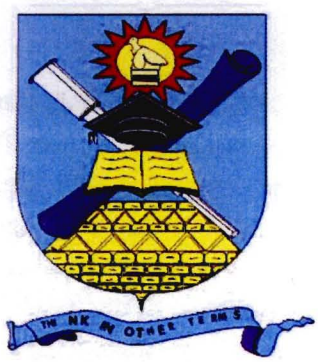
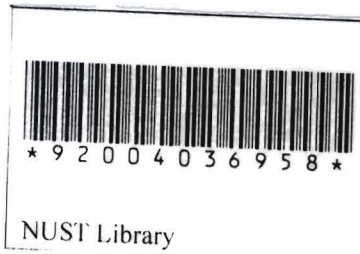


NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

FACULTY OF APPLIED SCIENCES

DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY

APPLICATION OF BIOCHEMICAL AND MOLECULAR TECHNIQUES IN THE CHARACTERISATION OF FLAVONOID PATHWAY MUTANTS IN *SORGHUM BICOLOR*



NGONIDZASHE MANGOMA

N0114332B

A dissertation submitted in partial fulfillment of the requirements of the Master of Science Degree in Applied Microbiology and Biotechnology

September 2013

ABSTRACT

Flavonoids confer important agronomic traits in developing and mature sorghum grain, and play a major role in human and animal nutrition. Sorghum lines that accumulate the desired spectrum of flavonoids may be identified through screening of large collections of mutants, and further developed through selective breeding. The aim of this project was to apply biochemical and molecular techniques in the characterisation of selected sorghum flavonoid pathway mutants. The DMACA stain assay and the Butanol-HCl assay were used to assay for condensed tannins in the grain, while 3-deoxyanthocyanidins and flavan-4-ols were assayed for spectrophotometrically. Furthermore, genomic DNA extracted from a subset of 11 selected landraces was used in PCR amplification of the sorghum *Anthocyanidin synthase* (*ANS*) and *Tannin 1* (*Tan1*) genes, and a putative sorghum vacuole transporter protein (*SbTT12*) gene. Restriction digestion analysis and sequencing was used to investigate sequence variations at the three *loci*. From the DMACA assay, 89 % of the brown lines, 4 % of the red lines and none of the white lines tested positive for condensed tannins in their grain, while only five landraces produced unusual DMACA staining patterns. Quantifiable condensed tannins were detected in all but just 3 of the brown lines tested, while only 3 red lines and none of the white lines had assayable condensed tannins in their grain. Levels of condensed tannins detected were in the range 0.1 – 1.8 AU at 550nm per gram of sorghum grain sample (butanol-HCl assay). The levels of flavan-4-ols and 3-deoxyanthocyanidins were generally low in all landraces. The *ANS*, *Tan1* and *SbTT12* genes were amplified in all selected samples as confirmed by agarose gel electrophoresis and sequencing. There was differential migration of *SbTT12* gene PCR products, with the PCR amplicons appearing slightly bigger in white accessions as compared to brown and red ones. This could be due to an insertion in the white accessions at this *locus*, rendering the gene defective. This phenomenon can be potentially used in the generation of sorghum mutants with novel flavonoid profiles by insertional mutagenesis targeting the *SbTT12* gene. However, restriction digestion analysis and sequencing did not reveal any relevant sequence variations at all three *loci* under study. Therefore, the differences in flavonoid profiles observed in the different landraces could be due to mutations at other *loci*, or due to a combination of genetic and environmental factors.