involved in the biosynthesis thereof in microbial systems or in plants with high productivity. The biosynthesis of such compounds starts with the symmetrical cleaving, in positions 7,8 and 7'8', of zeaxanthin by means of a dioxygenase (CCD, Carotenoid Cleavage Dioxygenase) (Fig. 1). The two products of the cleavage of zeaxanthin, i.e. 3-OH-β-cyclocitral and crocetin dialdehyde, are dehydrogenated and glycosylated in order to produce, respectively, picrocrocin and crocins. The glucosyltransferases responsible for the glycosylation reaction have been described in saffron and *Gardenia* (5) (6). With regard to the first biosynthetic step, some authors have reported that an enzyme called ZCD cleaves zeaxanthin symmetrically at positions 7,8/7',8', producing crocetin dialdehyde (7), which is an observation contradicted by subsequent works (8). The modelling of the three-dimensional structure of ZCD has shown that ZCD is an incomplete enzyme, devoid of blade VII of the conserved *β-propeller* structure (9), consistent with the absence of enzymatic activity of ZCD (8). Consequently, it is not currently possible by biotechnological methods to produce the apocarotenoid compounds of saffron, and in particular crocetin and crocins, since the identification of the first enzyme of the biosynthetic pathway, i.e. CCD, which is responsible for the symmetrical cleaving in positions 7,8 and 7',8' of zeaxanthin, is still uncertain.

The object of the present invention is to provide a new carotenoid dioxygenase and methods for the biological production in microorganisms and plants of compounds of high added value derived from saffron.

Summary

In one aspect, there is provided a nucleotide sequence of SEQ ID No. 1 coding for a carotenoid dioxygenase enzyme able to cleave carotenoids symmetrically in positions 7,8 and 7,'8' with the production of crocetin dialdehyde.

In another aspect, there is provided an enzyme coded from the nucleotide sequence as described herein, having the amino acid sequence of SEQ ID No. 2.

In yet another aspect, there is provided a method for the production of crocetin dialdehyde and/or crocetin in microorganisms that accumulate zeaxanthin, comprising a step of expression in said microorganisms of the enzyme coded by the nucleotide sequence of SEQ ID No. 1.

In yet another aspect, there is provided a method for the production of crocetin in vegetable cells that express zeaxanthin, comprising a step of expression in said cells of the enzyme coded by the nucleotide sequence of SEQ ID No. 1.

In yet another aspect, there is provided use of an enzyme coded by the nucleotide sequence of SEQ ID No. 1 or of an enzyme having SEQ ID No. 2 to cleave symmetrically the

molecule of zeaxanthin in positions 7,8 and 7,'8' with the production of crocetin dialdehyde and/or crocetin.

Description

The above-mentioned and further objects of the invention, as will become clear from the following description, are achieved by means of a gene coding for a Carotenoid Dioxygenase enzyme, an enzyme coded in this way, and methods for producing crocin precursors as claimed in the corresponding independent claims.

The present invention will be better understood from the following detailed description of some preferred embodiments, provided with reference to the accompanying drawings, in which the corresponding figures show:

Fig. 1 Biosynthetic pathway of apocarotenoids in saffron. Proposed biosynthetic pathway for the biosynthesis of the apocarotenoids. Zeaxanthin is cleaved in positions 7,8 and 7',8' by the activity of a CCD. The cleavage product C_{20}, crocetin dialdehyde, is converted into crocetin from an aldehyde dehydrogenase, then into crocin by the activity of at least two UDPG glycosyltransferases. The product C_{10}, 3-OH-β-cyclocitral, is converted into picrocrocin from a UDPG-glycosyltransferase, and then into safranal.