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Evolution of phosphoenolpyruvate carboxylase activity and lipid content during seed maturation of two spring rapeseed cultivars (*Brassica napus* L.)

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Abstract

Phosphoenolpyruvate carboxylase (PEPc: EC 4.1.1.31) activity was monitored during seed maturation of two varieties (Hybridol and Pactol) of rapeseed (*Brassica napus* L.), widely cultivated in Tunisia. In the Hybridol variety, PEPc activity did not exceed 5 μmol h^{-1} per gram of fresh weight (FW) during the first stages of maturation. It then highly increased to reach more than 30 μmol h^{-1} g^{-1}/FW. On the contrary, in the Pactol variety, the evolution of PEPc activity showed a classical curve, i.e. an increase during the most active phase of lipid accumulation in maturating seeds, followed by a rapid decrease until the end of seed maturation. In both varieties, the seed oil was characterised by a high content of oleic acid ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic acids ($C_{18:3}$). Saturated fatty acids were also present, although decreasing with maturation course. The analysis of the triacylglycerols (TAG) showed that trioleoylglycerol (OOO) and dioleoyllinoleoylglycerol (OOL) were the major species (ca. 35% and ca. 25% of the total respectively). The evolution pattern of fatty acids and TAG contents was similar to that of PEPc activity. Taken together, our findings suggest that PEPc may be involved in fatty acid and triacylglycerol biosynthesis during seed maturation of both rapeseed varieties. ***To cite this article: K. Sebei et al., C. R. Biologies 329 (2006).***

Résumé

Évolution de l'activité de la phosphoénolpyruvate carboxylase et des teneurs en lipides au cours de la maturation des graines de deux variétés de colza de printemps (*Brassica napus* L.). Nous avons suivi l'évolution d'une enzyme, la phosphoénolpyruvate carboxylase (PEPc : EC 4.1.1.31), chez une plante en C3, le colza (*Brassica napus* L), au cours de la maturation des graines de deux variétés de printemps, Hybridol et Pactol. Nous avons trouvé que les deux variétés se comportent différemment. Chez Hybridol, l'activité de la PEPc n'augmente que faiblement pendant les quatre premiers stades de maturation, puis fortement pour atteindre son maximum au stade VI. Au contraire, chez Pactol, l'activité de la PEPc augmente régulièrement au cours de la maturation, pour atteindre un maximum au stade V. Dans les deux variétés, l'activité a chuté fortement au dernier stade de maturation. On a remarqué que l'évolution des teneurs en acides gras monoinsaturés (AGMI) et polyinsaturés (AGPI) et des teneurs

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en triacylglycérols (TAG) suit l'évolution de la PEPc. On pense que la PEPc intervient dans la biosynthèse des acides gras et leur accumulation sous forme de lipides de réserve : TAG. En effet, ce rôle a été confirmé chez certaines plantes oléagineuses. ***Pour citer cet article : K. Sebei et al., C. R. Biologies 329 (2006).***

Keywords: *Brassica napus*; PEPc; Acides gras; Huile; Maturation; Graines

Mots-clés : *Brassica napus* ; PEPc ; Fatty acid ; Oil ; Maturation ; Seeds

1. Introduction

Phosphoenolpyruvate carboxylase (PEPc: EC 4.1.1.31) catalyses the irreversible β-carboxylation of phosphoenolpyruvate in the presence of HCO_3^- to yield oxaloacetate and inorganic phosphate Pi [1,2]. PEPc catalyses the first step in the fixation of atmospheric CO_2 during C_4 photosynthesis [3]. This enzyme has been extensively studied in C_4 and crassulaceae (CAM) plants. C_3 plants contain less, yet appreciable, amounts of PEPc, which is found in virtually all organs and may play multiple physiological roles [4]. The properties and functions of PEPc in C_3 and non-photosynthetic tissues of C_4 and CAM plant are less well understood [1]. One role is the so-called anaplerotic function, which consists in the replacement of oxaloacetate in the tricarboxylic acid cycle, whenever the demand for carbon skeletons for amino acid biosynthesis is high. There is also increasing evidence for the presence of PEPc in seeds of different plant species [2]. Smith et al. [5] demonstrated that malate supports very high rates of fatty acid synthesis by isolated leucoplasts prepared from developing Castor oilseeds. Based on the measurement of localized $NADP^+$ malic enzyme, they proposed that malate might be a key carbon source for fatty acid synthesis in vivo. PEPc may convert PEP into malate, which is then utilized by the leucoplast for fatty acid biosynthesis [1]. Developing *Brassica napus* seeds and microspore-derived embryos contain relatively high PEPc activity during their most active phase of oil synthesis [1]. In oleaginous plants, the PEPc is active, in particular during development of seeds [6]. Its activity and concentration increase have been observed during the most active phase of lipid accumulation during the course of castor seeds maturation [7]. In spite of the high importance of this enzyme in lipid and fatty acids synthesis, PEPc was less studied in the oleaginous plants [8].

Rapeseed is an important potential source of edible unsaturated oils. It is one of the most oleaginous plants cultivated in the world and it offers several advantages in the application of biotechnical approaches to improve oil composition [9,10]. Sebei et al. [11] reported that two Tunisian rapeseed varieties (Hybridol and Pactol) contained more than 60% of oleic acid, 18% of linoleic acid, and 8% of linolenic acid. Fatty acid composition varied from variety to variety and changed according to environmental conditions [12,13]. Only few information dealing with lipid accumulation in seeds, all along the course of maturation in relation with PEPc activity are available, and none concerns rapeseed.

The present investigation focuses on the time–course changes in (*i*) PEPc activity during maturation of seeds from two varieties (Hybridol and Pactol) of rapeseed (*Brassica napus* L.), and (*ii*) the accumulation of total lipids, fatty acids and triacylglycerols. The two rapeseed cultivars used here are widely cultivated in Tunisia, owing to their high seed oil yield.

2. Material and methods

2.1. Culture conditions and plant sampling

The study was carried out on the two rapeseed varieties, Hybridol and Pactol ('double zero'). The plants were grown in a parcel at the National Institute of Agronomic Research of Tunisia (INRAT), under natural conditions and were irrigated with tap water twice a week until flowering stage. During this study, seven samples (one sample per week for each variety) were achieved, starting with the apparition of siliques (30 days following flowering) until the end of seed maturation. It is noteworthy that sampling of Pactol seeds occurred earlier than that of Hybridol seeds because of its precocity.

2.2. Total lipid extraction

Total lipid extraction was performed according to Folch [14]. This extraction was used to determine fatty acid and triacylglycerol composition. The seed oil content was determined using the Soxhlet extraction according to the official method [15]. From each cultivar, 50 g were ground and than extracted with petroleum ether in a Soxhlet apparatus for 6 h. Petroleum ether was then evaporated under reduced pressure using a ro-