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

By

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Submitted in partial fulfilment of the requirements for the degree

PhD

Food Science

In the

Department of Food Science
Faculty of Natural and Agricultural Sciences
University of Pretoria
South Africa

March 2012

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.

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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

ACKNOWLEDGEMENTS

I would like to acknowledge all those people who have helped make this thesis possible.

Firstly, words cannot express the gratitude I have for my supervisors Prof. John R. N. Taylor and Dr Janet Taylor for all their guidance, encouragement, constructive criticism and patience. Their devotion to cereal science, academic excellence and to their students is inspiring.

Thanks are due to Dr Zuo-Zhao of Pioneer Hi-Bred International, USA, and Dr Luke Mehlo of the CSIR BioSciences, South Africa, for the transgenic sorghums. Dr Dirk Hays of the Texas Agricultural Experiment Station Sorghum Improvement Program, Texas A&M University, for the high protein digestibility mutant sorghums.

Special thanks goes to: Alan Hall and Chris van der Merwe (Laboratory for Microscopy and Microanalysis, University of Pretoria) for microscopy training and guidance, Theresa de Beer for taking responsibility for part of my lecturing load and Susan Appelcryn for helping to obtain many of the articles needed.

I would like to express my gratitude to Erika Jordaan and colleagues at the Department of Biotechnology and Food Technology (Tshwane University of Technology), and the staff and postgraduate students at the Department of Food Science (University of Pretoria), for their continued support, encouragement and friendship.

The Bill and Melinda Gates Grand Challenges 9 and Africa Biofortified Sorghum Project are thanked for funding this project through a sub-grant to the Africa Harvest Biotechnology Foundation International. The South African National Research Foundation and Tshwane University of Technology, Faculty of Science Research and Innovation Committee, are thanked for additional funds.

Finally, I thank my family and friends for all their love and support.

ABSTRACT

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

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Laura Suzanne da Silva

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-, ɣ- 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 ɣ-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-, ɣ- and δ-kafirin sub-classes and removal of the tannin trait, resulted in TG with high cooked protein digestibility (±80%), improved Amino Acid Score (0.8) and Protein Digestibility Corrected Amino Acid Score (0.7) compared to the non-TG null controls (±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 ɣ- 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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