Impact of *in vitro* induced epigenetic variation on the nutritional value of three Ghanaian sweet potato genotypes: implications on biofortification

By

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A thesis submitted for the partial fulfilment of the requirements of the Master of Biotechnology (Plant Biotechnology)

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2016

Declaration

I declare that this thesis is a record of 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 and belief, 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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15th December 2016

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Preface

This research was performed over 10 months as part of a Master of Biotechnology (Plant Biotechnology). In accordance with the requirements of the program, the research is presented in the format of a manuscript for submission to a peer-reviewed scientific journal. I have chosen to follow the format of Plant Cell Tissue and Organ Culture. My co-authors for the manuscript are Dr Carlos M. Rodríguez López, Dr Marian D. Quain, and Dr Sunita Ramesh. Dr Sunita Ramesh and Dr Marian Quain are my main supervisors who assisted with experimental design, ordering of the reagents and consumables, providing the protocols used except for MSAP, and reviewing drafted versions of the manuscript. Dr Carlos M. Rodríguez López assisted with the protocols used in MSAP, statistical analysis of the MSAP data, and interpretation of data.

The manuscript in this thesis is intended as the first draft of a manuscript for future publication. The word count for the manuscript (excluding references and supplementary material) is 7000. Appendix 1 of this thesis contains a more complete set of acknowledgements than was possible to include within the manuscript itself. Appendix 2 contains supplementary data. I have followed the instructions of the authors for Plant Cell Tissue and Organ Culture, except that 1.5-cm margins at the top, bottom and right-hand side of the page, and a 3.5-cm margin on the left-hand side of the page was allowed for the binding of the manuscript. I have also included figure legends in the manuscript for easy access to examiners in order to satisfy the thesis guidelines for the Master of Biotechnology (Plant Biotechnology) program.

Manuscript

Title: Impact of *in vitro* induced epigenetic variation on the nutritional value of three Ghanaian sweet potato genotypes: implications on biofortification

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Key words Biofortification, DNA methylation, Epigenetics, Field-maintained, *In vitro*, Micropropagation, Orange Fleshed Sweet Potato, Sweet potato, Virus-free

Abstract

Biofortification aims to increase crop nutritional value to combat nutrient deficiency. Due to the prevalence of viruses, healthy cultivars of biofortified genotypes are produced through micropropagation techniques. However, during micropropagation, plants are exposed to conditions that could induce somaclonal variation, and result in phenotypic changes affecting the crop's nutritional value. Currently, sweet potato (*Ipomoea batata*) is biofortified for enhanced beta-carotene content to alleviate Vitamin A Deficiency (VAD). Undesired somaclonal abnormalities acquired during *in vitro* culture could alter key nutrients such as beta-carotene, protein, or zinc. Therefore, it is important to ensure the clonal fidelity of the micropropagated biofortified lines. This study assessed the extent of *in vitro* induced epigenetic variation in the genome of meristem-cultured plants, and its correlation with the nutritional composition in three Ghanaian sweet potato genotypes (Bohye, Ogyefo and Otoo). Micropropagated plants presented no observable leaf and storage root abnormalities, but relatively lower levels of iron, protein, zinc, and glucose. Methylation Sensitive Amplification Polymorphism analysis showed a high level of *in vitro* induced molecular variation in micropropagated plants. Ogyefo showed the least viral incidence and epigenetic differentiation but the most profound nutritional changes, while Bohye showed the highest epigenetic variability. Further analysis indicated that epigenetic, rather than genetic, accounts for most of the observed variability. Taken collectively, this study offers an insight into the impact of micropropagation in methylation profiles, and its correlation to root quality in the improved sweet potato genotypes. The implications of these results to the ongoing bio-fortification projects are also discussed.

Abbreviations

AMOVA Analysis of molecular variance

ANOVA Analysis of variance

CFT Confined Field Trials

CIP International Potato Centre

CRI Crops Research Institute

CSIR Council for Scientific and Industrial Research

CTAB Cetyltrimethylamonium bromide

DAP Days After Planting

EDTA Ethylenediaminetetraacetic acid

FM Field-Maintained

HPLC High Pressure Liquid Chromatography

ICGEB International Centre of Genetic Engineering and Biotechnology

MSAP Methylation Sensitive Amplification Polymorphism

MSL Methylation Susceptible Loci

NCBI National Centre for Biotechnology Information

NML No-Methylation Loci

NIRS Near Infrared Spectrophotometry

OFSP Orange Fleshed Sweet potato

PTC Plant Tissue Culture

RCBD Randomized Complete Block Design

SSA Sub-Saharan Africa

VAD Vitamin A Deficiency

VF Virus-free

WAP Weeks After Planting