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GENETIC ENGINEERING OF MAIZE WITH THE TRANSCRIPTION FACTOR *OSMYB4* FOR TOLERANCE TO LOW TEMPERATURE

V. NATOLI*, S. CORNETI*, M. MATTANA**, I. CORAGGIO**, R. TUBEROSA*

*) Department of Agroenvironmental Science and Technology, University of Bologna, Via Fanin 44, 40127 Bologna, Italy - roberto.tuberosa@unibo.it
**) Institute of Crop Biology and Biotecnology, IBBA - CNR, Via Bassini 15, 20133 Milano, - mattana@ibba.cnr.it

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A major constraint faced in maize cultivation is the extensive damage caused by late cold events frequently occurring at high latitudes or with very early sowing, especially in condition of high light intensity. Early sowing in maize also allows to reduce the negative effect on grain yield of mid-summer drought episodes. The rice gene *Osmyb4* encodes for a transcription factor involved in the cold acclimation pathway. When expressed into Arabidopsis, *Osmyb4* increases chilling and freezing tolerance (Vannini et al., 2004, Plant J, 37, 115-127). In this work, *Osmyb4* was genetically engineered in corn in order to explore the possibility to improve its level of tolerance to cold. We transformed three maize genotypes (bo21, B73A and GS3) with a biolistic protocol applied to type–II callus obtained from immature embryos. We used a mixture of two plasmid constructs, one carrying the *Osmyb4* gene and one carrying the selectable marker (*bar*) and the reporter (*gus*) genes both driven by a constitutive promoter (ubiquitin). Two different constructs containing *Osmyb4* were prepared with either an inducible (Cor15) or a constitutive (ubiquitin) promoter. The selection for transgenic events was carried out by cultivation of calli in media containing Bialaphos (3 mL/L) for 90 d followed by testing for Gus expression. All Gus-positive calli were then regenerated. Out of several hundreds of independently treated calli, transformation frequency was 1.13% for bo21 and 0.13% for GS3. We did not recover any transgenic event from B73A callus and from the constructs with *Osmyb4* under constitutive promoter. The transgenic nature of the regenerants was confirmed by PCR. Southern analysis is in progress to verify the integrity of the transgene in each transformation event. Three out of 20 independent transgenic events were finally selected (R0 plants) based on a check for integrity of the gene and promoter sequences. The corresponding selected R1 progenies were subjected to a further PCR-based check to confirm the integration of the gene. Then the progenies were tested for cold-response, at 2 °C for 4 h, total RNA was extracted and used for RT-PCR to assess the level of transgene expression. Out of the three selected events, only one of them showed a strong and rapid induction of the transgene expression. The selected event (bo21-E-14-2) was further investigated as to its cold response by subjecting the plants to two different cold-stress treatments. Plants (the selected transformed event and the wild type, bo21) were treated either at 2 °C for three days or 2 °C for six days. Plants were allowed to recover at 24 °C for five days with 16 h of photoperiod. The shorter cold treatment did not affect the integrity of the leaf tissues in bo21-E-14-2, while the wild-type showed clear tissue necrosis; the longer cold treatment induced leaf damages in both wild type and bo21-E-14-2, but the damage was more evident in the control. After the recovery period only the transformed plants survived and were able to recover. After exposure to cold, leaf tissue of bo21-E-14-2 showed a substantial increase in sugar and praline content as compared to the control. Preliminary results indicate that bo21-E-14-2 is also able to withstand a drought stress sufficient to cause irreversible damage in control plants. Further experiments have been planned to better ascertain the level of tolerance to drought of bo21-E-14-2.