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ScienceDirect

Phytochemistry 69 (2008) 684–691

PHYTOCHEMISTRY

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Lipid characterization of a wrinkled sunflower mutant

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Received 3 August 2007; received in revised form 27 September 2007
Available online 19 November 2007

Abstract

As part of a sunflower mutagenesis program carried out to obtain lines with fatty acid profiles in their oils, the half-palmitic CAS-7 line, with ca. 14% palmitic acid content, was isolated. Attempts to obtain a homozygotic line proved to be futile due to the lack of growth of the seedlings 10–12 days after germination. At this age, the seedlings stop growing, displayed a lack of chlorophyll and poor linolenic acid content, a fatty acid intimately linked to photosynthetic membranes. Accordingly, this line has only been maintained through heterozygotic seeds. Likewise, the cotyledons of seeds from this line with medium levels of palmitic acid present a characteristic wrinkled phenotype. In the oil of these seeds, the triacylglycerol content displayed a reduction of approximately 57% with respect to the control line, although a similar reduction was not observed in the polar lipids. Furthermore this mutant has 40.0% of trilinolein, the higher content found until today in sunflower seeds. These data indicate that the CAS-7 mutant possesses a multiple phenotype having a reduced triacylglycerol seed content, a modified intraplastidial fatty acid synthesis, together with a seedling blocked growth and poor green colour and reduced chloroplast development.

Keywords: *Helianthus annuus*; Fatty acids; Lipid; Mutant; Seed; Wrinkled; Linolenic; Palmitic

1. Introduction

In plants, fatty acid biosynthesis up to oleic acid occurs in the chloroplasts or plastids depending on whether the tissue is green or not (Harwood, 1996). The activity of a type II dissociable fatty acid synthetase (FAS) enzyme complex is responsible for the biosynthesis of this fatty acid. The sequential actions of FASIII and FASI produce the palmitoyl-acyl carrier protein (palmitoyl-ACP), which is then lengthened by the action of the FASII complex to stearoyl-ACP. These three enzymatic FAS complexes only differ in one enzyme, the ß-keto-acyl-ACP synthetase (KAS). Finally, stearoyl-ACP can be desaturated to oleoyl-ACP by stearoyl-ACP desaturase (SAD).

Palmitic, stearic and oleic acids can be exported to the cytoplasm following their hydrolysis due to the action of the acyl-ACP thioesterases. Thus, the interaction between the enzymes responsible for the final activities in the main pathway, FASII and SAD, and the acyl-ACP thioesterases would determine the amount of each fatty acid that is exported to the cytoplasm. Accordingly, these enzymes influence the final fatty acid composition of the oilseed. Once acyl-ACPs are hydrolized, acyl-CoA synthase incorporates acyl molecules to the pool of acyl-CoA (Roughan and Slack, 1982), and oleic acid, once incorporated into phosphatidyl-choline, can be desaturated in the endoplasmic reticulum to linoleic and linolenic acids, by the action of the oleate desaturase and the linoleate desaturase, respectively, and latter on incorporated to the acyl-CoA pool. The resulting acyl-CoAs are substrates for the synthesis of complex lipids, mainly triacylglycerols in seeds or galactolipids in green tissues.

In order to study lipid biosynthesis and its regulation, Arabidopsis mutants in which lipid synthesis is affected have been used. These mutants display modifications in

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Abbreviations: ACP, acyl carrier protein; DAS, days after sowing; FAS, fatty acid synthetase; KAS, ß-Keto-acyl-ACP synthase; SAD, stearoyl-ACP desaturase; TAG, triacylglycerol.

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0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.09.026

the fatty acid or lipid content of their tissues, or in the synthesis of specific lipids (Gallardo et al., 2001; Lemieux et al., 1990; Routaboul et al., 1999). In certain mutant lines, steps in the main pathways of fatty acid biosynthesis are affected, such as the ß-keto-acyl-ACP synthetase (Wu et al., 1994), stearoyl-ACP desaturase (Lightner et al., 1994), acyl-ACP thioesterases (Bonaventure et al., 2003) or oleate desaturase (Horiguchi et al., 2001). These mutants displayed certain modifications in their fatty acid composition and, in the *wrinkled*1 mutant in particular, the glycolytic pathway is affected and the plants display reduced lipid content and a modified fatty acid composition (Gallardo et al., 2001).

Mutant sunflower (*Helianthus annuus* L.) lines have been described with an increase in palmitic acid (e.g. CAS-5 with a 28% palmitic acid in the seed oil) or stearic acid content (e.g. CAS-3 with a 26% of stearic acid) (Osorio et al., 1995; Fernández-Martínez et al., 1997). The biochemical characterization of high-palmitic sunflower lines demonstrated a reduction in KASII activity and elevated thioesterase activity with respect to palmitoyl-ACP (Martínez-Force et al., 1999). Additionally, there is a higher content of uncommon fatty acids derived from the intraplastidial accumulation of palmitic acid, palmitoleic and asclepic acids in the high-palmitic sunflower lines.

Here we describe the selection and characterization of a mutant sunflower obtained by sodium azide mutagenesis that accumulates palmitic and some palmitoleic acids in the seed lipids. This mutant displays a wrinkled seed phenotype and deficient synthesis of linolenic acid during seedling growth.

2. Results

2.1. Mutant selection

Mutated seeds were germinated and sown in the field, and the first generation plants were self-fertilized. The seeds from these plants were collected to select the mutants and the fatty acid composition of the M_2 generation was analyzed. In one of the capitula analyzed, seeds were identified with palmitic acid content higher than the normal. Indeed, when all the seeds from this plant were analyzed, two classes of seeds could be distinguished based on the percentage of palmitic acid that was found in their oil (Fig. 1): (i) seeds with between 6% and 10% of palmitic acid that are denominated here as low-palmitic; and a second class (ii) with a palmitic acid content between 10% and 20% that we called medium-palmitic. Indeed, in none of the seeds were the values of the high-palmitic CAS-5 and CAS-12 mutant lines reached, these mutants having been selected previously and containing about 30% palmitic acid (Álvarez-Ortega et al., 1997). In the first batch of 271 seeds analyzed, 92 seeds were considered as medium-palmitic and these new mutant seeds were named CAS-7.

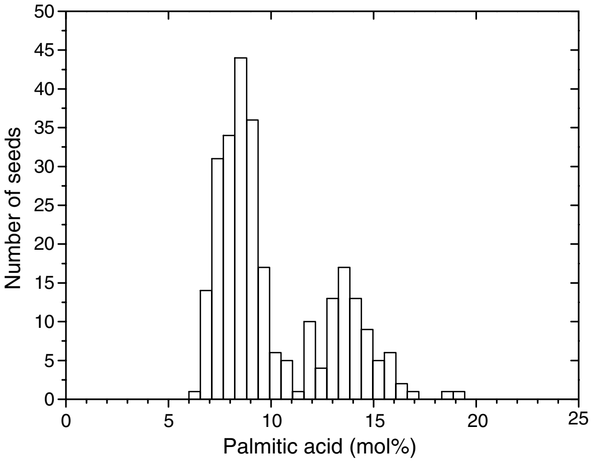

Fig. 1. Histogram of the palmitic acid content in the seeds of the original capitula from which the CAS-7 mutant was identified.

In order to fix the characteristics of this mutant, the seeds with the highest palmitic content were sown. During this selection process it was noted that the seeds with the highest palmitic acid content had problems in germinating and that their growth ceased at 10–12 days after sowing (DAS). More precisely, the cotyledons and hypocotyls of these medium-palmitic seeds remained pale yellow,[1] similar to an etiolated seedling, whilst the seeds with lower palmitic content germinated and grew without any problem. The phenotypic differences associated with the medium-palmitic acid seeds were readily apparent at 5 DAS (Fig. 2A). The three seedlings shown with low-palmitic acid levels displayed a green and healthy vegetative aspect whereas the three seedlings with medium-palmitic acid levels suffered retarded growth and their cotyledons remained pale yellow (below), as was also evident at 12 DAS (Fig. 2B). When the control RHA-274 plants were compared with the medium-palmitic acid plants (Fig. 2C), the control plants are clearly taller and with a greater leaf span. When the germinating CAS-7 mutant seedlings were examined more closely, the lack of normal green colouring and of secondary roots can be more clearly appreciated (Fig. 2D).

During the process of fixation, medium-palmitic acid seeds were only found in some capitula that descended from the subpopulation of low-palmitic seeds that had higher values of palmitic acid. In no case were seeds obtained with values of palmitic acid higher than those observed in the initial M_2 generation.

The fatty acid composition of both classes of seeds, low and medium-palmitic acid, was obtained from various plants of the heterozygotic CAS-7 line during the fixation process (Table 1). As a control, the fatty acids composition of the parental RDF-1-532 and the standard RHA-274 lines are also shown. As can be observed, the increase in

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[1] For interpretation of color in Fig. 3, the reader is referred to the web version of this article.