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Identification and characterization of putative zaxinone synthase enzymes in tomato

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Abstract

The cleavage of carotenoids, mediated by CCDs (Carotenoid Cleavage Dioxygenases) enzymes, provides the biosynthesis of apocarotenoids, a wide class of metabolites that includes phytohormones, signal molecules, chromophores and aroma constituents. In addition to the five CCD subfamilies identified and characterized so far (NCED, CCD1, CCD4, CCD7, CCD8), a sixth subfamily, named ZAS (zaxinone synthase), has recently been identified in *Oryza sativa*. The rice genome encodes four homologs, called *OsZAS*, *OsZAS1b*, *OsZAS1c* and *OsZAS2*. Among these, only the *OsZAS* and *OsZAS2* functions were investigated: both enzymes cleave the apocarotenoid β-apo-10'-zeaxanthinal (C27) derived from zeaxanthin (C40), at the C13-C14 double bond, yielding zaxinone (C18). Zaxinone is an important growth regulating apocarotenoid metabolite in rice; it is involved in arbuscular mycorrhizal symbiosis and in the interaction with parasitic weeds, also influencing strigolactones levels in roots and exudates. Zaxinone has also been detected in *Solanum lycopersicum*, prompting our interest in exploring its function and biosynthetic pathway in this species.

We have identified three orthologs of the rice ZAS enzyme in tomato genome, named *SlZAS*, *SlZAS-like 1* and *SlZAS-like 2* through a series of bioinformatical analysis. We decided to evaluate their enzymatical functions trough different experimental strategies: both heterologous expression in bacteria followed by

in vitro assays and reverse genetic approaches, generating knock-out tomato plants in our genes of interest using CRISPR/Cas9 technique.

In vitro assays were conducted to evaluate the enzymatic activities of tomato ZAS enzymes, focusing on their ability to cleave apo-10'-zeaxanthinal to produce zaxinone. Preliminary results indicate that only SlZAS enzyme catalyzes this reaction.

Using CRISPR/Cas9 technique, tomato plants (*var*. MoneyMaker) edited in *SlZAS* gene have been obtained. Phenotypic, biochemical, and molecular characterization of *zas loss-of-function* mutants (T_4 generation) revealed reduced shoot growth and a less developed root system compared to wild-type plants. In addition, we have obtained T_0 generation plants edited in *SlZAS-like 1* and *SlZAS-like 2*, which are currently undergoing molecular screening.

Further investigations and analyses will elucidate the enzymatic functions of these genes and clarify the zaxinone biosynthetic pathway also in tomatoes, a globally important agricultural species. Understanding these physiological processes is crucial for advancing tomato cultivation in terms of food safety.

Keywords: apocarotenoids, genome editing, tomato, zaxinone synthase, zaxinone.

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