Genetic engineering of grain sorghum (Sorghum bicolor (L.) Moench) for nutritional quality improvement

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Abstract PhD thesis

Sorghum (Sorghum bicolor L. Moench), the fifth most important cereal crop in the world, is one of the most important staple crops in Africa and South Asia and represents the only viable food grain for many of the world’s most food insecure people. However, sorghum grain shares the typical nutritional deficiencies of cereal grains, a low content of several essential amino acids. Therefore, a diet based mostly on sorghum, is not adequate to meet the nutritional growth or maintenance requirements for children and adults and partly causes malnutrition for 100 million people, especially 30-50 million children under 5 years of age. Almost another 200 million are at risk (http://www.ahbfi.org/ABS.pdf). Therefore, sorghum grain with enhanced protein quality would contribute significantly to improving the nutritional value of the diets of people as well as livestock dependent on sorghum as a major protein source.

In the present study, an improved in vitro regeneration system suitable for Agrobacterium-mediated transformation was developed. The improvements focused on limiting the production of phenolic compounds and the use of suitable culture vessels for each developmental stage in plant regeneration from immature embryo derived calli. The addition of AC in the callus induction medium reduced the production of black pigments; however it also inhibited the callus formation on immature embryo explants. Cold pre-treatment of the immature seeds from which embryo explants were excised had a positive effect on both explant survival and callus formation. A one-day 4°C treatment of immature seeds significantly improved the callus formation from immature embryos and reduced the need for frequent subculture. Petri dishes with ventilation were suitable for the callus induction phase, but not for plant regeneration. Regeneration of plants could be improved by using disposal plastic boxes (250 ml volume) instead of Petri dishes. Our improved tissue culture system increased Agrobacterium-mediated transformation efficiency of sorghum from 0.07% to 5%.

The genes encoding a methionine-rich 15+10kDa zein protein (15 kDa β-zein fused with a 10 kDa δ-zein; provided by Dr. R. Amir, Migal Galilee Technology Centre, Israel) and a lysine-rich barley chymotrypsin inhibitor CI-2 (provided by Prof. P. Shewry, Rothamsted, UK) under the control of a seed specific zein promoter were introduced into sorghum genotype Sensako 85/1191. The stable insertion and expression of the transgenes was proved by PCR, Southern, Northern and Western analysis and their transmission to progeny through segregation analysis. Transgenes were inherited in a Mendelian fashion and were integrated at a single locus in the majority of the transgenic lines.

The expression of the 15+10kDa zein or the CI-2 protein resulted in raised seed methionine and lysine concentrations. Quantification of methionine or lysine in T2 seeds of transgenic plants showed that, the methionine or lysine content increased approximately 40% or 33%, respectively, compared to the non-transgenic control.