Metabolic screening of carotenoids and elucidation of a carotenoids metabolic pathway in sweetpotato (*Ipomoea batatas*)

Muhammad Zubair Khan*[1,2], Miho Takemura[2], Takashi Maoka[3], Motoyasu Otani[2] and Norihiko Misawa[2]

[1] Department of Biotechnology, Faculty of Sciences, University of Kotli AJK 11100 Pakistan (Corresponding Author's Current Affiliation)

[2] Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi-shi, Ishikawa 921-8836, Japan

[3] Research Institute for Production Development, Shimogamo-morimotocho, Sakyo-ku, Kyoto 606-0805, Japan

*Corresponding Author email: zubairgenes@gmail.com

Background

Sweetpotato [*Ipomoea batatas* (L.) Lam], also described as "sweet potato", belongs to the family Convolvulaceae and occupies the seventh position among the food crops of the world after wheat, rice, maize, potato, barley, and cassava. Sweetpotato has not been fully evaluated for its primary and secondary metabolites in Pakistan. It is a source of food supply to combat malnutrition in the developing nations, since the tuberous roots (tubers) are enriched with starch and dietary fiber, along with carotenoids, anthocyanin, ascorbic acid, potassium, calcium, iron, and other bioactive ingredients. Despite of having high rank among the world food crop plants, no studies on local germplasm have been made regarding its variation and utilization in conventional and molecular plant breeding. The genetic studies on this species are exhausting, since it is difficult to generate seeds and to evaluate the effects of polyploidy on the genome. Complex structure of its genome also manifests self and cross-incompatibility causing barrier for genetic studies on important agronomical characters.

Carotenoids, the visible colors of life are the 40-carbon isoprenoids synthesized naturally by fungi, bacteria, algae, cyanobacteria and lower and higher plants. They play numerous metabolically active roles in living organisms. All carotenoids show antioxidants activities appearing in a variety of colors in red, yellow and orange and are divided into two broad classes of carotenes and xanthophylls. Carotenes and xanthophylls are used as natural pigments in food, food supplements, nutraceuticals, pharmaceutical and cosmetic industry and other biotechnological purposes. Sweetpotato is highly rich in β-carotene (pro-vitamin A) along with variable amounts of other carotenoids in its tubers. Molecular mechanism of the carotenoids accumulation in sweetpotato has not yet been fully studied. The present study encompassed metabolic screening of carotenoids, structural and functional analyses of cyclases genes and elucidation of a comprehensive carotenoids metabolic biosynthesis pathway in sweetpotato for the first time.

Materials and Methods

Two sweetpotato (*I. batatas*) cultivars, WS and W71, were selected for carotenoid pigments extraction and analysis by HPLC-PDA-HRMS. Carotenoid composition in the leaves and tubers of sweetpotato cultivars was calculated based on the HPLC chromatograms. 3'and 5'-RACE PCRs were carried out to isolate full length cDNA sequences of carotenoids cyclases genes from selected cultivars. Isolated carotenoids cyclases genes were sequenced, aligned for phylogenetic relationship, functionally identified by *E. coli* heterologous gene complementation expression system and submitted to DDBJ & GenBank. For complementary expression of the two IbLCYb and IbLCYe genes, they were cloned simultaneously into pETDuet and named pETD-IbLCYb/IbLCYe. Each plasmid was introduced into a lycopene-producing *Escherichia coli* [BL21 (DE3)], which carried the plasmid pACCRT-EIB for the expression of the crtE, crtB, and crtI genes for lycopne synthesis from the bacterium *Pantoea ananatis.*

Results

Carotenoids Composition of Sweetpotato Cultivars WS and W71

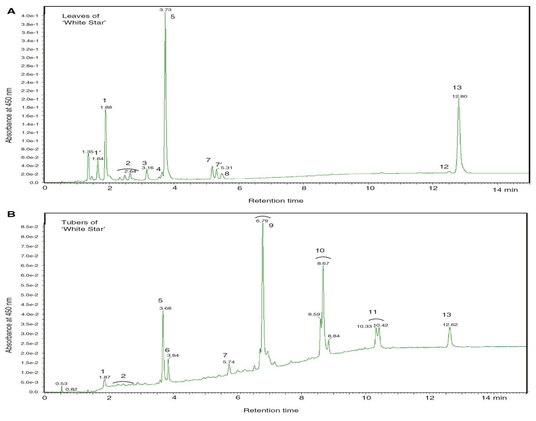

Figure 1. HPLC chromatograms of carotenoids extracted from the leaves (A) and tubers (B) of sweetpotato (*Ipomoeoa batatas*) cultivar WS.
Identification of peaks: 1', neochrome; 1, neoxanthin; 2, violaxanthin (including auroxanthin); 3, antheraxanthin; 4, zeaxanthin; 5, lutein; 6, ipomoeaxanthin A; 7, 9-cis-lutein, 7', 13-cis-lutein; 8, β-cryptoxanthin; 9, β-carotene-5,6,5',8'-diepoxide (stereoisomers); 10, β-carotene-5,8,5',8'-diepoxide (stereoisomers); 11, β-carotene-5,8-epoxide (stereoisomers); 12, α-carotene; 13, β-carotene.

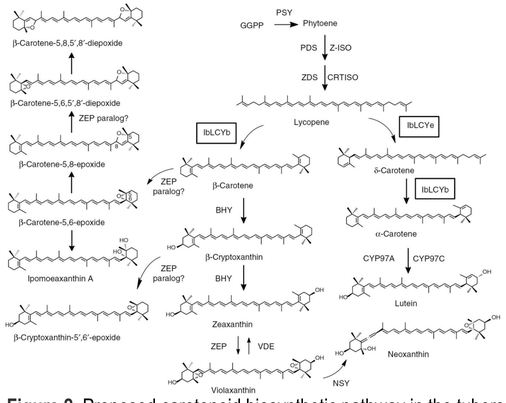

Figure 2. Proposed carotenoid biosynthetic pathway in the tubers of sweetpotato cultivars WS and W71.
Carotene cyclases elucidated in this work are boxed. GGPP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; CRTISO, Carotenoid isomerase; LCYb, lycopene β-cyclase; LCYe, lycopene ε-cyclase; CYP97A/C, heme-containing cytochrome P450-type carotene ε- ring hydroxylases ;BHY, Non-heme di-iron type carotene β-ring hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NSY, neoxanthin synthase.

Table 1. Carotenoid content and composition in WS and W71 cultivars of sweetpotato, where 'nd', represents 'not detected'

	WS leaf	W71 leaf	'White Star' tuber	W71 tuber
Total carotenoid (μg/g FW)	34	170	0.5	4.0
Carotenoid composition	%	%	%	%
β-Carotene	30.7	31.1	7.3	59.3
α-Carotene	0.6		nd	nd
β-Cryptoxanthin	2.4			3.2
Zeaxanthin	0.7			trace
Lutein	43.2	46.8	13.7	2.0
cis-Lutein	3.3	3.3		
Antheraxanthin	1.8			
Violaxanthin (+ Auroxanthin)	12.3	3.5	0.9	
Neoxanthin (+ Neochrome)	3.6	6.4	1.5	
β-Carotene-5,8-epoxide	nd	nd	9.4	4.6
β-Carotene-5,6,5',8'-diepoxide	nd	nd	35.7	9.2
β-Carotene-5,8,5',8'-diepoxide	nd	nd	25.1	13.8
β-Cryptoxanthin-5',6'-epoxide	nd	nd	nd	2.2
Ipomoeaxanthin A	nd	nd	4.5	nd
Others	1.4	8.9	1.9	5.7

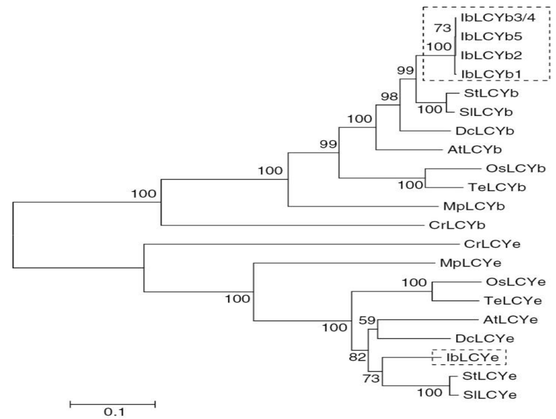

Figure 3. Phylogenetic tree of various lycopene β-cyclases (LCYb) and lycopene ε-cyclases (LCYe) including the newly isolated sweetpotato carotene cyclases. Two and three lycopene β-cyclase gene (IbLCYb) sequences were, respectively isolated from WS, named IbLCYb1 and IbLCYb2, and from W71, designated IbLCYb3, IbLCYb4 and IbLCYb5. IbLCYb3 and IbLCYb4 were found to encode the same deduced amino acid sequences. A single sequence designated IbLCYe, was isolated from both WS and W71.

GenBank accession numbers of the aligned cyclases include: Arabidopsis LCYb (AtLCYb), U50739; carrot LCYb (DcLCYb), DQ192190; wheat LCYb (TaLCYb), JN622196.1; rice LCYb (OsLCYb), XP_015627235; tomato LCYb (SlLCYb1), EF650013;potato LCYb (StLCYb), XP_006364433; liverwort LCYb (MpLCYb),AB794089; Chlamydomonas LCYb (CrLCYb), AY860818; Arabidopsis LCYe (AtLCYe), U50738.1 carrot LCYe (DcLCYe), DQ192192.1; wheat LCYe (TaLCYe), EU649787; rice LCYe (OsLCYe), XP_015622198; tomato LCYe (SlLCYe), Y14387; potato LCYe (StLCYe), XP_006353544.1; liverwort LCYe (MpLCYe), AB794090; Chlamydomonas LCYe (CrLCYe), AY606130.

Figure 4. Tuberous roots of sweetpotato (*Ipomoea batatas* L.)
Photo Courtesy : Professor Norihiko Misawa

Conclusions

Metabolic analysis of the carotenoids accumulated by tuberous roots (tubers) of sweetpotato cultivars WS and W71 showed that rare carotenoids unique to sweetpotato, having a 5,6-epoxy-β-ring and/or a 5,8-epoxy-β-ring in their structures exist. Based on the content and the composition of carotenoids analyzed in WS and W71, a comprehensive carotenoids metabolic biosynthetic pathway was elucidated for the first time in sweetpotato. This pathway is a new horizon in better understanding and future manipulation of carotenoids metabolism at molecular level and to combat malnutrition of secondary metabolites through useful bio-fortifications.

Recommendations and Future Projection

Present results will help in molecular cloning and elucidation of the carotenoids biosynthesis genes and their metabolic pathway engineering either using foreign ketolase genes or by RNAi based silencing and genome editing for enhanced production of indigenous and novel carotenoids in sweetpotato and other related species to be possibly used in agricultural, nutritional, pharmaceutical, and cosmetic industries. Presently, locally selected high yielding cultivars from Pakistan are also being screened for carotenoids potential under HEC SRGP for future molecular plant breeding through pathway engineering & gene editing.

Literature Cited

Khan M Zubair, Takemura M, Maoka T, Otani M, and Misawa N (). Carotenoid analysis of sweetpotato *Ipomoea batatas* and functional identification of its lycopene β- and ε-cyclase genes. Z. Naturforsch. C 2016; 71, 9-10: 313-322.

Maoka T, Akimoto N, Ishiguro K, Yoshinaga M, Yoshimoto M. Carotenoids with a 5,6-dihydro-5,6-dihydroxy-beta-end group, from yellow sweet potato "Benimasari", Ipomoea batatas Lam. Phytochemistry 2007;68, 1740–5.

Acknowledgements

i) Author is highly thankful to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Japan for funding this research through MEXT Research Scholarship Program.

ii) The authors are grateful to Mr. Mitsuo Hashimoto for his technical support in growing the sweetpotato plants.