

Transgenic sorghum: Effects of altered kafirin synthesis on kafirin polymerisation, protein quality, protein body structure and endosperm texture

By

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DECLARATION

I hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other university or institution of higher learning.

Laura Suzanne da Silva

DEDICATION

I dedicate this thesis to my wonderful family. To my husband, José, for his continued support, understanding and patience over the last six years. To my sons, Daniel and Nathan, for ensuring that play time and walks at the Botanical Gardens were non-negotiable.

Perseverance is the hard work you do after you get tired of
doing the hard work you already did.

Newt Gingrich

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ABSTRACT

Transgenic sorghum: Effects of altered kafirin synthesis on kafirin polymerisation, protein quality, protein body structure and endosperm texture

By

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Supervisor: Prof. J.R.N. Taylor

Co-supervisor: Dr J. Taylor

Transgenic (TG) sorghum genotypes with altered kafirin synthesis were developed by the Africa Biofortified Sorghum Project, employing recombinant DNA technology, with the aim of improving the protein nutritional quality of the grain. In this project, the effects of altered kafirin synthesis on kafirin polymerisation, protein quality, protein body structure and endosperm texture in different TG lines were investigated.

The first generation of TG lines were in a type II low-tannin sorghum background. Altered synthesis of different major kafirin sub-classes (α -, γ - and δ -kafirin) was targeted. Some TG lines had improved lysine content (3.17 g/100 g protein) and moderate (55%) to high (74%) cooked *in vitro* protein digestibility, compared to the parent (2.05 g/100 g protein; 47.4%, respectively). This is of significance as tannins reduce protein digestibility, by complexing with the proline-rich kafirins. Transmission electron microscopy revealed that the improved protein quality traits were associated with floury endosperm texture and irregular protein body structure. Irregular protein bodies were 2-3 μm in diameter, with few to numerous invaginations, compared to normal protein bodies. The high digestibility TG line also had a unique dense protein matrix, with occasional thick dark-staining inclusions. It appears that reduced kafirin synthesis, specifically γ -kafirin, has a major effect on the protein body structure, which in turn results in changes in protein digestibility and endosperm structure.

To further improve the protein quality and poor endosperm texture of the first generation of TG lines, improved non-tannin sorghums were transformed to suppress kafirin synthesis, or they were back-crossed into TG lines with improved protein quality. Co-suppression of the α -, γ - and δ -kafirin sub-classes and removal of the tannin trait, resulted in TG with high cooked protein digestibility ($\pm 80\%$), improved Amino Acid Score (0.8) and Protein Digestibility Corrected Amino Acid Score (0.7) compared to the non-TG null controls ($\pm 50\%$, 0.4 and 0.2, respectively). However, these high-protein quality lines still had a floury endosperm texture. They also had irregular shaped protein body structure, as described previously. When fewer kafirin sub-classes were suppressed (only γ - and δ -kafirin) the endosperm was corneous with normal protein body structure, but the improvement in cooked protein digestibility was less. Apparently, co-suppression of several kafirin sub-classes is required to obtain high-protein quality sorghum, but this seems to result in floury-type grain endosperm.

Further work conducted on the high digestible TG line revealed that the proportion of kafirin-1, extracted with 60% tert-butanol alone, was greatly increased. However, the total amount of kafirin remained unchanged. Also, the kafirin was much less polymerised by disulphide bonding, and there was evidence of compensatory synthesis of other kafirin proteins. Hence, the mechanism for the increased protein digestibility of TG lines is probably related to their lower levels of disulphide-bonded kafirins, allowing better access of proteases. This work appears to confirm that disulphide bond formation in kafirin is responsible for the reduced protein digestibility of cooked sorghum.

Since grain hardness is an important grain quality attribute, playing a major agronomic role, in sorghum processing and in the end-use quality of sorghum-based foods, further research should focus on transforming sorghum to have both improved protein nutritional quality and good grain endosperm texture.

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