleic acid	Transcript	
					DELTA9	
	phenotype		production	content		
Santiago	[LO]	Novartis	Hybrid selfing	38%	+	+
Trisun 870	[HOAC]	Mycogen	Hybrid selfing	91%	+	–
Olbaril	[HOAC]	Pioneer	Hybrid selfing	84%	+	–
HOC 97	[HOAC]	Monsanto	Line selfing	84%	+	–
HOC B	[HOAC]	Monsanto	Line selfing	86%	+	–
HOC 98	[HOAC]	Monsanto	Line selfing	85%	+	–
HOC 500K	[HOAC]	Monsanto	Line selfing	85%	+	–
Ha OI 9	[HOAC]	CSIC	Line selfing	86%	+	–
BD 70080	[LO]	Monsanto	Line selfing	36%	+	+
BE 78078	[HOAC]	Monsanto	Line selfing	85%	+	–
BD 70032	[LO]	Monsanto	Line selfing	35%	+	+
BE 73201.1	[HOAC]	Monsanto	Lines selfing	84%	+	–
BE 73201.2	[HOAC]	Monsanto	Line selfing	83%	+	–
BE 73201.4	[HOAC]	Monsanto	Line selfing	86%	+	–
BE 73201.5	[HOAC]	Monsanto	Lines selfing	85%	+	–
90 R 19	[LO]	Inra	Line selfing	23%	+	+
63 B	[LO]	Inra	Line selfing	29%	+	+
83 HR 4	[LO]	Inra	Line selfing	25%	+	+
83 HR 4 x HOC	[LO] x [HOAC]	Inra	Line crosses	61%	+	–
Ha 342 x RHA 345	[HOAC] x [HOAC]	Inra	Lines crosses	77%	+	–
Ha 342 x OPA 2	[HOAC] x [HOAC]	Inra	Lines crosses	81%	+	–
63 A x HOC B	[LO] x [HOAC]	Inra	Line crosses	78%	+	–

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

Table 3. *Prediction of classes for RI lines according to two loci: oleHL and suppressor loci. Sup0 corresponds to a null allele on [HOAC].*

Genotypes		OAC classes	
Locus oleHL	Locus Supole	[LO]	[HOAC]
oleLOR	Sup0	[LO]	
oleLOR	Supole	[LO]	
oleHOS	Sup0		[HOAC]
oleHOS	Supole	[LO]	