MYBLEU OVEREXPRESSION IN CITRANGE CARRIZO IMPROVES TOLERANCE TO HYPOXIC STRESS


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Classical breeding and genetic transformation have been used to improve deficit oxygen tolerance in different crops. However, by classical breeding successes have been achieved only in rice (*Oryza sativa*). The Italian citrus cultivation is, in some areas, performed on soils characterized by a content of clay and/or loam in excess compared to optimal levels, determining the inability to eliminate water excesses during years of heavy and prolonged rainfall. In this kind of soils, humidity retention, flooding and/or waterlogging, represent a serious environmental stress that can lead to anaerobic conditions affecting many plant processes. Anaerobiosis may cause premature senescence which results in leaf chlorosis, necrosis, defoliation, cessation of growth and in extreme cases even plant death.

A large number of Myb transcription factors are involved in plant responses to abiotic and biotic stresses. The Mybleu ectopic expression in *Arabidopsis thaliana* plants positively affects cellular metabolism and increases tolerance to oxygen deficit. This Myb transcription factor, isolated from rice (a monocot, herbaceous), is able to activate the anaerobic response pathways in Arabidopsis (a dicot, herbaceous). In order to clarify the mechanisms involved in the response to oxygen deficit and to improve tolerance to flooding in citrus species, we overexpressed Mybleu in Carrizo citrange, one of the most popular citrus rootstocks, by *Agrobacterium tumefaciens* mediated transformation.

Analysis by polymerase chain reaction (PCR) and sequencing showed that stable integration of the transgene in the plant genome was obtained in the 71.6% of the analysed plants. Real-time PCR performed on a random selection of transgenic plants lines confirmed the expression of the inserted transgene and allowed a classification of the transgenic plants based on the different expression levels of the transgene. Wild type and transgenic lines (both low and high expressing lines) were tested for their tolerance to hypoxia/anoxia by physiological, biochemical and molecular analyses. The results indicated that high expressing plants are more tolerant to hypoxia than wild type and low expressing plants.

Our findings on Carrizo citrange confirm previous results of improved tolerance in Arabidopsis by Mybleu supporting the hypothesis of a conservation of the regulatory pathways involved in the anaerobic response among species. Therefore, our results indicate that the overexpression of Mybleu may represent a powerful tool to improve hypoxic stress tolerance in crops.