MOLECULAR CHARACTERIZATION AND AGRONOMIC BIOFORTIFICATION OF ZINC AND β-CAROTENE IN SELECTED SWEET POTATO GERMPLASM

By

KHANDAKAR ABU MD MOSTAFIZAR RAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To my lovely parents and my beloved wife and daughter who always kept me in their prayer to achieve my goal

and

To my family members

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Professor Dato' Abdul Shukor bin Juraimi, PhD
Faculty : Agriculture

Micronutrient deficiencies coupled with malnutrition affect key development processes including poor physical and mental development in children. Health impacts of micronutrient deficiency are not always acutely visible; it is therefore sometimes termed 'hidden hunger'. Sweet potato (*Ipomoea batatas* L., family: Convolvulaceae) is one of the most traditional root crops in many countries including Bangladesh and Malaysia. Screening and molecular characterization using genetic markers for determining the short duration, Zn and β-carotene enriched sweet potato are very effective tools to confirm this species' diversity. In this regard, the present study we investigated the molecular characterization and agronomic bio-fortification of zinc for higher beta carotene in selected sweet potato germplasm and varieties. Three independent experiments were conducted following randomized complete block design (RCBD) to determine molecular characterization and agronomic bio-fortification of zinc for higher β-carotene. In the first experiment, genetic diversity of 20 sweet potato germplasms were evaluated for their morphological and antioxidative properties. The higher individual tuber root weights (about 0.4 kg) were recorded in SP14 (BARI Misti Alu 14), SP15 (BARI Misti Alu 15), SP16 (BARI Misti Alu 16) and SP20 (VitAto) and it ranges from 0.13 kg to 0.4 kg. Result showed significant germplasm variation in β-carotene content among 20 sweet potato germplasms and it ranges from 0.03 to 27.47 mg/100g fresh weight in SP03 (BARI Misti Alu 03) and SP16 (BARI Misti Alu 16), respectively. The PCA analysis showed that, PC1 accounted from 42.2 percent of the overall variability across the collected germplasm. Considering the phenotypic performance and the correlation matrix of the traits, five germplasm such as SP002 (BARI Misti Alu 02), SP014 (BARI Misti Alu 14), SP015 (BARI Misti Alu 15), SP016 (BARI Misti Alu 16) and SP020 (VitAto) found promising for future breeding programs towards development of carotenoid enriched sweet potato germplasm and β-carotene content of those five germplasm were 13.59, 14.833, 12.35, 11.13 and 13.433 mg/100 g fresh weight. In the second experiment, genetic diversity of 20 sweet potato germplasms were accomplished using simple sequence repeats (SSRs) marker. A total of 64 alleles were generated by

the 20 primers throughout the 20 germplasm samples, with locus IBS97 having the highest number of alleles (5) whereas locus IbU33 had the fewest alleles (2). The Polymorphism Information Content (PIC) values for the loci IbL46 and IBS97 varied from 0.445 to 0.730. IBS97 has the highest number of effective alleles (3.704), compared to an average of 2.520. The sweet potato germplasm included in this study had a broad genetic base. SP01 (BARI Misti Alu 01) vs. SP09 (BARI Misti Alu 09) and SP12 (BARI Misti Alu 12) vs. SP18 (Gendut) germplasm pairings had the greatest genetic distance (GD=0.965), while SP01 (BARI Misti Alu 01) vs. SP02 (BARI Misti Alu 02) germplasm couples had the least genetic diversity (GD =0.093). Twenty germplasms were classified into two groups in the UPGMA dendrogram, with 16 germplasms classified as group "A" and the remaining four germplasms, SP10 (BARI Misti Alu 10), SP18 (BARI Misti Alu 18), SP19 (Kuala bikam2), and SP20 (VitAto) classified as group "B". In the third experiment, both soil and foliar application of Zn were evaluated on growth, yield attributes, bio-chemical characteristics and nutrient content in tuber of five germplasm of sweet potato. There were two doses of Zn applied in soil such as 0 and 2.5 kg/ha and four doses of Zn applied as foliar includes 0, 15, 30, and 45 ppm. The growth, yield contributing characters and yield were increased by Zn irrespective of method of application. The nutrient contents and the bio-chemical compounds were also found to be increased by Zn application. Amino acid content in sweet potato were influenced by foliar application of Zn but not with soil application. The Zn content in the tuber were also found to be increased either through soil or foliar application. It was evident that, the effects of Zn application on different studied parameters were more conspicuous as foliar than those of soil application. Therefore, the present study gives the new insights of screening the collected sweet potato germplasm based on their genetic potentiality of tuber yield, β-carotene content, and ability of Zn biofortification. Among the germplasms studied, VitAto can be planted for achieving the highest yield (26.2 t/ha), BARI Misti Alu 15 for higher protein (Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, and Tryptophan), and while BARI Misti Alu 2 and (BARI Misti Alu 14 for higher β-carotene (13.912 and 14.833 mg/100 g fresh weight, respectively).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN MOLEKULAR DAN BIOFORTIFIKASI AGRONOMI ZINK DAN BETA KAROTENA BAGI GERMPLASMA TERPILIH UBI KELEDEK

Oleh

KHANDAKAR ABU MD MOSTAFIZAR RAHMAN

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Kekurangan mikronutrien dan malnutrisi merupakan antara punca utama kerencatan tumbesaran fizikal dan mental kanak-kanak. Walau bagaimanapun, impak kesihatan berpunca daripada kekurangan mikronutrien ini biasanya tidak ketara menyebabkan ia dikenali sebagai "kelaparan yang tersembunyi". Ubi keledek (*Ipomoea batatas* L., family: Convolvulacea) merupakan salah satu tanaman akar tradisional di banyak negara termasuk Bangladesh dan Malaysia. Satu cara yang efektif dalam menentukan kepelbagaian spesis ubi keledek singkat masa yang diperkaya dengan Zn dan beta-karotena adalah melalui saringan dan pencirian molekular menggunakan penanda genetik. Oleh itu, kajian ini meneroka pencirian molekular dan biofortikasi agronomi zink bagi germplasma terpilih dan varieti ubi keledek dengan beta karotena yang tinggi melalui tiga eksperimen individu menggunakan randomized complete block design (RCBD). Dalam eksperimen pertama, kepelbagaian genetik 20 ubi keledek telah dinilai dari segi morfologi dan perihal antioksida. Berat ubi per individu yang tinggi (kira-kira 0.4 kg) telah dicatatkan oleh SP14 (BARI Misti Alu 14), SP15 (BARI Misti Alu 15), SP16 (BARI Misti Alu 16) dan SP20 (VitAto) dengan perbezaan jumlah antara 0.13 kg hingga 0.40 kg. Hasil eksperimen menunjukkan perbezaan yang signifikan terhadap isi kandungan beta karotena dalam 20 germplasma ubi keledek dengan berat segar 0.03 sehingga 27.47 mg/100 g berat segar untuk SP03 (BARI Misti Alu 03) dan SP16 (BARI Misti Alu 16). Analisis Principle Component Analysis (PCA) menunjukkan bahawa 42.2 peratus dari keseluruhan kepelbagaian germplasma adalah dipengaruhi oleh PC1. .Berdasarkan persembahan fenotip dan korelasi ciri-ciri, lima germplasma iaitu SP002 (BARI Misti Alu 02), SP014 (BARI Misti Alu 14), SP015 (BARI Misti Alu 15), SP016 (BARI Misti Alu 16) dan SP020 (VitAto) menunjukkan potensi untuk diperkenalkan dalam program pembiakkan bagi menghasilkan germplasma varieti ubi keledek yang tinggi dengan karotenoid. Kandungan berat segar beta karotena lima germplasma tersebut adalah 13.59, 14.83, 12.35, 11.13 dan 13.43 mg/100 g. Dalam eksperimen kedua, kepelbagaian genetik 20 germplasma ubi keledek telah dikenalpasti menggunakan penanda *simple sequence repeats* (SSRs). Sejumlah 64 alel telah dihasilkan dari 20 sampel germplasma menggunakan 20 pasangan primer. Daripada

jumlah tersebut, lokus IBS97 mempunyai bilangan alel yang paling tinggi (5 alel), manakala lokus IbU33 mempunyai bilangan alel yang paling rendah (2 alel). Jumlah Isi Kandungan Polimorfisme (PIC) untuk lokus IbL46 dan lokus IBS97 berbeza dari 0.445 hingga 0.730. IBS97 mempunyai jumlah alel efektif yang paling tinggi iaitu 3.704 berbanding purata sebanyak 2.520. Germplasma ubi keledek yang telah digunakan dalam kajian ini mempunyai kepelbagaian genetik yang luas. Kombinasi germplasma SP01 (BARI Misti Alu 01) vs. SP09 (BARI Misti Alu 09) dan SP12 (BARI Misti Alu 12) vs. SP18 (Gendut) mempunyai jarak genetik yang paling jauh (GD=0.965). Manakala, kombinasi germplasma SP01 (BARI Misti Alu 01) vs. SP02 (BARI Misti Alu 02) mempunyai kepelbagaian genetik yang paling rendah (GD=0.093). Dua puluh germplasma telah dibahagikan kepada dua buah kumpulan dalam dendrogram UPGMA di mana 16 germplasma telah diklasifikasikan sebagai kumpulan "A" dan lebihan empat germplasma, SP10 (BARI Misti Alu 10), SP18 (BARI Misti Alu 18), SP19 (Kuala bikam2) dan SP20 (VitAto) telah diklasifikasikan sebagai kumpulan "B". Dalam eksperimen ketiga, aplikasi Zn terhadap kedua-dua tanah dan dedaun telah dilakukan dan penilaian terhadap tumbesaran, hasil, ciri-ciri biokimia dan kandungan zat ubi dalam lima germplasma ubi keledek telah direkodkan. Dua dos Zn yang digunakan dalam tanah adalah 0 dan 2.5 kg/ha, manakala empat dos yang digunakan pada dedaun adalah 0, 15, 30 dan 45 ppm. Kedua-dua cara aplikasi Zn menunjukkan peningkatan terhadap tumbesaran, ciri-ciri hasil dan hasil ubi keledek. Selain itu, kandungan zat dan kompaun biokimia juga meningkat selepas aplikasi Zn. Kandungan asid amino juga dipengaruhi oleh aplikasi Zn terhadap dedaun tetapi tidak dipengaruhi oleh aplikasi Zn ke atas tanah. Kandungan Zn dalam ubi juga meningkat sama ada melalui aplikasi Zn terhadap tanah atau dedaun. Bukti-bukti telah menunjukkan bahawa kesan aplikasi Zn adalah lebih ketara melalui aplikasi terhadap dedaun berbanding tanah berdasarkan pelbagai parameter yang telah dikaji. Oleh itu, kajian ini memberi perspektif baru terhadap penyaringan germplasma ubi keledek berdasarkan potensi genetik ubi, isi kandungan beta karotena dan kebolehupayaan biofortifikasi Zn. Berdasarkan semua germplasma yang telah dikaji, VitAto boleh ditanam untuk mengeluarkan hasil ubi yang paling tinggi (26.2 t/ha), BARI Misti Alu 15 boleh ditanam untuk hasil protein yang tinggi (Isoleucine, Leucine, Lycine, Methionine, Phenylalanine dan Tryptophan) dan BARI Misti Alu 2 dan BARI Misti Alu 14 boleh ditanam untuk menghasilkan ubi dengan beta karotena yang tinggi (masing-masing mempunyai 13.912 mg/100 g dan 14.833 mg/100 g berat segar).

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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
OFSP	Orange-Fleshed Sweet Potato
SSR	Simple Sequence Repeats
CIP	International Potato Centre
MS	Moderately Smooth
ddNTPs	dideoxynucleotide
SNP	Single Nucleotide Polymorphism
AFLP	Amplified Fragment Length Polymorphism
RAPD	Randomly Amplified Polymorphic DNA
SAMPL	Selective Amplification of Microsatellite Polymorphic Loci
ANOVA	Analysis of variance
ISSR	Inter Simple Sequence Repeats
RCBD	Randomized complete block design
DAF	DNA Amplification Fingerprinting
ppm	Part per million
HPLC	High Performance Liquid Choromatography
PGPR	Plant Growth Promoting Bacteria
ROS	Reactive Oxygen Species
SAS	Statistical Analysis Software
BHT	Butylated Hydroxytoluene
PCA	Principle Component Analysis
LMV	Length of Main Vine
EDTA	Cetyltrimethylammonium Bromide
TAE	Tris acetate EDTA

PIC	Polymorphism Information Content
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
g	Gram
Kg	Kilogram
µ mole	Micromole
%	Percentage
℃	Degree Celsius
µ	Micron
cm	Centimeter
g	gram
m	meter
L	Liter
mg	Milligram
MARDI	Malaysian Agricultural Research and Development Institute
TCRC	Tuber Crops Research Center
BARI	Bangladesh Agricultural Research Institute
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 Research background

Sweet potato (*Ipomoea batatas* L. Lam) is a nutritious food crop that belongs to the Convolvulaceae family locally known as 'keledek' in Malaysia. It originated from Central America and North-western parts of South America. It is an important food crop, ranking third in production value and fifth in caloric value for human diets around the world (Muñoz-Rodríguez *et al*., 2018). In Malaysia, sweet potato ranked second largest tuber crops next to cassava (Yusoff *et al*., 2018). It is one of the popular crops grown by small farmers for the fresh market. Several regions in Malaysia namely Perak, Kelantan and Terengganu are engaged in sweet potato cultivation. In Malaysia, the Department of Agriculture Peninsular Malaysia reported that a total of 4,634.79 metric tons sweet potato production was recorded (Muhammad *et al*., 2021). In addition to its nutritional benefits, the crop is easy to grow even on less fertile soils, has a short growing season and is resistant to various biotic as well as abiotic constraints. The presence of several vitamins, amino acids and minerals as well as carbohydrates makes it a food with great nutritional value (Dong *et al*., 2017). In developing countries, it plays an important role in food as well as nutritional security where it is a source of beta carotene that promotes good health.

Sweet potato is one of Malaysia's most popular freshly consumed tuber crops. Several sweet potato varieties, particularly orange-fleshed contain high amounts of β-carotene, starch, dietary fiber, minerals, vitamins (particularly vitamins C, B6 and folate), as well as several antioxidants (Neela and Fanta, 2019). Orange- fleshed sweet potato has a high concentration of beta carotene which provides vitamin A and is used for the treatment of vitamin A deficiency especially for children. For children aged between six months to five years, vitamin A supplementation improves their immune system, intervenes in disease reduction reducing infant mortality by 24 % and saving more than 600,000 lives each year in low and middle-income countries (Imdad *et al*., 2017). Low *et al*., (2017) stated that yellow and orange-fleshed sweet potato cultivars have high beta-carotene content, and their consumption encourages the fight against vitamins deficiency. Orange-fleshed sweet potato is an excellent source of the provitamin A, β -carotene. In addition to being rich in β -carotene, it contains significant amounts of fat, protein, carbohydrate, dietary fibre, other micronutrients and some phytonutrients (Amagloh *et al*., 2021). Therefore, orange-fleshed sweet potato is a staple food that can provide a supply of vitamin A and energy to people in resource-poor developing countries. (Lakhawat, 2018).

Crop production increased significantly with the use of micronutrients through their specific functions (Zewide *et al*., 2021). Zinc is one of them which plays an important metabolic role in the growth and development of plants and is therefore known as an essential micronutrient or trace element (Mousavi *et al*., 2013). It stimulates the biosynthesis of growth hormone, starch formation and seed production and their maturation. In plants, Zn found in the form of Zn^{2+} that has a physiological function in all living systems (Khan *et al*., 2019), such as facilitating the formation of protein, maintaining biological membranes' functional and structural integrity, gene expression and energy production, Krebs cycle and enzyme's structure and also improve crop production. Qualitative and quantitative crop production depends heavily on Zn concentration in soil (Mousavi *et al*., 2013). Zinc is usually required in small amounts, but it is essential for several plant physiological passages to work (Yosefi *et al*., 2011). Zinc is needed in the carbonic enzyme, present in every photosynthetic tissue that is required for chlorophyll biosynthesis (Khan *et al*., 2019).

Agronomic biofortification reported to increase micronutrient concentrations temporarily by enhancing fertilization in productive parts of plants (Siwela *et al*., 2020). It may increase the levels of iron, zinc, iodine and selenium in edible portion of the plant. In addition, to adopt the agronomic farming technique in increasing micronutrient accumulation, the timing of foliar spray is found to be important. Zinc is applied as $ZnSO_4$, ZnO, or as synthetic chelates to crops (White and Broadley, 2009). The Zn concentrations in the edible part of crops depend on the ability of the crops to acquire Zn from Zn-fertilizer and accumulate it in their edible portions (White and Brown, 2010). In addition to raise Zn levels in food and feed, the bio-fortification strategy also promises to raise crop yields in infertile soils.

Although sweet potato is an important crop, its genome has not yet been fully sequenced. This is due to the complex genomes of sweet potatoes that are difficult to assemble. Sweet potatoes are hexaploid and highly polymorphic with very large genome size (Wu *et al*., 2018). However, the origin of polyploidy (autopolyploidy, allopolyploidy) in sweet potatoes is still unclear (Wu *et al*., 2018). RAPD and AFLP markers were used for sweet potato genetic linkage (Monden and Tahara, 2017). Simple-sequence repeats (SSR) markers were used to assess diversity and characterization of sweet potato (Zhang *et al*., 2016). Recently, genome sequencing and assembly has been used for research on the sweet potato genome. Thus, sweet potatoes have been studied in many ways, from molecular markers to sequencing (Kin *et al.*, 2018; Isobe *et al.*, 2019; Gao *et al*., 2020).

1.2 Problem statement

Mitigation of micronutrient deficiencies has progressed with interventions such as dietary supplementation, plant breeding, and plant genetic engineering. Zinc deficiency is the most prevalent micronutrient deficiency in humans, negatively affecting at least one-third of the world's population (Wang *et al*., 2020) and vitamin A deficiency mainly affects children in under developing countries (Wirth *et al*., 2017). Globally, it is estimated that around 30% of children <5 years of age are deficient in vitamin A and around 2% of all deaths are attributable to vitamin A deficiency in this age group. The dark, orange-fleshed sweet potato is reported to have a beta-carotene content that can reach 350% or more of the recommended daily allowance (Tumwegamire *et al*., 2011). It is an excellent source of β-carotene and can be promoted to alleviate vitamin A deficiency. Zn is a micronutrient present in sweet potatoes but found at a very low amount. So, Zn biofortified sweet potatoes varieties help to increase the amount of Zn in sweet potatoes as a result we can develop sweet potatoes varieties enriched with β-carotene and Zn content.

Sweet potato as a food security crop is especially popular among farmers with limited resources, and is increasingly being recognized as having an important role in improving national and household food security, health and livelihoods of poor families (Shikuku *et al*., 2019). Orange-fleshed sweet potato is an excellent source of β-carotene, and can be promoted to alleviate vitamin A deficiency. To be successful in reducing vitamin A malnutrition, a biofortified crop such as the orange-fleshed sweet potato must be high yielding and profitable to the farmer, be shown to be efficacious and effective at reducing vitamin A malnutrition, and be acceptable to both farmers and consumers in target region. For subsistence farming, planting high yielding cultivars, using improved agronomic practices and the availability of agricultural inputs are important to increase production (Bashir & Schilizzi, 2013).

In Malaysia, sweet potato research is mainly focused on the development of specific germplasm of sweet potato with high yield and quality. Studies have shown that foliar application of Zn is effective in increasing the bioavailability of Zn, while little is known about its agronomic bio-fortification in beta-carotene-rich sweet potatoes. Thus, molecular characterization of the cultivated beta-carotene rich sweet potato and subsequent bio-fortification with Zn through agronomic means would ensure food and nutritional security in the developing countries including Bangladesh.

1.3 Objectives

The study aimed to investigate molecular characterization and agronomic biofortification of zinc for beta carotene rich sweet potato varieties/germplasm.

The specific objectives of the study were:

i. To determine the molecular characterization of selected sweet potato germplasm
ii. To evaluate high beta carotene rich sweet potato germplasm from collected sweet potato germplasm
iii. To investigate bio-fortification of sweet potato through agronomic approach

1.4 Hypotheses

i. Ho: Selected sweet potato germplasm do not exhibit different molecular characterization
Ha: Selected sweet potato germplasm exhibit different molecular characterization

ii. Ho: There are no high beta carotene rich sweet potato germplasm from collected sweet potato germplasm
Ha: There are high beta carotene rich sweet potato germplasm from collected sweet potato germplasm

iii. Ho: There are no bio-fortification of sweet potato through agronomic approach
Ha: There are bio-fortification of sweet potato through agronomic approach

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