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Ectopic expression of a stress-inducible glycosyltransferase from saffron enhances salt and oxidative stress tolerance in *Arabidopsis* while alters anchor root formation

Q1 Oussama Ahrazem[a,b], Angela Rubio-Moraga[a], Almudena Trapero-Mozos[a], María Fernanda López Climent[c], Aurelio Gómez-Cadenas[c], Lourdes Gómez-Gómez[a,*]

[a] *Instituto Botánico, Departamento de Ciencia y Tecnología Agroforestal y Genética, Facultad de Farmacia, Universidad de Castilla-La Mancha, Campus Universitario s/n, 02071 Albacete, Spain*

Q2 [b] *Fundación Parque Científico y Tecnológico de Albacete, Spain*

[c] *Universitat Jaume I, Department of Agricultural and Environmental Sciences, 12071 Castelló de la Plana, Spain*

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ABSTRACT

Glycosyltransferases play diverse roles in cellular metabolism by modifying the activities of regulatory metabolites. Three stress-regulated UDP-glucosyltransferase-encoding genes have been isolated from the stigmas of saffron, UGT85U1, UGT85U2 and UGT85V1, which belong to the UGT85 family that includes members associated with stress responses and cell cycle regulation. *Arabidopsis* constitutively expressing *UGT85U1* exhibited and increased anchor root development. No differences were observed in the timing of root emergence, in leaf, stem and flower morphology or flowering time. However, salt and oxidative stress tolerance was enhanced in these plants. Levels of glycosylated compounds were measured in these plants and showed changes in the composition of several indole-derivatives. Moreover, auxin levels in the roots were higher compared to wild type. The expression of several key genes related to root development and auxin homeostasis, including *CDKB2.1*, *CDKB2.2*, *PIN2*, *3* and *4*; *TIR1*, *SHR*, and *CYCD6*, were differentially regulated with an increase of expression level of *SHR*, *CYCD6*, *CDKB2.1* and *PIN2*. The obtained results showed that *UGT85U1* takes part in root growth regulation via auxin signal alteration and the modified expression of cell cycle-related genes, resulting in significantly improved survival during oxidative and salt stress treatments.

1. Introduction

Q3 Plant secondary metabolite UDP-dependent glycosyltransferases (UGTs) catalyze the transfer of a carbohydrate from an activated donor sugar onto small molecule acceptors by the formation of a glycosidic bond [1,2]. Glycosylation changes the stability and/or solubility of the aglycone, and may even create a higher diversity due to differential and multiple conjugations. The range of sugar acceptors in plants is thought to be very diverse, but the exact substrates are known for only a few of the UGTs, and there is a lack of strict correlation between the structural identity and substrate specificity [3]. Considering the huge number of different glycosides found in plants, high specificity would indicate the requirement of many more UGTs than actually identified [4]. As observed by in vitro glycosylation tests, most UGTs are indeed regiospecific rather than substrate-specific. This confers plants with a certain degree of flexibility and enables them to respond to rapidly changing environmental conditions or evolutionary tendencies [5]. For instance, several UGTs are highly inducible by both abiotic as well as biotic stress factors [6–12], indicating an important stress related function [13,14]. Previously described plant acceptor molecules for glycosylation include hormones (such as auxin, ABA, cytokinin, SA, and brassinosteroids), secondary metabolites (monolignols, anthranilate, caffeic acid, and flavonoids), and xenobiotics [15]. These glycosylation reactions are an important feature in the regulation of the activity of these signalling molecules and defence compounds. Therefore, UGTs might play important roles in maintaining cell homeostasis, regulating plant growth and improving their defence responses to stressful environments.

Drought is one of the greatest worldwide environmental constraints for agriculture [16] and there is great emphasis on identifying novel stress responsive genes from stress tolerant

* Corresponding author. Tel.: +34 967599200.
E-mail address: Marialourdes.gomez@uclm.es (L. Gómez-Gómez).

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species since understanding their role will help to improve the tolerance of stress susceptible crops [17]. In addition, roots serve as the major interface between the plant and various biotic and abiotic factors in the soil environment: by both sensing and responding to environmental cues, roots enable plants to overcome the challenges posed by their sessile status. Faster and more extensive root growth would be important for good plant growth under the most adverse soil conditions [18] and such a root system would enable plants to extract more soil water and nutrients [19]. Root system architecture exploitation and modification in crops may enable plants to make more efficient use of existing soil nutrients and increase stress tolerance, improving yields while decreasing the need of heavy fertilizer application [20]. Of the factors controlling total root architecture, lateral root formation and growth are an important component for the adaptability of the root system to its environment.

Crocus sativus (saffron) is mainly cultivated because of its long red stigmas which produce and store significant quantities of carotenoid derivatives, formed from the oxidative cleavage of β-carotene and zeaxanthin [21,22]. The dried stigmas of *C. sativus*, are one of the oldest natural food additives used as a flavour and colouring agent. Several agronomic traits make saffron suitable as a subsistence crop for rural farmers. It is drought tolerant, is propagated vegetatively by corms, grows well on low-nutrient soils and requires minimal resource input to cultivate [23]. By contrast to dicots that often produce a primary tap root that produces lateral roots, the fibrous root system of *C. sativus* is similar to those from *Oryza sativa* and *Zea mays*, which are predominantly composed of adventitious roots also called nodal roots or crown roots, which develop from the stem [24], or from the corm, a modified stem, in the case of saffron. It appears that crown-root initiation is controlled somewhat similarly to lateral-root initiation even though these two root types develop from shoot and root tissues, respectively [25]. Auxin induces lateral-root formation in dicots and adventitious root formation in grasses [26]. Crown root initiation requires auxin biosynthesis, transport, and signalling. However, it is not yet clear if auxin controls the differences in morphology seen in dicots versus monocots.

In this study, cDNAs encoding three uridine diphosphate glycosyltransferase (UGT) paralogous, assigned the names UGT85U1, UGT85U2 and UGT85V1, have been isolated from saffron. These UGTs were regulated under different stress conditions in stigmas and roots, and ectopic expression of UGT85U1 in *Arabidopsis* resulted in plants with enhanced salt and oxidative stress tolerance compared to the wild type. Further, the transgenic plants had enhanced levels of auxin which was correlated to a specific phenotype characterized by the development of anchor in roots and a reduction of root length.

2. Materials and methods

2.1. Plant material

For this study, *Arabidopsis thaliana* Col-0 plants and *C. sativus* obtained from the Botanical Garden of Castilla-La Mancha (Albacete, Spain) were used. Plant tissues from *C. sativus* and *A. thaliana* plants were independently harvested and frozen in liquid nitrogen and stored at −80 °C until required. Stigmas were collected at seven developmental stages defined according to Rubio et al. [21].

Seeds of *Arabidopsis* wild type ecotype Columbia (Col), and transgenic lines were sown in pots containing vermiculite and watered with nutrient solution under controlled environment with 16 h light/8 h dark cycles at 22 °C.

2.2. Stress treatments in C. sativus

To determine stress-induced gene expression in stigmas, red stigmas were collected from young flowers and transferred to 24-well-plates containing 1 ml water supplemented with abcisic acid (ABA) (100 μM), indole acetic acid (IAA) (100 μM), 1-N-naphthylphthalamic acid (NPA) (100 μM), 0.2 μl/ml methyl jasmonate (MtJA), an elicitor suspension from *Fusarium oxysporum* (fungi), NaCl (200 mM), Sorbitol (200 mM) or distilled water that was used as a control; and incubated under normal conditions (16 h light/8 h dark cycles at 22 °C). After 24 h stigmas were removed from the different solutions, immediately frozen in liquid nitrogen and stored at −80 °C until required. Heat stress was applied by rising the temperature in the growth chamber to 38 °C for 6 h. Cold stress was applied by incubation of the stigmas at 10 °C for 6 h to the stigma samples which were thereafter immediately frozen in liquid nitrogen and stored at −80 °C until required. For the dehydration experiments detached red stigmas on Whatman No. 3MM paper (Whatman International, Maidstone, England) were subjected to dehydration at 60% relative humidity and 22 °C for 22 h in Petri dishes, under dim light (100 lx). The wounding experiments were done with a sterile needle, and the samples collected 24 h later.

Stress experiments were also done with corms (10 corms from experiment) growing in perlite and irrigated during one week with NaCl (300 mM), only water, irrigated with H_2O_2 (10 mM) and in the presence of elicitor suspension from *F. oxysporum* (fungi). The root tissue was collected and analyzed.

2.3. Stress treatments in Arabidopsis

To determine the salt-stress sensitivity of *Arabidopsis* seedlings to NaCl, 6-d-old seedlings of the wild type and *UGT85U1* transgenic plants grown on MS agarose-medium were transferred to liquid MS medium supplemented with different concentrations of NaCl (50, 150, and 200 mM) and grown at 22 °C under long day conditions. After 6 d, the survival rate of the 12-d-old seedlings was scored in three independent experiments (30 seedlings per experiment). Plants were also transferred to MS plates containing 100 mM NaCl, 100 mM KCl and 100 mM Sorbitol under the same conditions.

H_2O_2 stress was imposed by transferring 10-d-old *UGT85U1* or wild-type seedlings from MS agarose-plates to liquid MS medium (30 seedlings per experiment) supplemented with 1 or 3 mM H_2O_2 for 5 d. Plants were also transferred to MS plates containing 1 mM H_2O_2 under the same conditions.

2.4. Auxin determination

Roots and aerial parts were collected from 50 *A. thaliana* seedlings growing for 10 days in MS-agarose medium plates. The tissue was weight, frozen and lyophilized. IAA was analyzed by LC/ESI–MSMS essentially as described in Durgbanshi et al. [27] with slight modifications. Briefly, lyophilized plant material was extracted in ultrapure water by using a tissue homogenizer (Ultra-Turrax, Ika-Werke, Staufen, Germany) after spiking with 10 ng of d2-IAA. After extraction and centrifugation, pH of the supernatant was adjusted to 3.0 and partitioned twice against diethyl-ether (Panreac, Barcelona, Spain). The organic layer was combined and evaporated in a centrifuge vacuum evaporator (Jouan, Saint-Herblain, France). The dry residue was thereafter resuspended in a water:methanol (9:1) solution, filtered and injected in a UPLCTM Acquity system (Waters, Milford, MA, USA). The analyte was then separated in a C18 column (Nucleodur C18, 1.8 μm 50 mm × 2.0 mm, Macherey-Nagel, Barcelona, Spain) using as solvents methanol and water supplemented with 0.01% acetic acid at a flow rate of 300 μl min^{-1}. The mass spectrometer, a triple quadrupole (Xevo TQ-S, Waters) was operated in negative

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