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GLOBE ARTICHOKE POLYPHENOL OXIDASE GENE MINING: CHARACTERIZATION AND EXPRESSION PROFILE WITH A VIEW OF THEIR KNOCKOUT TO AVOID CAPITULA BROWNING AFTER CUTTING

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The cutting of globe artichoke capitula (*Cynara cardunculus* var. *scolymus* L.) activate the polyphenol oxidases (PPOs), which in turn catalyze the oxidization of polyphenols and cause their browning. The latter has a negative impact on capitula quality for both industrial transformation and fresh consumption. The knockout of *PPO* genes based on CRISPR genome editing represents a promising strategy to avoid undesired browning of the capitula, while preserving their content in beneficial phenolic compounds and reducing the need for physical and chemical treatments in the food industry. However, the application of gene editing in globe artichoke is hampered by its recalcitrance to in vitro regeneration, most likely due to oxidative necrosis.

Thanks to the recent availability of a high quality, annotated and anchored globe artichoke genome, 11 *PPO* genes were identified on the base of homology-based characterization.

The analysis of cis-acting elements in *PPO* promoters highlighted a gene regulatory and functional profile associated to biological processes related to plant growth, development and response to stresses. Phylogenetic analysis identified their structural and functional conservation with Asteraceae *PPOs* involved in plant response to different physical stimuli.

The genomic diversity of the identified *PPOs* was investigated across the varietal types: ‘Spinoso di Palermo’, ‘Violetto di Sicilia’, ‘Violetto di Toscana’ and ‘Romanesco C3’, and spotted a range of 600-700 variants/varietal type, some of which with high impact on gene translation.

Through RTqPCR the expression pattern of the globe artichoke *PPO*s was assessed in different plant tissues upon wounding, and *PPO6* and *PPO11* were found the most implied in capitula browning with *PPO1, PPO2* and *PPO5* playing a minor role. On the other hand, in calli underwent to oxidative browning the *PPO5*, *PPO6*, *PPO7* and *PPO11* were up-regulated in respect to green calli.

Agrobacterium tumefaciens-mediated transformations were performed in the view of setting up a *PPO*-based gene editing approach in the species. With the goal to improve the regeneration efficiency and simultaneously introduce the editing in a targeted genomic site, leaf explants were transformed by co-culture with a vector containing growth regulators (*WUS* and *BBM*) and the CRISPR/Cas9 system targeting the *PDS* gene. Side by side, in order to skip the regeneration step, shoot apical meristems were transformed by micro-injection and co-culture using two constructs respectively containing *GFP* and *GUS* reporters. At last, in order to assess the way of producing T-DNA free edited plants, procedures for protoplasts isolation and their transformation with CRISPR/Cas9 ribonucleoproteins are in progress.

The efficiency of the adopted transformation protocols is undergoing.