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A NOVEL CAROTENOID CLEAVAGE DIOXYGENASE CATALYZES THE FIRST DEDICATED STEP IN SAFFRON CROCIN BIOSYNTHESIS

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Saffron stigmas are used as a spice since the Greek-Minoan civilization, due to their content of different valuable metabolites that include three apocarotenoids (crocetin, picrocrocin and safranal) that contribute to the red color, bitter taste and aroma of the stigmas and whose synthesis occurs during early stigma development. This pathway is initiated by a carotenoid cleavage reaction catalyzed by a CCD (Carotenoid Cleavage Dioxygenase). Conflicting data have been reported about the identity of this enzyme and its substrate (b-carotene vs zeaxanthin). Using RNA-Seq of 6 different saffron stigma developmental stages, from immature to fully developed, we identified 7 different *CCDs*, of which two, *ZCD-1* and *ZCD-2*, are expressed during early and late stigma development, respectively. All identified CCDs were expressed in *E. coli* cells engineered to accumulate different carotenoids. ZCD-1 cleaved zeaxanthin, but not lycopene or b-carotene at the 7,8 and 7'8' positions, yielding crocetin dialdehyde, identified through high resolution mass spectrometry and co-migration with authentic standards. This activity was confirmed through transient expression in maize kernels, where the synthesis proceeds to crocetin, thanks to an endogenous aldehyde dehydrogenase activity. In vitro reactions performed with different substrates gave a clear idea of the stereospecificity of the enzyme. In contrast to a previous report, *ZCD-2* did not show any cleavage activity, consistent with it being an N-truncated CCD, lacking one blade of the β-propeller structure conserved in all CCDs. ZCD-1 has a cytoplasmic localization, suggesting that it may cleave carotenoids localized in the chromoplast outer envelope. These results suggest that ZCD-1 is the enzyme responsible for the cleavage step leading saffron apocarotenoid biosynthesis.