The molecular basis of the high linoleic acid content in Petunia seed oil: analysis of a seed-specific linoleic acid mutant

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Abstract
From a random transposon mutagenesis experiment, using Petunia line W138, a seed-specific linoleic acid mutant was isolated. The tagged gene was cloned and identified as a microsomal Δ12 desaturase. Expression of the gene, however, was constitutive and not, as might have been expected, seed-specific. Moreover, self-fertilized homozygous mutants still contain 40% 18:2 in the seed lipid fraction. This suggests that at least two (seed-specific) Δ12 desaturase genes are responsible for the high linoleic acid content in Petunia seed oil. Five members of the microsomal Δ12 desaturase gene family have been identified and isolated. Data are presented on the molecular characterization and tissue-specific expression of these genes, which suggest that, in Petunia, the flux through the prokaryotic and eukaryotic pathways of lipid synthesis might be different from the situation found in Arabidopsis.

Introduction
Petunia line W138 contains more than 200 copies of the 284-bp transposable element dTphI. The element is so active that virtually any gene in the Petunia genome will be hit by a dTphI insertion at a frequency of about 1 in 1000 plants [1]. Transposon-saturation mutagenesis in Petunia hybrida has proven a powerful tool to generate mutants disturbed in the synthesis and/or regulation of seed lipids [2]. From a random-transposon-mutagenesis experiment, 49 out of 1638 putative transposon-induced mutants had a significant deviation from the average values for oil content and fatty acid profile in their seeds. Mutant 629 was studied in more detail. The seed fatty acid profile of this line showed 30% 18:1 and 60% 18:2, as compared with 10% 18:1 and 80% 18:2 found in wild-type Petunia seeds. Line 629 is heterozygous for the mutation, which results in an altered lipid phenotype exclusively in seeds. Self-fertilized homozygous mutants still contain 40% 18:2 in the seed lipid fraction [2].

Results and discussion

Line 629 contains a mutated microsomal Δ12 desaturase (fad2)
Based on the decreased linoleic acid level of mutant 629 we designed degenerate fad2 primers, corresponding to highly conserved regions from other plant microsomal fad2 sequences. PCR amplification on genomic DNA of line 629 using a degenerate fad2 and a dTphI primer confirmed that mutant 629 contains a dTphI transposon insertion in a fad2 gene. We established unambiguously that this gene, designated desA, represents a fad2 locus, by heterologous expression in the yeast Saccharomyces cerevisiae. PCR analysis showed that progeny of mutant 629 with altered linoleic acid profiles either contain a dTphI insertion or dTphI footprints of 7 or 8 bp in desA. Expression in yeast showed that these mutant footprint proteins were inactive and therefore cannot be responsible for the remaining 40% 18:2 found in the seed lipid fraction of the homozygous mutant progeny.

The Petunia fad2 gene family
Genomic Southern-blot analyses suggested the existence of at least five fad2 genes. Using degenerate fad2 primers and genomic DNA walking (using the Universal Genome-Walker Kit, Clontech), five fad2 genes (desA, B, C, D and E) have now been identified and completely or partially sequenced. The five Petunia fad2 genes have highly homologous coding regions (> 90% identical at the amino acid level) but unique 5' and 3' untranslated regions. No introns were found in the coding regions. Both desA and desE, however, contain large introns (4.2 and 4.4 kb) in their 5' untranslated region. The desA gene is transcribed from at least two different promoters, one residing 4.2 kb upstream from the start ATG, the other residing within the intron (Figure 1).

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5' and 3' RACE (rapid amplification of cDNA ends) experiments showed that desA is expressed

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in all tissues examined (leaves, seeds and corolla), desB and desD are inactive in these tissues, and desE is expressed in leaves only. desC appears to be a pseudogene with several deletions and insertions. These results, however, do not explain the remaining 40% 18:2 in the seed lipid fraction of homozygous desA mutants. It is possible that (i) the RACE experiments do not give conclusive information on the fad2 expression patterns, (ii) there are still other seed-specific Petunia fad2 genes responsible for the 40% 18:2, (iii) in the mutant lines the desA function is taken over by one or more of the other fad2 genes, or (iv) in Petunia, 50% of the total 18:2 in seed lipids is synthesized in the chloroplast by the action of the plastidial \( \omega-6 \) desaturase. This latter possibility is, however, different from the situation in Arabidopsis where the plastidial \( \omega-6 \) desaturase does not contribute to oleate desaturation in seeds [3]. Further experimentation will be required to discriminate between these possibilities.

References

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Substrate specificity, regioselectivity and cryptoregiochemistry of plant and animal \( \omega-3 \) fatty acid desaturases
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Abstract
In order to define the substrate requirements, regiochemistry and cryptoregiochemistry of the \( \omega-3 \) fatty acid desaturases involved in polyunsaturated fatty acid formation, the genes Fad3 and fat-1 from Brassica napus and the nematode Caenorhabditis elegans respectively were expressed in baker's yeast (Saccharomyces cerevisiae). Various fatty acids, including deuterium-labelled thiafatty acids, were supplied to growing cultures of transformed yeast. The results from GC-MS analysis of the desaturated products indicate that both the plant and animal desaturases act on unsaturated substrates of 16–20 carbons with a