Prunus Hexokinase 3 genes alter primary C-metabolism and promote drought and salt stress tolerance in Arabidopsis transgenic plants

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Abstract

Hexokinases (HXKs) and fructokinases (FRKs) are the only two families of enzymes in plants that have been identified as able to phosphorylate Glucose (Glc) and Fructose (Fru). Glc can only be phosphorylated in plants by HXKs, while Fru can be phosphorylated by either HXKs or FRKs. The various subcellular localizations of HXKs in plants indicate that they are involved in diverse functions, including anther dehiscence and pollen germination, stomatal closure in response to sugar levels, stomatal aperture and reducing transpiration. Its association with modulating programmed cell death, and responses to oxidative stress and pathogen infection (abiotic and biotic stresses) also have been reported. To extend our understanding about the function of HXK-like genes in the response of Prunus rootstocks to abiotic stress, we performed a detailed bioinformatic and functional analysis of hexokinase 3-like genes (HXK3s) from two Prunus rootstock genotypes, 'M.2624' (Prunus cerasifera Ehrh × P. munsoniana W.Wight & Hedrick) and 'M.F12/1' (P. avium L.), which are tolerant and sensitive to hypoxia stress, respectively. A previous large-scale transcriptome sequencing of roots of these rootstocks, showed that this HXK3-like gene that was highly induced in the tolerant genotype under hypoxia conditions. In silico analysis of gene promoters from M.2624 and M.F12/1 genotypes revealed regulatory elements that could explain differential transcriptional profiles of HXK3 genes. Subcellular localization was determinates by both bioinformatic prediction and expression of their protein fused to the green fluorescent protein (GFP) in protoplasts and transgenic plants of Arabidopsis. Both approaches showed that they are expressed in plastids. Metabolomics analysis of Arabidopsis plants ectopically expressing Prunus HXK3 genes revealed that content of several metabolites including phosphorylated sugars (G6P), starch and some metabolites associated with the TCA cycle were affected. These transgenic Arabidopsis plants showed improved tolerance to salt and drought stress under growth chamber conditions. Our results suggest that Prunus HXK3 is a potential candidate for enhancing tolerance to salt and drought stresses in stone fruit trees and other plants.