The Effect of Phytate reduction on Sorghum (*Sorghum bicolor* L. Moench) grain Germination

By

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Declaration

I declare that this thesis is a record of an original work and contains no material, which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

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Josephine Amedu
9 December 2016

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Preface

This research was carried out over a twelve month period as part of the Master of Biotechnology (Plant Biotechnology) program. Following the requirements of the program, the research is presented in the format of a manuscript for submission to the peer-reviewed scientific journal, Journal of Experimental Botany. However, margins, font size, spacing as well as inserted figures and tables have been customized to satisfy the thesis guidelines for the program.

My co-authors for the manuscript are Professor Rachel Burton, Professor Aba Daniel and Dr. Natalie Betts. Professor Burton was the overall supervisor of this project, developed most protocols used in this project and reviewed drafts of manuscripts; Professor Daniel helped design the field sampling protocol while Dr. Betts oversaw the laboratory experiments as well as data analysis.

The manuscript in this thesis is intended as the first draft for future publication. The word count for the manuscript is 6592, excluding references, appendices and supplementary material.

Highlights

Germination processes in sorghum, with emphasis on endosperm modification and cell wall structure remodelling, are more similar to barley than previously reported.

Abstract

Sorghum quality is improved by reducing anti-nutritional components, including phytates that sequester cations such as iron, zinc and calcium, to make nutrients more bioavailable for absorption. The current study investigated the quality and germination of a transgenic variety developed by the Africa Biofortified Sorghum project, aimed at developing sorghum varieties with reduced phytate content. However, results showed a significantly higher phytate content in transgenic grains ($p<0.05$) when compared with the wild type (WT). Furthermore, phytate in transgenic grains was less susceptible to degradation over 96 hrs of germination when compared with WT. Further study focused exclusively on WT grain where starch degradation was limited in the first 72 hrs but significantly increased by 96 hrs. This decrease in starch content strongly correlated ($r^2=0.93$) with α-amylase activity that peaked at 115 CU/g at 96 hrs. (1,3;1,4)-β-glucan levels changed a little during germination, remaining at approximately 0.5% (w/w) even in the presence of increased beta-glucanase activity. Fluorescent microscopy showed that (1,3;1,4)-β- glucan and arabinoxylan around the pericarp, aleurone layer and embryo changed marginally over 96 hrs of germination. While treatment with GA repressed α-amylase activity, starch degradation patterns resembled untreated samples. GA induced lower, but same secretion patterns of endo-(1,3;1,4)-β-glucanase as untreated samples but delayed degradation pattern of (1,3;1,4)-β-glucan. These results suggest that the germination process in sorghum grain may be more similar to events in barley than previously reported.

Keywords: Phytate, germination, ABS, cell wall degradation, gibberellic acid, *Sorghum*

Abbreviations

ABS; Africa Bio-fortified Sorghum

AX; Arabinoxylan

BC; Backcross

CU; Ceralpha Units

GA; Gibberellic acid

Hrs; Hours

Ins; Inositol

IMP; Inositol monophosphate

IPK1; Inositol-pentakisphosphate 2-kinase

ITPK; Inositol tris/tetrakisphosphate kinases

LPA; Low phytic acid

MIK; *Myo*-inositol kinase

MIPS; *Myo*-inositol-3-phosphate synthase

PCR; Polymerase chain reaction

2PGK; 2-phosphoglycerate kinase

WT; Wild type