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Metabolic engineering of Vit C: Biofortification of potato

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Vitamin C (ascorbic acid) is an essential component for collagen biosynthesis and also for the proper functioning of the cardiovascular system in humans. Unlike most of the animals, humans lack the ability to synthesize ascorbic acid on their own due to a mutation in the gene encoding the last enzyme of ascorbate biosynthesis. As a result, vitamin C must be obtained from dietary sources like plants. In this study, we have developed two different kinds of transgenic potato plants (*Solanumtuberosum* L. cv. Taedong Valley) overexpressing strawberry GalUR and mouse GLoase gene under the control of CaMV 35S promoter with increased ascorbic acid levels. Integration of the these genes in the plant genome was confirmed by PCR and Southern blotting. Ascorbic acid(AsA) levels in transgenic tubers were determined by high-performance liquid chromatography(HPLC). The over-expression of these genes resulted in 2-4 folds increase in AsA intransgenic potato and the levels of AsA were positively correlated with increased geneactivity. The transgenic lines with enhanced vitamin C content showed enhanced tolerance to abiotic stresses induced by methyl viologen(MV), NaCl or mannitol as compared to untransformed control plants. The leaf disc senescence assay showed better tolerance in transgenic lines by retaining higher chlorophyll as compared to the untransformed control plants. Present study demonstrated that the over-expression of these gene enhanced the level of AsA in potato tubers and these transgenics performed better under different abiotic stresses as compared to untransformed control.

We have also investigated the mechanism of the abiotic stress tolerance upon enhancing the level of the ascorbate in transgenic potato. The transgenic potato plants overexpressing GalUR gene with enhanced accumulation of ascorbate were investigated to analyze the antioxidants activity of enzymes involved in the ascorbate-glutathione cycle and their tolerance mechanism against different abiotic stresses under *invitro* conditions. Transformed potato tubers subjected to various abiotic stresses induced by methyl viologen, sodium chloride and zinc chloride showed significant increase in the activities of superoxide dismutase(SOD, EC 1.15.1.1), catalase, enzymes of ascorbate-glutathione cycle enzymes such as ascorbate peroxidase(APX, EC 1.11.1.11), dehydroascorbate reductase(DHAR, EC 1.8.5.1), and glutathione reductase(GR, EC 1.8.1.7) as well as the levels of ascorbate, GSH and proline when compared to the untransformed tubers. The increased enzyme activities correlated with their mRNA transcript accumulation in the stressed transgenic tubers. Pronounced differences in redox status were also observed in stressed transgenic potato tubers that showed more tolerance to abiotic stresses when compared to untransformed tubers. From the present study, it is evident that improved to lerance against abiotic stresses in transgenic tubers is due to the increased activity of enzymes involved in the antioxidant system together with enhanced ascorbate accumulated in transformed tubers when compared to untransformed tubers. At moment we also investigating the role of enhanced reduced glutathione level for the maintenance of the methylglyoxal level as it is evident that methylglyoxal is a potent cytotoxic compound produced under the abiotic stress and the maintenance of the methylglyoxal level is important to survive the plant under stress conditions.