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Overexpression of the *IbMYB1* gene in an orange-fleshed sweet potato cultivar produces a dual-pigmented transgenic sweet potato with improved antioxidant activity

Sung-Chul Park[a,b], Yun-Hee Kim[c], Sun Ha Kim[a], Yu Jeong Jeong[d], Cha Young Kim[d], Joon Seol Lee[e], Ji-Yeong Bae[f], Mi-Jeong Ahn[f], Jae Cheol Jeong[a], Haeng-Soon Lee[a,b] and Sang-Soo Kwak[a,b,*]

[a]Plant Systems Engineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea
[b]Department of Green Chemistry and Environmental Biotechnology, University of Science & Technology (UST), Daejeon, Korea
[c]Department of Biology Education, College of Education, and Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Korea
[d]Infection Control Material Research Center, Bio-materials Research Institute, KRIBB, Jeongeup, Korea
[e]Bioenergy Crop Research Center, National Institute of Crop Science, RDA, Muan, Korea
[f]College of Pharmacy and Research Institute of Life Sciences, Gyeongsang National University, Jinju, Korea

Correspondence
*Corresponding author,
e-mail: sskwak@kribb.re.kr

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The R2R3-type protein IbMYB1 is a key regulator of anthocyanin biosynthesis in the storage roots of sweet potato [*Ipomoea batatas* (L.) Lam]. Previously, we demonstrated that *IbMYB1* expression stimulated anthocyanin pigmentation in tobacco leaves and Arabidopsis. Here, we generated dual-pigmented transgenic sweet potato plants that accumulated high levels of both anthocyanins and carotenoids in a single sweet potato storage root. An orange-fleshed cultivar with high carotenoid levels was transformed with the *IbMYB1* gene under the control of either the storage root-specific sporamin 1 (*SPO1*) promoter or the oxidative stress-inducible peroxidase anionic 2 (*SWPA2*) promoter. The *SPO1-MYB* transgenic lines exhibited higher anthocyanin levels in storage roots than empty vector control (EV) or *SWPA2-MYB* plants, but carotenoid content was unchanged. *SWPA2-MYB* transgenic lines exhibited higher levels of both anthocyanin and carotenoids than EV plants. Analysis of hydrolyzed anthocyanin extracts indicated that cyanidin and peonidin predominated in both overexpression lines. Quantitative reverse transcription-polymerase chain reaction analysis demonstrated that *IbMYB1* expression in both *IbMYB1* transgenic lines strongly induced the upregulation of several genes in the anthocyanin biosynthetic pathway, whereas the expression of carotenoid biosynthetic pathway genes varied between transgenic lines. Increased anthocyanin levels in transgenic plants also promoted the elevation of proanthocyanidin and total phenolic levels in fresh storage roots. Consequently, all *IbMYB1* transgenic plants displayed much higher antioxidant activities than EV plants. In field cultivations, storage root yields varied between the transgenic lines. Taken together, our results indicate that overexpression of *IbMYB1* is a highly promising strategy for the generation of transgenic plants with enhanced antioxidant capacity.

Abbreviations – ANS, anthocyanidin synthase; AsA, L-ascorbic acid; bHLH, basic helix-loop-helix; CHI, chalcone isomerase; CHS, chalcone synthase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DW, dry weight; EV, empty vector; F3H, flavonoid 3-hydroxylase; HPLC, high-performance liquid chromatography; LMO, living modified organism; PAL, phenylalanine ammonia-lyase; PAP1, PRODUCTION OF ANTHOCYANIN PIGMENT1; PDS, phytoene desaturase; RT-qPCR, quantitative reverse transcription-polymerase chain reaction; SHM, sinhwangmi; SPO1, sporamin 1; SWPA2, sweet potato anionic peroxidase 2; SZM, sinzami; TF, transcription factor; UV, ultraviolet.

Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam] is one of the most nutritionally important root crops worldwide, especially in parts of Asia and Africa. It is widely used not only as a major food source but also as an important industrial raw material for the production of animal feed, alcohol and various antioxidants such as anthocyanin and carotenoids (Oki et al. 2002, Teow et al. 2007). The sweet potato flesh color depends on pigmentation from carotenoids or anthocyanin. These antioxidants are of particular interest because they perform protective roles in combating oxidative stress, which is implicated in aging and the development of a number of diseases, including cancer (Chen et al. 2005, Luceri et al. 2008). As a consequence, recent attention has focused on orange- or purple-fleshed sweet potato cultivars that have naturally high levels of carotenoids or anthocyanins, respectively, and are popular among consumers.

In higher plants, natural purple, orange and green pigmentations are controlled by the relative quantities of anthocyanins, carotenoids and chlorophyll, respectively. These compounds are not only necessary for maintaining plant health and performance but are also considered to be phytonutrients that are beneficial for human health (Tanaka et al. 2008). Plant anthocyanins are elevated in response to developmental, environmental and stress stimuli, including temperature, nutrient status, wounding, pathogen infection, water stress and various light conditions (Christie et al. 1994, Chalker-Scott 1999, Steyn et al. 2002). Anthocyanins have antioxidant properties and free radical-scavenging capacities and can inhibit the growth of tumor cells (Chen et al. 2005). Anthocyanin biosynthesis is transcriptionally regulated by a combination of three components: an R2R3-MYB protein, a basic helix-loop-helix (bHLH, MYC) protein and WD40-type co-regulators (WD40) (Ramsay and Glover 2005). MYB transcription factors (TFs) play particularly pivotal roles in the transcriptional regulation of secondary metabolism in plant flavonoid biosynthetic pathways. For example, maize purple plant (Cone et al. 1993), Arabidopsis PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1)/AtMYB75 and PAP2/AtMYB90 (Borevitz et al. 2000), grape VlMYBA (Kobayashi et al. 2002) and apple MdMYB10 (Espley et al. 2007, Espley et al. 2013) all control anthocyanin biosynthesis. The overexpression of PAP1 in transgenic Arabidopsis stimulated the upregulation of a number of genes in the anthocyanin biosynthesis pathway, including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and dihydroflavonol 4-reductase (DFR) (Borevitz et al. 2000, Tohge et al. 2005). The apple *MdMYB10* gene was found to control anthocyanin biosynthesis in apple fruit skin and in the flesh of a red-fleshed cultivar (Espley et al. 2007). In sweet potato, Mano et al. (2007) reported that the R2R3-type IbMYB1 from a purple-fleshed sweet potato variety (cv. Ayamurasaki) was responsible for purple pigmentation in the flesh of storage roots. IbMYB1 exhibits an expression pattern that correlates with the expression of anthocyanin pathway genes and is able to activate the promoters of a number of these genes (Mano et al. 2007).

Plants do not generally accumulate carotenoids in their roots; however, the storage roots of sweet potato and carrot are exceptions that serve as good sources of carotenoids, especially β-carotene. In plants, carotene is a precursor for abscisic acid biosynthesis and is also essential for photosynthetic processes. In animals, carotene provides the major dietary source of provitamin A; this is essential, as animals and humans are unable to synthesize vitamin A (Tanaka et al. 2008). Vitamin A deficiency is a serious global problem, especially in Africa and South-East Asia. The World Health Organization estimates that vitamin A deficiency affects an estimated 190 million preschool children and 19.1 million pregnant women worldwide. Furthermore, night blindness is estimated to affect approximately 15 million preschool children and pregnant women (WHO 2009).

Transgenic approaches, aimed at providing dietary improvement, have been used to increase successfully anthocyanins or flavonoids in useful crop plants such as tomato (Butelli et al. 2008) and apple (Espley et al. 2013). Transgenic *MYB10* apple exhibited increased levels of anthocyanin, flavonoids and proanthocyanidins through increased transcription of related genes, and β-carotene content also increased in the peel and cortex of *MYB10* fruit (Espley et al. 2013). Overexpression of the maize TFs *Lc* (*bHLH*) and colorless1 (*MYB*) in tomato fruit produced elevated flavonoid levels but no increase in anthocyanin levels (Bovy et al. 2002). Another transgenic tomato, in which the *DET1* gene was downregulated, exhibited increased flavonoid and carotenoid contents (Davuluri et al. 2005). Moreover, a *DET1* knockout mutant had increased anthocyanin content in light conditions (Mustilli et al. 1999). A black-colored (peel and flesh) anthocyanin-rich tomato was developed through overexpression of the *Delila* (*bHLH*) and *Rosea1* (*MYB*) TFs from *Antirrhinum majus* (Butelli et al. 2008). Although sweet potato is recognized as an important healthful and useful root crop plant, the metabolic engineering of various pigment antioxidants in transgenic plants has not been substantially investigated to date.

In a previous study, we demonstrated that expression of *IbMYB1* under the control of a storage root-specific sporamin 1 (*SPO1*) promoter or an oxidative stress-inducible peroxidase anionic 2 (*SWPA2*) promoter regulated phenylpropanoid pathway genes such as *PAL*, *CHS*, chalcone isomerase (*CHI*), flavonoid 3-hydroxylase (*F3H*), *DFR* and anthocyanidin synthase (*ANS*) in tobacco leaves and Arabidopsis (Kim et al. 2010, Chu et al. 2013). In this study, we generated dual-pigmented transgenic sweet potato plants by overexpressing the *IbMYB1* gene, under the control of the sweet potato *SPO1* or *SWPA2* promoters, in a β-carotene-rich orange-fleshed sweet potato. Expression of the *IbMYB1* gene caused activation of anthocyanin synthesis genes in the transgenic sweet potato, thereby conferring high levels of antioxidant activity in the storage roots.

Materials and methods

Plant materials and expression vectors

Wild-type sweet potato cultivars Sinzami (SZM) and Sinhwangmi (SHM) were obtained from the National Institute of Crop Sciences, Rural Development Administration, Korea. SZM produces high amounts of anthocyanin (approximately 3.7 mg g^{-1} dry weight [DW]) but a low level of total carotenoids (approximately 10 µg g^{-1} DW). The SHM cultivar has 18× higher carotenoid levels than SZM but no detected anthocyanin in the storage root flesh (see Fig. S1). We isolated the *IbMYB1* gene from the anthocyanin-rich sweet potato SZM cultivar and constructed vectors as previously described (Kim et al. 2010).

Sweet potato transformation and regeneration

Embryogenic calli from a orange-fleshed sweet potato (SHM) were used for *Agrobacterium*-mediated transformation, as described previously (Lim et al. 2004). The transformed cells were selected on MS (Murashige and Shoog) medium containing 400 mg l^{-1} cefotaxime and 100 mg l^{-1} kanamycin and were subcultured to fresh medium at 3 week intervals. Regenerated plantlets were transferred to pots for further experiments.

Sample preparation for experiments

All storage root samples used in this study were obtained in 2013 from field-cultivated plants. On the day of harvest, approximately 200 g of storage roots (five roots from each line) were washed, towel dried, peeled and sliced and immediately frozen at −80°C, before lyophilization. Lyophilized sweet potato samples were ground into a fine powder and stored at −80°C until required for further experiments. All analyses were conducted in biological triplicate.

Analysis of anthocyanins

Anthocyanins were extracted from 0.2 g of finely ground plant material, as described by Chu et al. (2013). Briefly, lyophilized samples were extracted with 1 ml of acidic methanol containing 1% HCl (v/v) for 18 h at room temperature with moderate shaking. Subsequently, 500 µl of the supernatant was mixed with 500 µl of MilliQ H_2O_2 (Millipore, Bedford, MA, USA) and 300 µl of chloroform to remove carotenoids. The supernatant (water–methanol phase) was determined spectrophotometrically at 530 and 657 nm. The acidic methanol extraction (100 µl) was hydrolyzed as described by Chu et al. (2013). The sample was added to 900 µl of solvent [95:5 (v/v), n-butanol (100%):HCl (36%)], and the mixture was boiled for 2 h to release core anthocyanidins. The sample was then dried in a speed vacuum at room temperature, the residue was dissolved in 100 µl of 0.1% HCl–methanol solvent [0.1% HCl in high-performance liquid chromatography (HPLC)-grade methanol] and the sample was centrifuged at 14 500 *g* for 10 min. Core anthocyanidin molecules were identified from the supernatant by HPLC analysis, as described by Chu et al. (2013). All chromatograms were monitored at 520 nm. Cyanidin and hydrolyzed peonidin-3-O-glucoside chloride (Sigma-Aldrich, St. Louis, MO) were used as standards for identification.

Analysis of carotenoids

Carotenoids were extracted from 100 mg of peeled sweet potato storage roots and analyzed using the Agilent 1100 HPLC system (Hewlett-Packard, Palo Alto, CA, USA), as previously described (Kim et al. 2012, Kim et al. 2013). The HPLC-DAD system was operated using CHEMSTATION software (Hewlett-Packard). Carotenoids were quantified by an external calibration method. The β-carotene, β-cryptoxanthin, lutein, violaxanthin and zeaxanthin standards were obtained from CaroteNature (Lupsingen, Switzerland). Under these conditions, the peaks of standard carotenoids at retention time (min) values were as follows: violaxanthin (peak: 11.5), lutein (peak: 23.3), zeaxanthin (peak: 26.6), β-cryptoxanthin (peak: 33.5) and β-carotene (peak: 39.2). Carotenoid contents were measured in a minimum of three different storage roots. All content levels were expressed as the mean (the average content in g DW) ± SD (the standard deviation) of two independent determinations from three individual storage roots.

Analysis of proanthocyanidin

Quantification of total proanthocyanidin from the empty vector (EV) line and *IbMYB1* transgenic plants was performed using the vanillin–HCl method, as described previously (Price et al. 1978). Lyophilized storage root flesh (100 mg) was extracted using 10 ml of absolute methanol for 20 min, with shaking. Samples were centrifuged at 2500 *g* for 10 min, and the supernatant was used for subsequent analysis. Supernatant (1 ml) was mixed with 5 ml of 1% (v/v) vanillin and 4% HCl solution in methanol and added to 5 ml of 4% HCl volumetric mix. Mixtures were incubated for 20 min at 30°C in a water bath. Absorbance of samples and blanks was assessed at 500 nm using an ultraviolet (UV)/Vis spectrophotometer. Proanthocyanidin concentrations were determined in triplicate.

Analysis of total phenolics

Total phenolic contents were spectrophotometrically analyzed according to the method of Ainsworth and Gillespie (2007). Total phenolic acid was extracted with 2 ml of ice-cold 95% (v/v) methanol from 20 mg of lyophilized storage root flesh. Following centrifugation at 13 000 *g* for 5 min, 100 µl of the supernatant was mixed with 200 µl of 10% (v/v) Folin–Ciocalteu reagent, and 800 µl Na_2CO_3 (700 m*M*) was added. The mixture was incubated at room temperature for 2 h, after which absorbance was determined at 765 nm. Total phenolics were calculated as chlorogenic acid equivalents using the regression equation between chlorogenic acid standards and absorbance at 765 nm.

Analysis of radical-scavenging activity

The radical-scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was analyzed as described by Kim et al. (2013). Four *IbMYB1* overexpression sweet potato storage roots (approximately 200 g) were prepared by boiling in water for 20 min and then cooling to room temperature. Boiled sweet potatoes were peeled and whole flesh lyophilized. Extracts were prepared in 95% methanol from unboiled and boiled storage root flesh from *IbMYB1* overexpression lines and EV control lines. Absorbance of the extract was determined at 517 nm. L-Ascorbic acid (AsA) was used as a standard for the calibration curve, and the DPPH radical-scavenging activities were calculated as AsA equivalents per gram of tested samples.

Gene expression analysis

Total RNA was extracted from the lyophilized storage root samples with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and then treated according to the manufacturer's instructions with DNase I (Takara, Kyoto, Japan) to remove any traces of genomic DNA. Total RNA (2 µg) was reverse transcribed using oligo-dT primers and an M-MLV cDNA synthesis kit (Clontech, Mountain View, CA, USA), according to the manufacturer's instructions. Gene-specific primers were designed using a stringent set of criteria to enable application of universal reaction conditions. The sequence and accession numbers for each primer pair were as indicated in Table S2. Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) analysis was carried out in 96-well plates with a CFX real-time PCR system and CFX system software (Bio-Rad, Hercules, CA, USA) using the EvaGreen-based PCR assay. Each reaction (final volume of 20 µl) contained 1 µl diluted cDNA, 10 µl of EvaGreen PCR Master Mix (Solgent, Daejeon, Korea) and 1 µl of each primer. The PCR conditions were as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 20 s, 60°C for 40 s and 72°C for 20 s. The melting curves were analyzed at 65–95°C after 40 cycles. Each RT-qPCR was performed in duplicate. ADP-ribosylation factor was used as the reference gene for quantification as its transcript level is constant throughout root and leaf tissues.

Field cultivation of transgenic sweet potato plants

Sweet potato field cultivation was conducted in 2013 at the living modified organism (LMO) field of the National Institute of Crop Science, Mokpo, Korea. Twenty-five transgenic lines were grown for 1 month. Stems were then cut at 15 cm lengths, and 40 stems of each line were transplanted by hand in the field with 25 cm between plants and 60 cm between rows. The experimental design comprised a randomized complete block with three replications. Nitrogen (55 kg ha^{-1}), phosphorus (63 kg ha^{-1}) and potassium (156 kg ha^{-1}) fertilizers were incorporated into the soil before transplanting; thereafter, plots were mulched with black polyethylene films. Other cultural management practices were performed in accordance with the standard sweet potato cultivation methods defined by the Rural Development Administration, Korea. At harvest time, the fresh weights of the above-ground shoots and storage roots were recorded.

Statistical analysis

Experimental data were analyzed by one-way ANOVA. Subsequent multiple comparisons were examined by using the Dunnett test. All statistical analyses were performed using the STATISTICAL PACKAGE for SOCIAL SCIENCES (SPSS 12), and statistical significance was set at $*P<0.05$ and $**P<0.01$.

Results

Generation of dual-pigmented transgenic sweet potato plants

To generate dual-pigmented sweet potato plants by genetic engineering, binary vectors containing the *IbMYB1* gene under the control of either the storage roots-specific *SPO1* promoter or the oxidative stress-inducible peroxidase anion 2 (*SWPA2*) sweet potato promoter were transformed into a β-carotene-rich sweet potato cultivar (SHM) (Fig. 1A). Transgenic sweet potato plants expressing *IbMYB1* under the control of the *SPO1* (referred to *SPO1-MYB* plants) or S*WPA2* promoter (*SWPA2-MYB* plants) and EV as control (EV) were successfully generated by *Agrobacterium*-mediated transformation. Each independent transgenic line was grown in a growth chamber and LMO field for 4 months. Putative transgenic sweet potato lines exhibited ectopic accumulation of anthocyanin that produced a deep or mild pigmentation in the storage roots. Ten of fifteen *SPO1-MYB* lines and three of five *SWPA2-MYB* lines accumulated anthocyanin in storage roots (see Fig. S2). Two transgenic lines each from *SPO1-MYB* (2 and 14) and *SWPA2-MYB* (1 and 3) were selected on the basis of their high anthocyanin and carotenoid levels and were used for further characterization (Fig. 1B, Table S3). Anthocyanin strongly accumulated in shoot xylem in *SWPA2-MYB* line l, but only weak purple pigmentation was noted in the xylem of the other transgenic lines compared with EV control plants (Fig. 1B). *SPO1-MYB* leaves (both lines) were similar to those in EV plants, whereas the *SWPA2-MYB* leaves showed slightly higher anthocyanin accumulations than those of EV plants. Starch is the major storage carbohydrate of sweet potato (Bovell-Benjamin 2007). We measured starch contents to determine whether increased anthocyanin content affects starch levels in the storage roots of *IbMYB1* transgenic plants. Starch contents (23–25%) of wild-type (SHM), EV and *IbMYB1* transgenic plants were not statistically different (data not shown).

Increased levels of anthocyanins and transcripts of anthocyanin biosynthetic genes

Anthocyanin levels in *IbMYB1* transgenic and EV storage roots were spectroscopically assessed (Fig. 2A). Total average anthocyanin content varied from 176.5 $\mu g\,g^{-1}$ DW in *SWPA2-MYB* line 3 to 1545.4 $\mu g\,g^{-1}$ DW in *SPO1-MYB* line 14, whereas the EV line storage roots produced no anthocyanin. HPLC chromatograms indicated that cyanidin and peonidin were the predominant core anthocyanidins recovered from the *SPO1-MYB* and *SWPA2-MYB* storage roots (Fig. 2B). These data indicate that the overexpression of *IbMYB1* in transgenic sweet potato promoted accumulation of cyanidin and peonidin anthocyanidins.

Quantitative RT-PCR analysis was used to analyze the transcription profiles of anthocyanin pathway

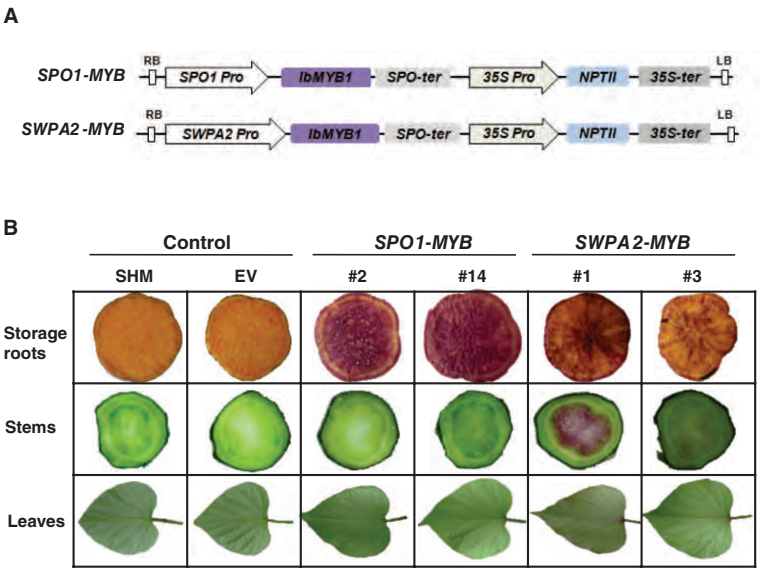

Fig. 1. Phenotypes of sweet potato lines overexpressing the *IbMYB1* gene. (A) Schematic diagram of the T-DNA region of the *SPO1-IbMYB1* and *SWPA2-IbMYB1* constructs used for plant transformation. LB and RB, left and right T-DNA borders, respectively; *35S* Pro, *CaMV 35S* promoter; *SPO1* pro, *sporamin 1* promoter. (B) Four selected *IbMYB1* overexpression lines, EV line and wild-type SHM line. Pictures of leaves, stems and storage roots were obtained from EV and *IbMYB1* transgenic lines.

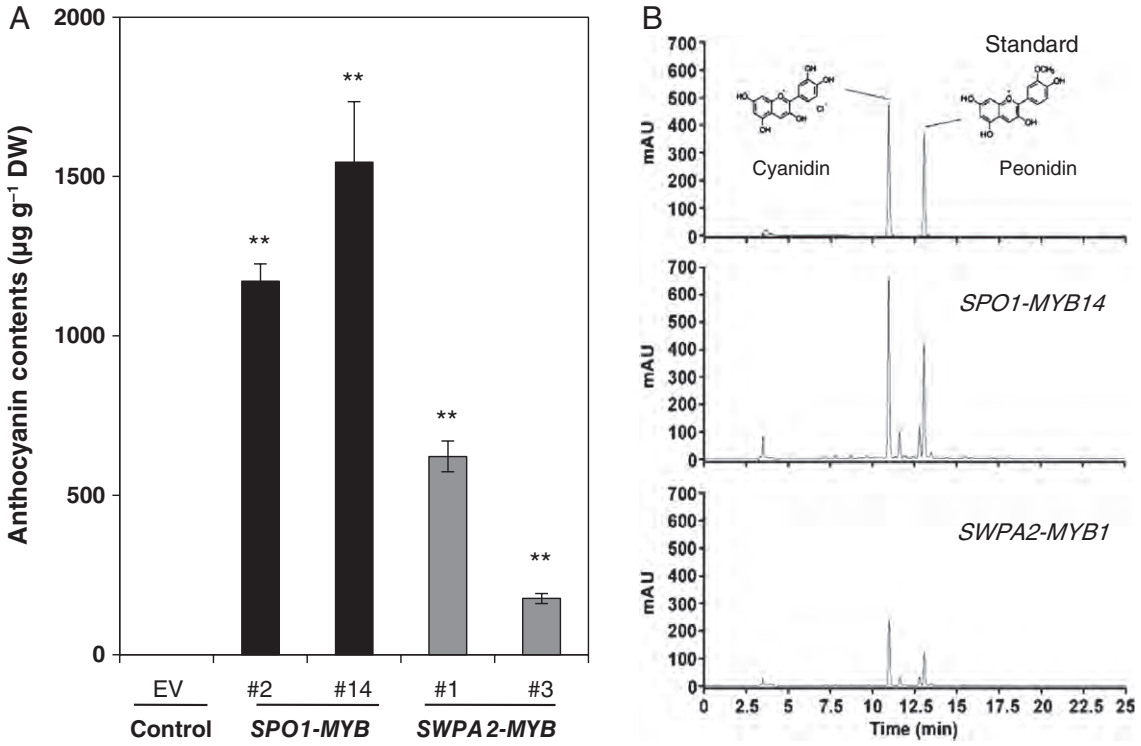

Fig. 2. Analysis of anthocyanin content in storage roots of transgenic sweet potato. (A) Analysis of total anthocyanin content. Data shown are the mean ± SD of two replicates from three individual storage roots. (B) HPLC analysis of anthocyanidins derived from acid hydrolysis of plant anthocyanins. Two anthocyanidin standards (cyanidin and peonidin) were used as controls.

genes in *IbMYB1* transgenic and EV plants and confirmed significant differences in the transgenic plants that correlated with increases in anthocyanin (Fig. 3). Expression of *IbMYB1* was higher in *SPO1-MYB* lines than in *SWPA2-MYB* lines, and no *IbMYB1* transcript was detected in EV line. Expression of the anthocyanin biosynthetic genes *PAL*, cinnamate 4-hydroxylase (*C4H*), *CHS*, *CHI*, *F3H*, UDP glucose: flavonoid-3-O-glucosyltransferase, *DFR* and *ANS* increased in storage roots in parallel with *IbMYB1* overexpression. As with *IbMYB1*, expression of anthocyanin biosynthetic genes was lower in *SWPA2-MYB* lines than in the *SPO1-MYB* lines, which correlated with the lower levels of anthocyanin accumulation in the *SWPA2-MYB* transgenic plants. This reflects the relative activities of the two promoters, with the *SPO1* promoter driving expression in storage roots more strongly than the *SWPA2* promoter. The high *IbMYB1* transcript levels produced under the control of the *SPO1* promoter likely caused the abundant anthocyanin accumulation in the *IbMYB1* transgenic sweet potato lines. These results indicate that expression of *IbMYB1* induces anthocyanin production in the storage roots of sweet potato by elevating the transcript levels of multiple anthocyanin biosynthetic genes.

Different levels of carotenoids and transcripts of carotenoid biosynthetic genes

To assess the effects of altered *IbMYB1* expression on the regulation of carotenoid biosynthesis, the carotenoid content of transgenic lines was analyzed in sweet potato storage roots by HPLC analysis. As the major carotenoid, β-carotene consisted of approximately 75–80% of the total carotenoids. *SPO1-MYB* roots contained similar total carotenoid levels to EV line (170.4 $\mu g\,g^{-1}$ DW) (Fig. 4A), with 151.4 and 154.4 $\mu g\,g^{-1}$ DW observed in lines 2 and 14, respectively. *SWPA2-MYB* lines 1 (225.6 $\mu g\,g^{-1}$ DW) and 3 (259.7 $\mu g\,g^{-1}$ DW) harbored higher carotenoid levels than the EV line. Levels of β-carotene were also similar in the EV and *SPO1-MYB* lines but elevated in the *SWPA2-MYB* lines (Fig. 4B). We performed qRT-PCR to compare the expression levels of eight key carotenoid metabolic pathway genes in the EV and *IbMYB1* transgenic lines (Fig. 4C). Transcript levels mostly differed between the EV and *IbMYB1* transgenic plants. With the exception of β-carotene hydroxylase, all genes exhibited lower expression in *SPO1-MYB* lines than in the EV line. By contrast, most of the carotenoid biosynthesis-related genes, such as phytoene desaturase (*PDS*), zeta-carotene desaturase, lycopene β-cyclase and

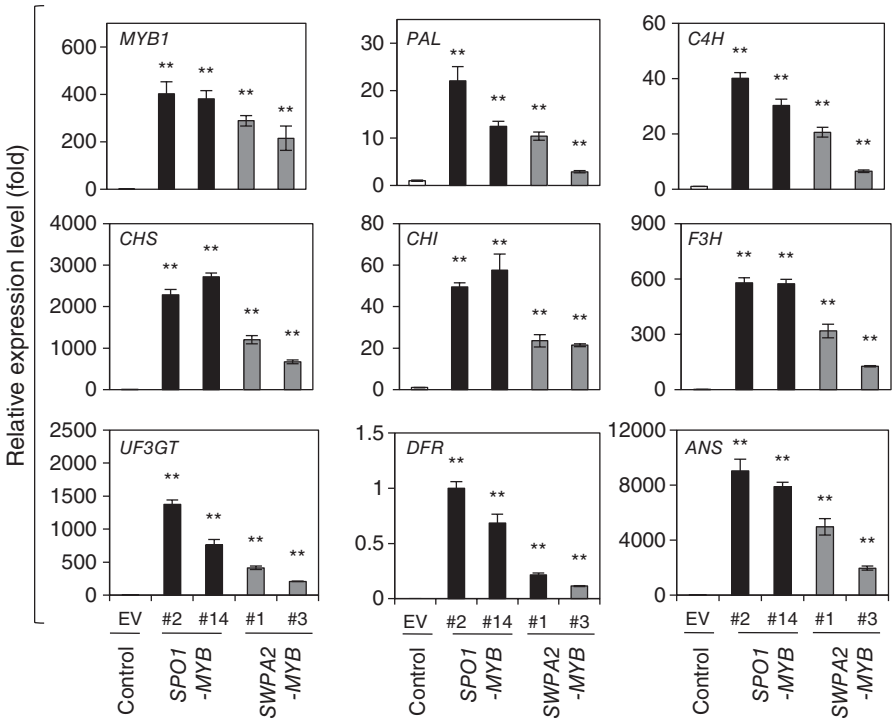

Fig. 3. Expression of *IbMYB1* and anthocyanin biosynthesis-related genes in storage roots of *IbMYB1* transgenic and EV plants. Data shown are the means ± SD of three technical qRT-PCR replicates from three individual storage roots.

lycopene ε-cyclase, were expressed at slightly higher levels in at least one of the *SWPA2-MYB* lines than in the EV line. These results suggest that *IbMYB1* expression might affect carotenoid biosynthesis-related gene expression and total carotenoid content.

Increased antioxidant activity in transgenic plants

In transgenic apple plants, an increase in anthocyanic color was accompanied by elevated concentrations of proanthocyanidins and various phenolic compounds (Espley et al. 2013). To evaluate whether similar effects were produced in transgenic sweet potato plants overexpressing *IbMYB1*, levels of proanthocyanidins and total phenolics were analyzed in the storage roots. As expected, proanthocyanidin and total phenolic levels increased significantly in response to *IbMYB1* overexpression, and the increases correlated with increases in anthocyanin content (Fig. 5). Levels of proanthocyanidins and total phenolics were higher in *SPO1-MYB* lines than in *SWPA2-MYB* lines, and EV line had very low levels of both proanthocyanidins and total phenolics.

To investigate antioxidant activity in transgenic sweet potato, we analyzed DPPH radical-scavenging activity in both fresh and boiled sweet potato storage roots (Fig. 6). Fresh transgenic sweet potato lines exhibited approximately 3–5-fold more DPPH activity than the EV line, with the highest level observed in *SPO1-MYB* line 14. All transgenic boiled sweet potato lines also had stronger DPPH radical-scavenging activities than the EV line.

Yield of *IbMYB1* transgenic sweet potato under field conditions

Field experiments were carried out from 2012 to 2013. In 2012, a preliminary experiment was conducted to evaluate dual-pigmented storage root phenotypes to select lines (data not shown). In 2013, the average yield of storage roots was assessed, and it varied between the transgenic lines. EV control plants produced 1083 kg per 10 ares (10 a^{-1}), and *SPO1-MYB* line 2, *SPO1-MYB* line 14 and *SWPA2-MYB* line 1 produced similar yields of storage roots (Fig. 7A, B). In *SWPA2-MYB* line 3, the yield was significantly greater, at levels approximately 2.2-fold higher than the EV lines (Fig. 7B). The average yields of the aerial parts of the EV and *IbMYB1* transgenic lines were similar in all lines (2083–2778 kg 10 a^{-1}) (Fig. 7C).

Discussion

Dietary intervention with nutritionally enriched food has the potential to improve life expectancy. Increased public

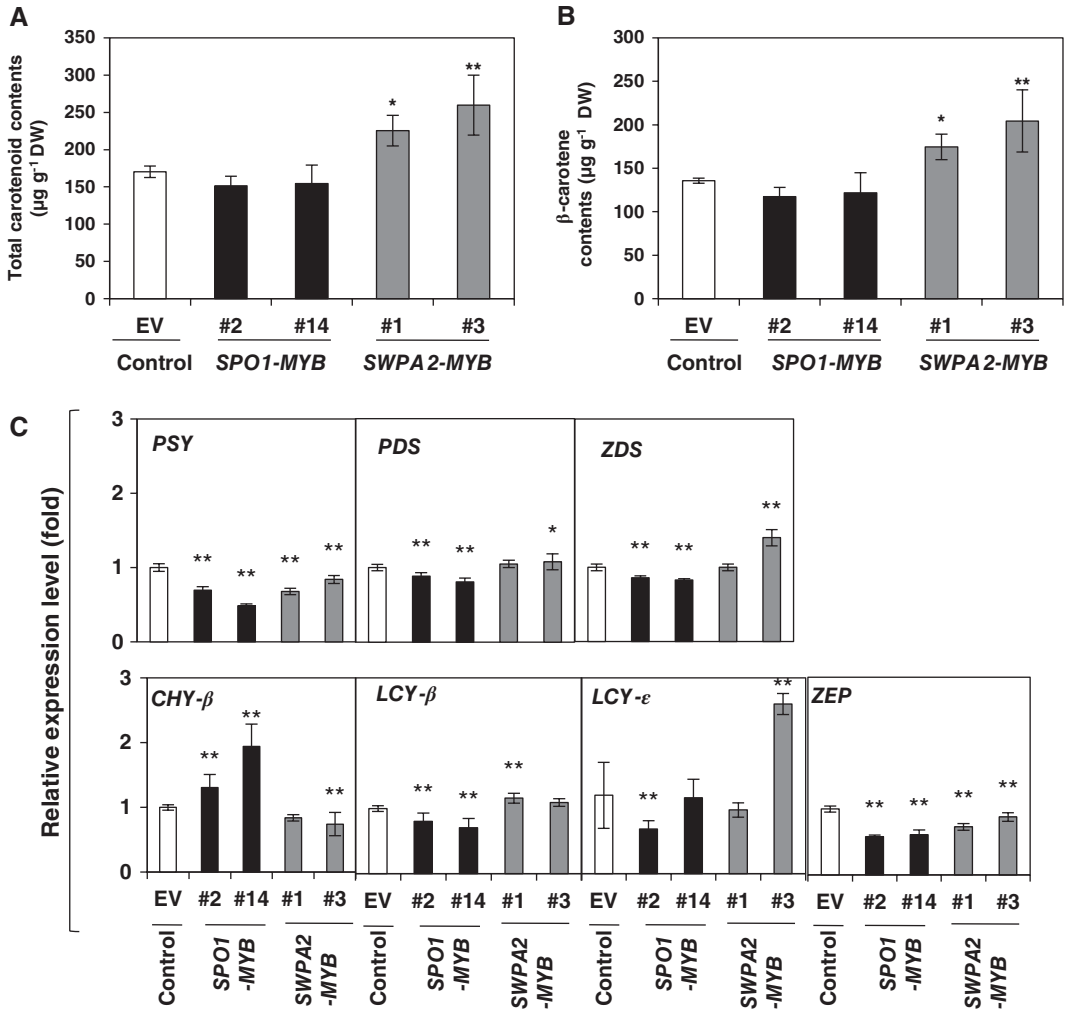

Fig. 4. Carotenoid contents and expression levels of carotenoid biosynthesis-related genes in storage roots of *IbMYB1* transgenic and EV plants. (A) Analysis of total carotenoid contents. (B) Analysis of β-carotene content by HPLC. (C) Expression of various carotenoid biosynthesis-related genes. Data shown are means $\pm$ SD of two replicates from three individual storage roots.

awareness of the nutritional benefits of antioxidants for human health has promoted scientific efforts to increase the levels of a variety of bioactive constituents in fruits and vegetables (Davies 2007, Pascual-Teresa and Sanchez-Ballesta 2008). While conventional breeding techniques may partially achieve this goal (Mayer et al. 2008, Whitty et al. 2013), the genetic diversity available within the sexually compatible species of any given crop limits the extent of improvement. The use of molecular breeding has great potential to increase the antioxidant and nutrient values of economically important crops and to allow development of nutritionally improved diets (Christou and Twyman 2004).

Sweet potato is a valuable source of β-carotene, which is a key precursor of vitamin A, and is thus an excellent candidate for nutritional enrichment. Recent research demonstrated that β-carotene-rich sweet potato improved the vitamin A status of primary school children (van Jaarsveld et al. 2005). Recently, the Centre for Science in the Public Interest (CSPI) in the United States reported that its nutritional content makes sweet potato an extremely healthy food. The high dietary fiber and low saturated fat levels found in sweet potato, alongside the abundance of small molecule antioxidants such as vitamin C and carotenoids, might be beneficial to human health (CSPI 2009). Sweet potato is also considered to be moderately tolerant to abiotic stress due to its naturally high levels of various antioxidants. In this study, we generated dual-pigmented sweet potatoes that harbored increased levels of anthocyanin (a hydrophilic antioxidant) in addition to β-carotene (a lipophilic antioxidant highly abundant in orange-fleshed sweet potato).

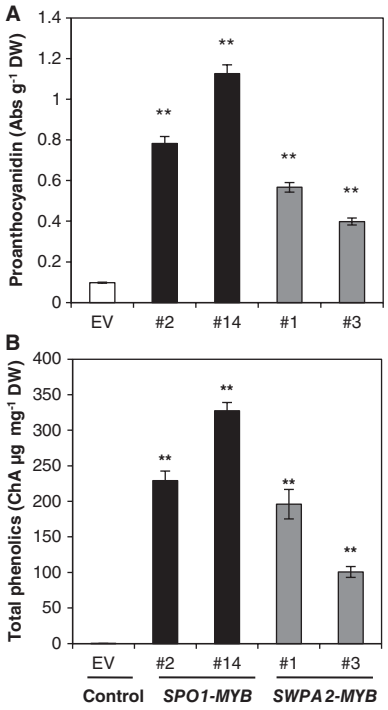

Fig. 5. Proanthocyanidine and total phenolic contents in the storage roots of *IbMYB1* transgenic and EV plants. (A) Analysis of total proanthocyanidin content by HPLC. (B) Analysis of total phenolic content. Data shown are means ± SD of three replicates from three individual storage roots.

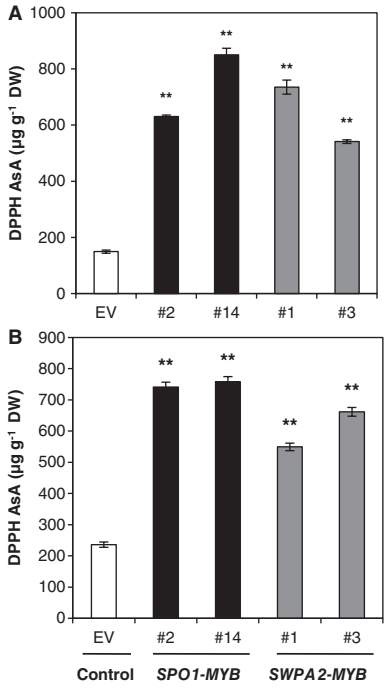

Fig. 6. DPPH radical-scavenging activities in the storage roots of *IbMYB1* transgenic and EV plants. (A) Peeled, unboiled storage roots. (B) Peeled, boiled storage roots. Data shown are means ± SD of three replicates from three individual storage roots.

Foods rich in both soluble and membrane-associated antioxidants are thought to offer the best protection against disease (Yeum et al. 2004). The important contribution of anthocyanin to the nutritional value of certain fruits and vegetables has led to attempts to induce or increase anthocyanin levels through metabolic engineering of the R2R3-MYB TF or anthocyanin biosynthesis pathway genes. In tomato, transgenic *Del* and *Ros1* overexpression stimulated an increase in anthocyanin biosynthesis and lycopene levels. When this high anthocyanin (delphinidin- and petunidin-base) transgenic tomato was fed to cancer-susceptible *Trp53* mice, the life span was extended by more than 1 month over the life span of mice fed with control tomatoes (Butelli et al. 2008). A transgenic apple overexpressing *MYB10* showed increased anthocyanin (cyanidin-base) levels in the cortex (Espley et al. 2013). In this study, our dual-pigmented sweet potatoes exhibited increased anthocyanin content in storage roots in addition to the high natural carotenoid content (Figs 2 and 4). Our *IbMYB1* transgenic sweet potatoes contained cyanidin- and peonidin-based anthocyanins (Fig. 2B). Previous studies examining transient expression of *IbMYB1* in tobacco leaves and Arabidopsis seedlings found cyanidin to be the main anthocyanin produced (Kim et al. 2010, Chu et al. 2013). Cyanidin and peonidin were previously observed in purple-fleshed sweet potato cultivars, with the anthocyanin levels varying greatly between cultivars (Lee et al. 2013). Chen et al. (2005) reported that black rice-derived cyanidin and peonidin induced apoptosis in vitro and inhibited tumor cell growth in vitro and in vivo. Our *IbMYB1* transgenic sweet potatoes also exhibited increased levels of proanthocyanidin and total phenolics, which led to high DPPH radical-scavenging activities in the fresh and boiled sweet potato storage roots (Figs 5 and 6). Taken together, these studies suggest that increased anthocyanin content in food might play a positive role in human health. Further work is required to assess the tolerance of the *IbMYB1* transgenic plants generated in this study to various biotic and abiotic stresses in order to determine their adaptability to harsh conditions.

We generated twenty putative *IbMYB1* transgenic lines. Among the transgenic plants, 5 of 15 *SPO1-MYB* lines and two of five *SWPA2-MYB* lines did not accumulate anthocyanin in storage roots (see Fig. S2), stems or leaves (data not shown). Different transgenic lines showed significantly different anthocyanin contents. We conducted Southern blot analysis to determine whether

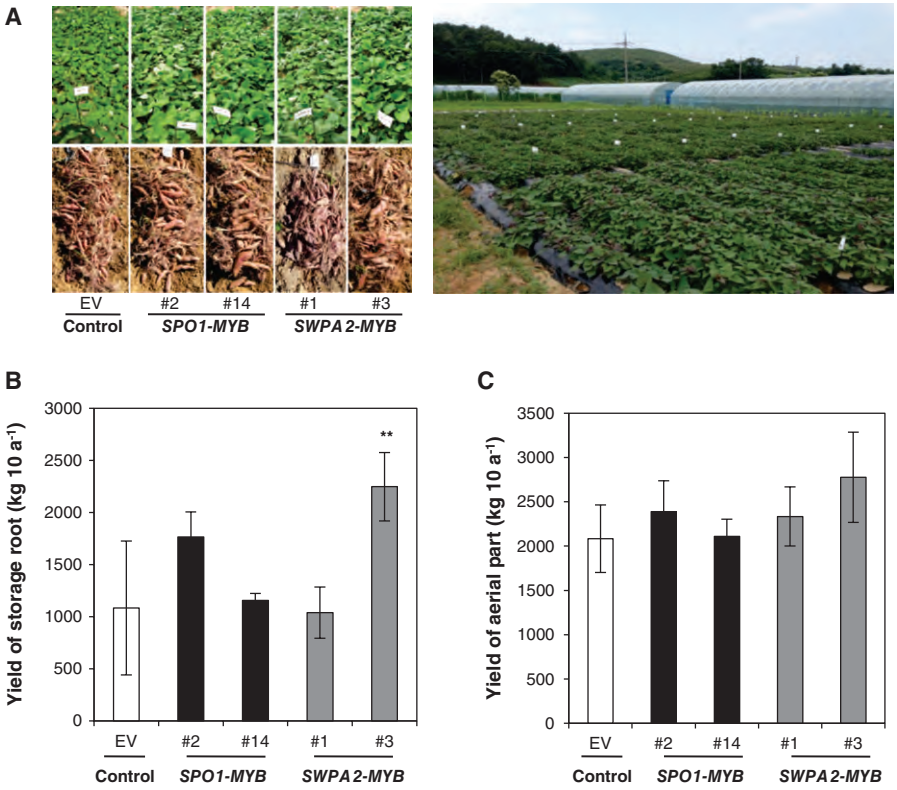

Fig. 7. Growth-related features of *IbMYB1* transgenic and EV sweet potato plants in field conditions. (A) A picture of aerial plant parts and storage roots. (B) Average yields of storage roots. (C) Average yields of aerial plant parts. Data shown are means ± SD of three row replicates (each row was planted with 40 individual plants).

there was a correlation between the integrated gene copy number, the *IbMYB1* transcript level and anthocyanin accumulation. The *SWPA2-MYB* lines 1 and 3 and the *SPO1-MYB* line 14 had different band patterns, indicating multiple gene copy numbers, whereas the *SPO1-MYB 2* line and the EV plant had single gene copy numbers (data not shown). This result suggested that gene copy number might affect anthocyanin accumulation in the storage roots of IbMYB1 transgenic sweet potato.

Interestingly, the *SPO1-MYB 14* line showed the highest anthocyanin content; however, the *SPO1-MYB 2* line showed the highest expression of *IbMYB1* and anthocyanin biosynthesis-related genes (Figs 2 and 3). Based on previous studies of apple and pear, we suggest that this phenomenon may depend on the developmental stage of the sweet potato storage root. Anthocyanin and anthocyanin biosynthetic genes were analyzed in red-fleshed apple during the five stages of fruit development at 40, 67, 102, 130 and 146 days after full bloom (DAFB). Anthocyanin content increased during early developmental stages, peaked at 130 DAFB and decreased at 146 DAFB. However, anthocyanin biosynthetic gene products were most abundant at 102 DAFB (Espley et al. 2007). A similar phenomenon was previously observed in the skin of pear (cv. Aoguan). Maximum anthocyanin accumulation was observed at 16 DAFB (Days after full bloom); however, the *PyMYB10* transcript level peaked at 10 DAFB and gradually decreased at 22 and 28 DAFB (Feng et al. 2010). In this study, we speculate on the basis of storage root weight that the developmental stage of the *SPO1-MYB 2* line might be earlier than that of the *SPO1-MYB 14* line. The anthocyanin content and expression of MYB TF and anthocyanin biosynthetic genes during storage root formation of sweet potato will be investigated in more detail in a future study.

In this study, we used two different promoters from sweet potato. The tissue-specific *sporamin* promoter was highly expressed in storage root. In addition, its expression is induced in leaves, stem and petiole by sucrose addition and in response to wounding stress (Hattori et al. 1991, Ohta et al. 1991, Yeh et al. 1997). The oxidative stress-inducible *SWPA2* gene encodes an anionic peroxidase in cell cultures of sweet potato, and its expression was strongly induced in response to various stresses, including wounding, chilling, sulfur dioxide

and ozone exposure and UV irradiation (Kim et al. 1999, Kim et al. 2007). The transgenic tobacco plants showed that the *SWPA2* promoter could be systemically induced by environmental stresses, including H_2O_2 exposure, wounding and UV irradiation (Kim et al. 2003). In our previous study, the *SWPA2-IbMYB1 Arabidopsis* line showed higher anthocyanin accumulation than *SPO-IbMYB1* lines in 8-day seedlings and 4-week-old rosette leaves (Chu et al. 2013). Interestingly, the results of this study showed that *SPO1-MYB* lines accumulate more anthocyanins in storage roots than *SWPA2-MYB* lines (Fig. 2A). The anthocyanin accumulation results suggest that the storage root-specific *sporamin* promoter is applicable to sweet potato systems for storage root targeting in transgenic plants.

SWPA2-MYB lines harbored increased levels of total carotenoids and β-carotene. An interaction between the phenylpropanoid and carotenoid pathways was observed when the light-signaling pathway regulatory gene, *DET1*, was suppressed using RNA interference method (Davuluri et al. 2005). Subsequent analysis demonstrated that *DET1* disruption had a widespread effect, which reflects the putative global nature of *DET1* as a core regulator of essential plant processes (Enfissi et al. 2010). The light hyper-responsiveness observed in *DET1* may result from the coordinated regulation of pathways related to high light conditions, such as those concerning carotenoid and flavonoid-type antioxidants. In a range of species, including Arabidopsis, *MYB* TFs were shown to be specific regulators of the flavonoid pathway, but without apparent links to carotenoid biosynthesis (Tohge et al. 2005). However, overexpression of *MYB* TFs in crops such as tomato and apple leads to increases in β-carotene and anthocyanin content. Our data suggest that the elevated anthocyanin pathway flux and highly pigmented phenotype are involved in the regulation of carotenoid production (Figs 2 and 4), despite little previous evidence for the coordinated regulation of the whole pathway or those components that lead to β-carotene production. Tomato *DET1* lines showed an increase in carotenoid levels but not in associated gene transcripts, and apple *MYB10* transgenic lines exhibited increased carotenoid levels and increased expression of synthetic genes such as *PDS* and phytoene synthase (Enfissi et al. 2010). These studies suggest that post-transcriptional regulation may be occurring in our *IbMYB1* overexpression lines. Lines with relatively high levels of anthocyanin (>1000 $\mu g\, g^{-1}$ DW) had reduced carotenoid content, whereas lines with <1000 $\mu g\, g^{-1}$ DW anthocyanin exhibited relatively high carotenoid levels (Figs 2 and 4, Table S3). Our results, therefore, suggest that carotenoid content may be dependent upon the quantity of anthocyanins or post-transcriptional regulation in the *IbMYB1* transgenic lines. Further work is required to elucidate fully the putative links between transcriptional control and overall changes in anthocyanin quantity.

With the exception of *SWPA2-MYB* line 3, which had an elevated yield, average storage root yields were similar between EV and *IbMYB1* transgenic sweet potato (Fig. 7B). Davuluri et al. (2005) reported that fruit-specific silencing of the *DET1* gene in tomato produced increased flavonoid and carotenoid levels but does not affect fruit yield. This indicates that higher flavonoid or anthocyanin might not directly affect yield. We will further access *SWPA2-MYB* line 3 to elucidate its agronomic characteristics including storage root yield.

In conclusion, many plant-breeding programs aim to enhance the nutritional content and/or yield of food crops. Our study showed that overexpression of the *IbMYB1* gene in a carotenoid-rich sweet potato cultivar produced dramatic increases in the levels of storage root anthocyanin alongside the originally high carotenoid levels. To our knowledge, there have been no previous examples where metabolic engineering has produced simultaneously high levels of both hydrophilic and lipophilic antioxidants in a single storage root. Our study is the first report in which sweet potato plants with dual-pigmented storage roots were successfully generated by transgenic approaches. Achieving this combination of anthocyanin and carotenoid traits with tissue-specific or stress-inducible expression systems might be challenging with conventional breeding. Therefore, our dual-pigmented transgenic plants represent a new production technology for sweet potato that may contribute to human health, particularly in developing countries, allowing sustainable development on marginal lands.

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References

Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2: 875–877

Borevitz JO, Xia Y, Blount J, Dixon RA, Lamb C (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12: 2383–2393

Bovell-Benjamin AC (2007) Sweet potato: a review of its past, present, and future role in human nutrition. Adv Food Nutr Res 52: 1–59

Bovy A, de Vos R, Kemper M, Schijlen E, Almenar Pertejo M, Muir S, Collins G, Robinson S, Verhoeyen M, Hughes S, Santos-Buelga C, van Tunen A (2002) High-flavonol tomatoes resulting from the heterologous expression of the maize transcription factor genes *LC* and *C1*. Plant Cell 14: 2509–2526

Butelli E, Titta L, Giorgio M, Mock H-P, Matros A, Peterek S, Schijlen EGWM, Hall RD, Bovy AG, Luo J, Martin C (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat Biotechnol 26: 1301–1308

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol 70: 1–9

Chen P-N, Chu S-C, Chiou H-L, Chiang C-L, Yang S-F, Hsieh Y-S (2005) Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis in vitro and suppress tumor growth in vivo. Nutr Cancer 53: 232–243

Christie P, Alfenito M, Walbot V (1994) Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. Planta 194: 541–549

Christou P, Twyman RM (2004) The potential of genetically enhanced plants to address food insecurity. Nutr Res Rev 17: 23–42

Chu H, Jeong JC, Kim W-J, Chung DM, Jeon HK, Ahn YO, Kim SH, Lee H-S, Kwak S-S, Kim CY (2013) Expression of the sweetpotato R2R3-type *IbMYB1a* gene induces anthocyanin accumulation in *Arabidopsis*. Physiol Plant 148: 189–199

Cone KC, Cocciolone SM, Burr FA, Burr B (1993) Maize anthocyanin regulatory gene *pl* is a duplicate of *c1* that functions in the plant. Plant Cell 5: 1795–1805

Davies KM (2007) Genetic modification of plant metabolism for human health benefits. Nutrigenomics 622: 122–137

Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J, Bramley PM, Pennings HMJ, Bowler C (2005) Fruit-specific RNAi-mediated suppression of *DET1* enhances carotenoid and flavonoid content in tomatoes. Nat Biotechnol 23: 890–895

Enfissi EMA, Barneche F, Ahmed I, Lichtlé C, Gerrish C, McQuinn RP, Giovannoni JJ, Lopez-Juez E, Bowler C, Bramley PM, Fraser PD (2010) Integrative transcript and metabolite analysis of nutritionally enhanced *DE-ETIOLATED1* downregulated tomato fruit. Plant Cell 22: 1190–1215

Espley RV, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. Plant J 49: 414–427

Espley RV, Bovy A, Bava C, Jaeger SR, Tomes S, Norling C, Crawford J, Rowan D, McGhie TK, Brendolise C, Putterill J, Schouten HJ, Hellens RP, Allan AC (2013) Analysis of genetically modified red-fleshed apples reveals effects on growth and consumer attributes. Plant Biotechnol J 11: 408–419

Feng S, Wang Y, Yang S, Xu Y, Chen X (2010) Anthocyanin biosynthesis in pears is regulated by a R2R3-MYB transcription factor PyMYB10. Planta 232: 245–255

Hattori T, Fukumoto H, Nakagawa S, Nakamura K (1991) Sucrose-induced expression of genes coding for the tuberous root storage protein, sporamin, of sweet potato in leaves and petioles. Plant Cell Physiol 32: 79–86

van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ, Benadé AJS (2005) β-Carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. Am J Clin Nutr 81: 1080–1087

Kim K-Y, Huh G-H, Lee H-S, Kwon S-Y, Hur Y, Kwak S-S (1999) Molecular characterization of two anionic peroxidase cDNAs isolated from suspension cultures of sweetpotato. Mol Gen Genet 261: 941–947

Kim K-Y, Kwon S-Y, Lee H-S, Hur Y, Bang J-W, Kwak S-S (2003) A novel oxidative stress-inducible peroxidase promoter from sweetpotato: molecular cloning and characterization in transgenic tobacco plants and cultured cells. Plant Mol Biol 51:831–838.

Kim Y-H, Lim S, Han S-H, Lee J-C, Song W-K, Bang J-W, Kwon S-Y, Lee H-S, Kwak S-S (2007) Differential expression of 10 sweetpotato peroxidases in response to sulfur dioxide, ozone, and ultraviolet radiation. Plant Physiol Biochem 45: 908–914

Kim CY, Ahn YO, Kim SH, Kim Y-H, Lee H-S, Catanach AS, Jacobs JME, Conner AJ, Kwak S-S (2010) The sweet potato *IbMYB1* gene as a potential visible marker for sweet potato intragenic vector system. Physiol Plant 139: 229–240

Kim SH, Ahn YO, Ahn M-J, Lee H-S, Kwak S-S (2012) Down-regulation of β-carotene hydroxylase increases β-carotene and total carotenoids enhancing salt stress tolerance in transgenic cultured cells of sweetpotato. Phytochemistry 74: 69–78

Kim SH, Ahn YO, Ahn M-J, Jeong JC, Lee H-S, Kwak S-S (2013) Cloning and characterization of an *Orange* gene that increases carotenoid accumulation and salt stress tolerance in transgenic sweetpotato cultures. Plant Physiol Biochem 70: 445–454

Kobayashi S, Ishimaru M, Hiraoka K, Honda C (2002) *Myb*-related genes of the Kyoho grape (*Vitis labruscana*) regulate anthocyanin biosynthesis. Planta 215: 924–933

Lee MJ, Park JS, Choi DS, Jung MY (2013) Characterization and quantitation of anthocyanins in purple-fleshed

sweet potatoes cultivated in Korea by HPLC-DAD and HPLC-ESI-QTOF-MS/MS. J Agric Food Chem 61: 3148–3158
Lim S, Yang K-S, Kwon S-Y, Paek K-Y, Kwak S-S, Lee H-S (2004) *Agrobacterium*-mediated genetic transformation and plant regeneration of sweetpotato (*Ipomoea batatas*). J Plant Biotechnol 31: 267–271
Luceri C, Giovannelli L, Pitozzi V, Toti S, Castagnini C, Routaboul J-M, Lepiniec L, Larrosa M, Dolara P (2008) Liver and colon DNA oxidative damage and gene expression profiles of rats fed *Arabidopsis thaliana* mutant seeds containing contrasted flavonoids. Food Chem Toxicol 46: 1213–1220
Mano H, Ogasawara F, Sato K, Higo H, Minobe Y (2007) Isolation of a regulatory gene of anthocyanin biosynthesis in tuberous roots of purple-fleshed sweet potato. Plant Physiol 143: 1252–1268
Mayer JE, Pfeiffer WH, Beyer P (2008) Biofortified crops to alleviate micronutrient malnutrition. Curr Opin Plant Biol 11: 166–170
Mustilli AC, Fenzi F, Ciliento R, Alfano F, Bowler C (1999) Phenotype of the tomato *high pigment-2* mutant is caused by a mutation in the tomato homolog of *DEETIOLATED1*. Plant Cell 11: 145–157
Ohta S, Hattori T, Morikami A, Nakamura K (1991) High-level expression of a sweet potato sporamin gene promoter: β-glucuroidase (GUS) fusion gene in the stems of transgenic tobacco plants is conferred by multiple cell type-specific regulatory elements. Mol Gen Genet 225: 369–378
Oki T, Masuda M, Furuta S, Nishiba Y, Terahara N, Suda I (2002) Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. J Food Sci 67: 1752–1756
Pascual-Teresa S, Sanchez-Ballesta M (2008) Anthocyanins: from plant to health. Phytochem Rev 7: 281–299
Price ML, Van Scoyoc S, Butler LG (1978) A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J Agric Food Chem 26: 1214–1218
Ramsay NA, Glover BJ (2005) MYB–bHLH–WD40 protein complex and the evolution of cellular diversity. Trends Plant Sci 10: 63–70
Steyn WJ, Wand SJE, Holcroft DM, Jacobs G (2002) Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol 155: 349–361
Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54: 733–749
Teow CC, Truong V-D, McFeeters RF, Thompson RL, Pecota KV, Yencho GC (2007) Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem 103: 829–838
The Center for Science in the Public Interest (2009) Sweet Potato Nutrition – Sweet Potato Ranks Number One in Nutrition. Available at http://www.foodreference.com/html/sweet-pot-nutrition.html (accessed 15 February 2014)
Tohge T, Nishiyama Y, Hirai MY, Yano M, J-i N, Awazuhara M, Inoue E, Takahashi H, Goodenowe DB, Kitayama M, Noji M, Yamazaki M, Saito K (2005) Functional genomics by integrated analysis of metabolome and transcriptome of Arabidopsis plants over-expressing an MYB transcription factor. Plant J 42: 218–235
Whitty CJM, Jones M, Tollervey A, Wheeler T (2013) Biotechnology: Africa and Asia need a rational debate on GM crops. Nature 497: 31–33
World Health Organization (2009) Global prevalence of vitaminA deficiency in populations at risk 1995–2005. Available at http://whqlibdoc.who.int/publications/2009/9789241598019_eng. pdf (accessed 15 February 2014)
Yeh KW, Lin MI, Tuan SJ, Chen YM, Lin CJ, Kao SS (1997) Sweet potato (*Ipomoea batatas*) trypsin inhibitors expressed in transgenic tobacco plants confer resistance against *Spodoptera litura*. Plant Cell Rep 16: 696–699
Yeum K-J, Russell RM, Krinsky NI, Aldini G (2004) Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma. Arch Biochem Biophys 430: 97–103

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Phenotype of wild-type sweet potato and antioxidant contents.

Fig. S2. Phenotypes of transgenic sweet potato storage roots.

Table S1. Carotenoid and total anthocyanin contents in storage roots of transgenic and nontransgenic sweet potato plants.

Table S2. Primer sequences used for expression analysis of sweet potato genes in this study.

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