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**ARTICLE**

# The role of ferritin in enhancing the stress tolerance of grapevine

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**ABSTRACT** In order to improve the stress tolerance of grapevine, transgenic plants were produced and regenerated from the anthers of a grapevine rootstock cultivar expressing the ferritin of *Medicago sativa*. Leaf disks of the plants were exposed to oxidative stress by intracellular ROS production using paraquat, a herbicide known to mediate superoxide radical formation in thylakoid membranes. The *Medicago* ferritin had a high expression level in the transgenic plants under normal condition, and it further increased about twofold under the applied stress condition. In response to the treatment, the transgenic plants showed enhanced stress tolerance as compared to the untransformed cultivar.
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**KEY WORDS**

Reactive Oxygen Species (ROS)
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transgenic plants
PCR,
gene expression

Environmental stresses cause yearly fluctuations in quantity and quality in crops, leading to substantial economical loss. Crossbreeding to obtain disease and stress resistant plants including grapes has been carried out for a long time. However, new grapevine varieties are difficult to introduce to the market due to the conservative nature of the consumers and hence the wine industry. Moreover, traditional breeding methods are time consuming and tedious procedures. As an alternative of crossbreeding, we attempted to increase stress resistance of this plant via molecular breeding approach. We have chosen to increase the intracellular level of ferritin, a protein having an important role in maintaining free iron pool in the cells. We aimed to decrease the free ferric ion concentration and hence decrease the possible occurrence of Fenton reaction converting hydrogen peroxide to hydroxyl radicals, which are the most reactive of all reactive oxygen species (ROS). Due to the fact that many stress factors act through ROS production and oxidative stress, the resulting transgenic plants are expected to show increased tolerance to a broad range of stress factors.

## Materials and Methods

For introducing the MsFerr into the genom of *Vitis* cultivar "Richter 110", a rootstock variety, the *Agrobacterium* mediated transformation of grapevine (Mozsár et al. 1998 and Oláh et al. 2003) was further optimized. Embryogen callus was generated from the anther of the grapevine and transformed using *Agobacterium tumefaciens* EHA105 strain harbouring plasmid vector pRok2MsFerr (Deák et al. 1999) containing the *Medicago* ferritin gene with a leader peptide rendering the expressed protein chloroplast-localized.

For assessment of stress response grapevine leaf disks were floated on water, containing paraquat at the indicated concentrations. After 3 h incubation in darkness, leaf disks were illuminated with 35 μmol $m^{-2}$ $s^{-1}$ PAR for 1h. Before measuring photochemical yield, samples were kept in the dark for 20 min. Maximum (potential) PS II quantum yield, $F_v/F_m$ was calculated as $(F_m - F_o)/ F_m$, from variable chlorophyll fluorescence parameters $F_o$ and $F_m$ (Schreiber et al. 1986), measured with Imaging-PAM (Heinz Walz GmbH, Germany) as described earlier (Hideg and Schreiber 2007). $F_v/F_m$ is shown either as images of the leaf disks from a single, typical experiment, or as values averaged from three repetitions. The latter are presented with standard deviations. From the leaf disks total RNA was isolated (Reid et al. 2006) for gene expression studies. The RNA was freed from residual DNA, reverse transcribed and real-time PCR was carried out using the corresponding kits from ABI (Foster City, CA, USA). Gene expression levels were determined and calculated relative to the actin gene as an internal control of constant expression (Reid et al. 2006)

The DFCI Vitis vinifera Gene Index (VvGI) EST database (http://compbio.dfci.harvard.edu/tgi/) was searched with plant ferritin sequences for finding grapevine ferritin mRNA sequences of significant expression level. Based on *in silico* investigation of the such sequences found, TC52333 and TC54876, both corresponding proteins are located in the chlo-

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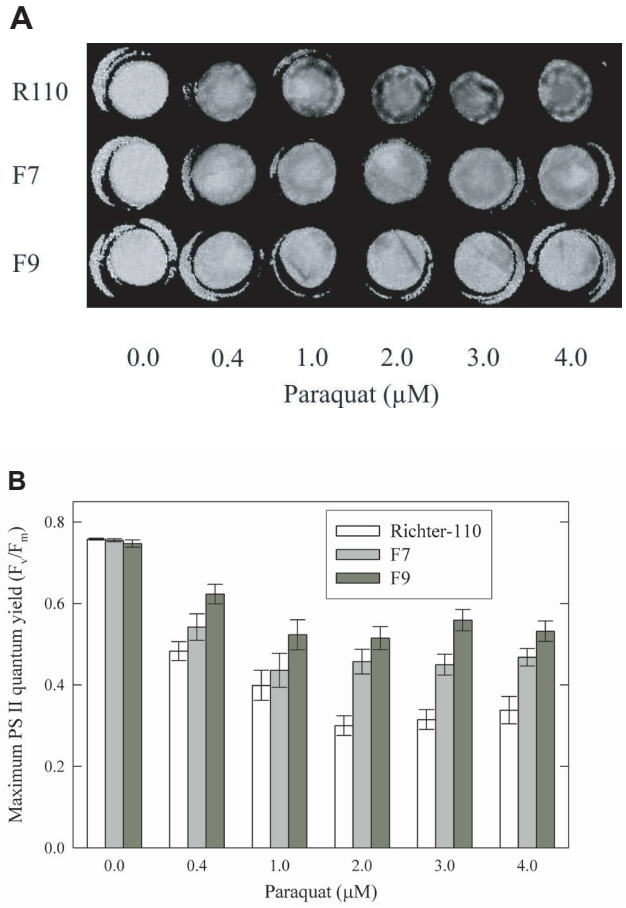


**Figure 1.** Grey-scale images (A) and values (B) of maximum PS II quantum yield, $F_v/F_m$ in grapevine leaf disks exposed to various concentrations of paraquat (see Materials and Methods for details). R110: "Richter 110" untransformed rootstock cultivar; F7, F9: transgenic plants.

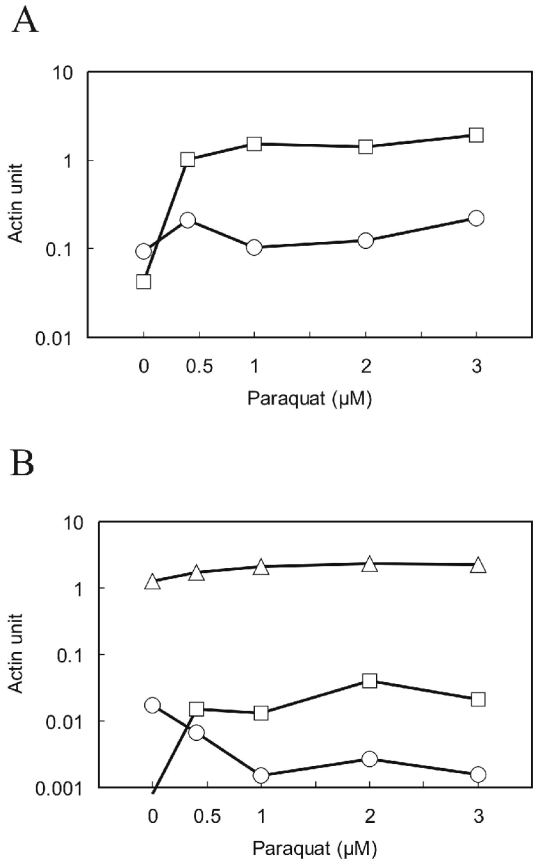


**Figure 2.** Gene expression levels of the two different *Vitis* ferritin genes (TC52333: circles and TC54876: squares ) and *M. sativa* ferritin gene (in F7, triangles) in grapevine leaf disks exposed to various concentrations of paraquat. The expression levels were determined in "Richter 110" control and F7 transgenic plant and are shown in terms of that of Actin.

roplast. Specific primer pairs for quantitative amplification of each sequences were designed and used in the quantitative real-time PCR (qPCR) reactions.

## Results

Transformed grapevine embryos showed developmental disorders after the *Agrobacterium* mediated transformation followed by one year of selection. Application of benzyladenin and removal of the plant roots proved to be the best of the tested alternative methods for obtaining normal shoot and root development (Oláh et al. 2003). Several independent embryos with normal morphologies could be obtained using this approach. All of these plants expressed MsFerr and were free of *Agrobacterium* as judged by conventional PCR. Experimental data on "Richter 110" and transgenic plants designated F7 and F9 will be shown below.

The control and transgenic plants were hardened and propagated in a greenhouse. Leaf disks were excised and exposed to paraquat. The photochemical activities in the leaf disks were determined after the treatment.

The "Richter 110" plants lost about half of their maximum photochemical yield upon exposure to 1 μM paraquat with further decrease at higher concentrations. In transgenic plants, however, the extent of the decrease was lower, and these leaf disks retained more than half of their $F_v/F_m$ even at 4 μM paraquat concentration (Fig. 1).

The ferritin expression levels were also determined in these paraquat treated leaf disks. We found that the expression level of MsFerr was more than tenfold higher than that of the *Vitis* genes in the transgenic plants in control conditions, and its expression further increased more than twofold under the applied oxidative stress (Fig. 2).

## Discussion

We have introduced the MsFerr to grapevine via *Agrobacterium* mediated transformation. As transgenic grapevine is

not easy to produce, we improved and optimized the methods for obtaining embryogenic callus from anthers, transformation of the calli and regeneration of embryos. The successful transformants showed abnormal morphology; therefore recovery of normal morphology was also necessary, followed by hardening the plants. The plant material was then propagated in a greenhouse.

QPCR investigation of ferritin gene expression revealed that the *Medicago* ferritin is highly expressed in the transgenic plants under control conditions, as compared to *Vitis* ferritins. Western blot results indicate that similar ratio of *Medicago* and *Vitis* ferritins can be observed at protein levels in the transgenic grapevine leafs (data not shown).

For assessment of stress resistance, leaf disks of "Richter 110" and transgenic plants were exposed to methyl-viologen (paraquat), an herbicide that generates superoxide radicals in the thylakoid membranes. One of the two *Vitis* ferritin genes was found to be overexpressed, while the other one got repressed under the applied paraquat treatment, which is in concert with the report on the differential expression of two maize ferritin genes under stress conditions (Fobis-Loisi et al. 1995). Still, the expression level of the MsFerr increased two to three fold under the applied conditions.

The stress response was monitored in the leaves by assessing the photochemical yield, as a sensitive and general indicator of intracellular oxidative damages. The photochemical yield of all cultivars decreased in response to paraquat treatment in a concentration dependent manner. However, this decrease was much less pronounced in transgenic plants expressing the MsFerr than in "Richter 110". Therefore we conclude that stress tolerance can be successfully enhanced in grapes via the applied approach. The overall intracellular concentration of ferritin molecules was increased dramatically by the introduction of the MsFerr construct. Due to the similar structure and function of the various plant ferritins, it seems likely that the increase in the resistance to oxidative stress can be attributed to quantitative, rather than qualitative differences between the ferritins in "Richter 110" and recombinant cultivars. This may open new routes to enhance resistance using the plants' overexpressed own ferritin genes, which can be easier to be accepted under the current concerns about transgenic plants.

Further abiotic stress tolerance experiments with transgenic grapevine plants are in progress in our laboratory.

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## References

Deák M, Horváth VG, Davletova S, Török K, Sass L, Vass I, Barna B, Király Z, Dudits D (1999) Plants ectopically expressing the iron-blinding protein, ferritin are tolerant to oxidative damage and pathogens. Nat Biotechnol 17:192-196

Hideg É, Schreiber U (2007) Parallel assessment of ROS formation and photosynthesis in leaves by fluorescence imaging. Photosynth Res 92:103-1084.

Oláh R, Szegedi E, Ruthner S, Korbuly J (2003) Thidiazuron-induced regeneration and genetic transformation of grapevine rootstock varieties. Vitis 42:133-136.

Reid KE, Olsson N, Schlosser J, Peng F, Lund ST (2006) An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biology 6:27.

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10:51-62.