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Anti-oxidative characterisation of NaN_3-induced common bean mutants

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ABSTRACT

The contents of total phenolics, total anthocyanins, total proanthocyanidins and antioxidant activities were measured using 2,2-diphenyl-1-picrylhydrazyl radicals scavenging activity, ferric reducing antioxidant power, lipid peroxidation inhibition ability and $ABTS^{-}$ radical-scavenging activity assays. These were compared in the seed coats of common bean (*Phaseolus vulgaris* L.) cultivar Hwachia and its eighteen NaN_3-induced mutants. NaN_3-induced mutants generally accumulated more total phenolics (19% more), total anthocyanins (65% more) and total proanthocyanidins (4% more) than Hwachia (containing 29.94 mg g^{-1} total phenolics, 0.31 mg g^{-1} total anthocyanins and 12.94 mg g^{-1} total proanthocyanidins, respectively). Anthocyanidins including delphinine, cyanidin and pelargonidin were detectable in seed coat, each with different levels depending on the tested accessions. Significant correlations (average of 0.847 across all comparisons, $p < 0.01$) were found between the tested antioxidant activities, total phenolics, total anthocyanins and total proanthocyanidins. Mutants SA-11-2, SA-13-2 and SA-34-2 that are enriched with antioxidants may be useful in food and other applications.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an annual legume belonging to the family *Fabaceae* and genus *Phaseolus*. It is the world's third most important food legume behind soybean and peanut, which provides protein, starch and dietary fibre to many people worldwide (Lin, Harnly, Pastor-Corrales, & Luthria, 2008). Common bean is also rich in phytochemicals with anti-oxidative potential, such as anthocyanins and proanthocyanidin, which exert beneficial effects on human health (Luthria & Pastor-Corrales, 2006; Macz-Pop, Rivas-Gonzalo, Pérez-Alonso, & González-Paramás, 2006; Rocha-Guzmán et al., 2007).

Both anthocyanins and proanthocyanidins are concentrated mostly in the seed coat of common bean seed (Aparicio-Fernández, Manzo-Bonilla, & Loarca-Piña, 2005; Madhujith & Shahidi, 2005). Anthocyanins are members of the flavonoids group that impart purple, red and blue colouration in plants (Castañeda-Ovando, de LourdesPacheco-Hernández, Elena Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). They are used widely as natural colourants in the food and pharmaceutical industry (Jing & Giusti, 2007; Wrolstad, Durst, & Lee, 2005). Proanthocyanidins are flavonoid polymers that present in the fruit, bark and seed of many plants, where they provide protection against predation (Xie & Dixon, 2005). They also give flavour and astringency to beverages such as teas, wines and fruit juices (Dixon, Xie, & Sharma, 2005). The roles of these two phytochemicals as medicinal agents are well recognised throughout the world, and they have been linked to a wide range of health benefits. Demonstrated benefits include protection against liver injury, reduction of blood pressure, improvement of eyesight and suppression of proliferation of cancer cells (Dixon et al., 2005; Kim et al., 2007; Lila, 2004).

Common bean grows fairly well all year round in Taiwan, but has very limited planting acreage due to its low yield when compared with other cultivated legumes. The produced fresh pods or immature seeds are mainly consumed as a vegetable. The harvested dry seeds are also used as a sweetened filling in steamed or baked cakes and beverage, or used as folk medicines. Recently, common bean has re-gained much interest, because it has been characterised as a healthy food by many nutritionists, due to its high phenolics and vitamin contents (Aparicio-Fernández et al., 2005; Broughton et al., 2003). However, there has been no bred cultivar through breeding programme reported, and only very limited landraces are available for common bean growers.

Chemical mutagenesis is an important way to cause genetic variation, and is well adopted for common bean breeding programmes (Blair et al., 2007; Sena, Barbosa, & Vieira, 1991). In 2005, Taiwan Agricultural Research Institute implemented a mutation programme on common bean and produced many NaN_3-induced mutants that differed in seed coat colour. The objective of the study was to investigate and compare the contents of several antioxidants including total phenolics, anthocyanins, proanthocyanidins and the antioxidant activity in the seed coat of NaN_3-induced mutants. The relationships between the tested antioxidants and antioxidant activity were also explored.

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2. Materials and methods

2.1. Seed materials

Seeds of common bean (*P. vulgaris* L.) cultivar Hwachia and it is eighteen NaN_3-induced mutants were obtained from the Taiwan Agricultural Research Institute (Wufeng, Taichung County, Taiwan, ROC) (Fig. 1). All the seeds were harvested in 2008 and stored at −4 °C until they were used for research. The seed coat colour of beans was described according to visual characteristics detailed by Durán et al. (2005). The dry weights of 100 randomly chosen seeds were determined and reported as g100 seeds^{-1}.

2.2. Chemicals

The Folin and Ciocalteu's phenol reagent, Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH·), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical anion ($ABTS^-$) and 2,2′-azobis-(2-methypropionamidine) dihydrochloride (AAPH) were purchased from Sigma Chemical Co. (St. Louis, Mo). Anthocyanin standards (cyanidin, delphinidin and pelargonidin) were purchased from Extrasynthese, Genau Cedex, France. All the other chemicals were purchased from Merck (Darmstad, Germany).

2.3. Determination of total phenolics

The seed coats of the tested beans were manually separated and ground until a fine homogeneous powder was obtained. Ground seed coat materials (1 g) were extracted with 20 ml 80% (v/v) methanol at 80 °C for 15 min, and the filtrate volume was re-fixed to 20 ml with extraction reagent, then it was shaken up and kept for later use. The content of total phenolics in extracts was estimated colourimetrically using the Folin–Ciocalteau assay. The absorption value was determined in 765 nm, with gallic acid used as a standard. The data was expressed as mg gallic acid equivalent per g seed coat dry weight.

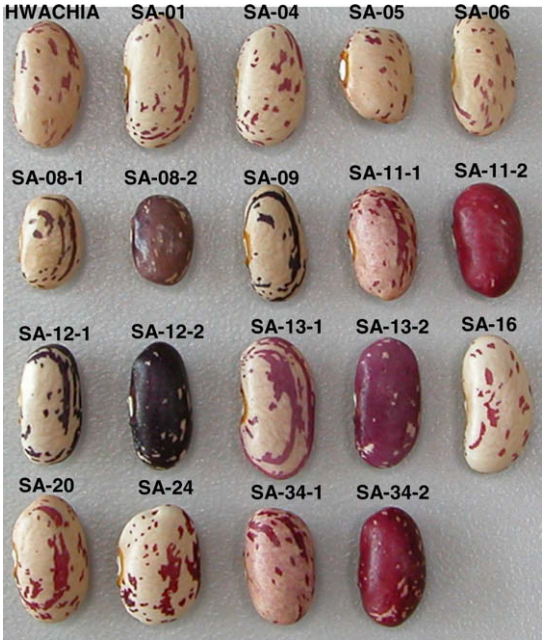

Fig. 1. Seed appearances of common bean variety Hwachia and its' NaN_3-induced mutants.

2.4. Determination of total anthocyanins and total proanthocyanidins

The contents of total anthocyanins and total proanthocyanidins were determined using the pH-differential spectrophotometric method detailed by Oki et al. (2002). Seed coat materials were extracted with methanol containing diluted HCl (1%) at 4 °C for 24 h. Part of methanol extract (5 ml) was further acidified with 2.5 ml 2.4 N HCl at 100 °C for 40 min to hydrolyse anthocyanidins from proanthocyanidin polymers. For monomeric anthocyanin determination, two dilutions were performed on each sample. The extracts were diluted with 0.025 M KCl (pH 1.0) and 0.4 M Na-acetate (pH 4.5), and absorbance measurements were taken at 530 and 700 nm using a spectrophotometer calibrated with distilled water as a blank. Proanthocyanidin was determined as the difference in anthocyanins content between acidified and non-acidified samples.

2.5. Determination of monomeric anthocyanidins

For measuring the levels of monomeric anthocyanidins, seed coat materials were extracted with methanol containing diluted HCl (1%) at 4 °C for 24 h. The extract (5 ml) was acidified with 2.5 ml 2.4 N HCl at 100 °C for 40 min. Prior to analysis, all the samples were filtered through a 0.45 μM membrane filter and concentrated at 30 °C in vacuo (Thermo SPD111V, USA). The concentrated extract was analysed using a high performance liquid chromatography (HPLC) (Waters 2996, USA) using ODS column (Inertsil ODS-3 column, 4.6 × 250 mm, 5 μm; Precolumn: Inertsil ODS-3 column, 4.6 × 33 mm, 5 μm, Japan). The flow rate was set at 1 ml/min using H_2O–acetic acid–methanol (69:10:21) with monitoring at 530 nm, and the column temperature was set at 30 °C. Anthocyanin contents were calculated by HPLC peak areas compared with external standard calibration curves. The linear standard calibration curves were generated by injecting 0.05–1 μg of purified delphinidin, cyanidin and petunidin (Extrasynthase, Genay Cedex, France) in 20 μl of diluted HCl–methanol (1%).

2.6. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical-scavenging activity

DPPH· radical-scavenging activity was assayed according to the method of Kim et al. (2006). One gramme of ground materials was mixed with 20 ml of 80% methanol. The mixture was shaken for 24 h at room temperature, filtered through Whatman No. 42 filter paper, and was freeze–dried at −40 °C until use. The samples were diluted to 1% in dimethyl sulfoxide (DMSO). DPPH· reagent contained 30 mg of DPPH· dissolved in 200 ml of pure ethanol and 200 ml of distilled water. For DPPH· test, 2.5 ml of DPPH· reagent was incubated with 250 μl sample (blank was DMSO) for 1 min, and the mixture was monitored at 517 nm, and trolox was used in the construction of the standard curve. The DPPH· radical-scavenging activity was expressed as trolox equivalent per unit dry weight.

2.7. Determination of ferric reducing antioxidant power (FRAP)

The FRAP of tested samples was determined according to the method of Mau, Chang, Huang, and Chen (2004) with some modifications. The extract (0.5 ml) was mixed with 2.5 ml of 66.7 mM sodium phosphate buffer (pH 6.6) containing 0.33% $K_3Fe(CN)_6$, and the mixture was incubated at 50 °C for 20 min. After 0.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 200*g* for 10 min. The upper layer (0.25 ml) was mixed with 4.15 ml of de-ionised water containing 0.1% ferric chloride, and the absorbance was monitored at 700 nm in a spectrophotometer, and trolox was used in the construction of the standard curve.

The reducing power was also expressed as trolox equivalent per unit dry weight.

2.8. Determination of lipid peroxidation inhibition

The ability of lipid peroxidation inhibition of the extract was determined according to the method described by Fukuzawa, Tokumura, Ouchi, and Tsukatani (1982) with some modifications. In brief, a mixture containing sample extract (125 μl), 500 μl lecithin (10 mg ml^{-1}), 300 μl phosphate buffer (20 mM, pH 7.4) was prepared. The oxidation was initiated by adding 25 μl $FeCl_3$ (25 mM) and 25 μl ascorbic acid (25 mM) to mixture and incubated at 37 °C for 1 h. After incubation, 20 μl BHT (0.2%) and 800 μl TBA (0.4%) were added to the mixture, and the mixture was incubated at 100 °C for 20 min. The pink pigment was extracted with same volume of *n*-butanol and its absorbance was monitored at 532 nm, and trolox was used in the construction of the standard curve.

2.9. Determination of $ABTS^{-}$ radical-scavenging activity

The $ABTS^{-}$ radical-scavenging activity assay is based on scavenging of $ABTS^{-}$. A solution of $ABTS^{-}$ was prepared in a 0.1 M saline phosphate buffer (pH 7.4, 0.15 M sodium chloride) (PBS) by mixing 2.5 mM AAPH with 2.0 mM $ABTS^{2-}$. The solution was heated for 16 min at 60 °C, protected from light, and stored at ambient temperature until used. Seed extract was dissolved in PBS at a concentration of 0.17 mg ml^{-1}. For measuring TEAC, 40 ml of the sample was mixed with 2.0 ml of $ABTS^{-}$ solution. Absorbance of the above solution was monitored at 734 nm, and trolox was used in the construction of the standard curve.

2.10. Statistical analysis

Data were analysed by analysis of variance using the Statistical Package for Social Science (SPSS 10.0 for Windows: SPSS INC., Chicago, Il, USA). Values were given as mean ± standard deviation (SD) and means were separated using a least significant difference (LSD) test. Correlation analysis was also used to characterise the relationships between the total phenolics, anthocyanins, proanthocyanidins and DPPH· radical-scavenging activity, FRAP, lipid antioxidant activity and TEAC.

3. Results and discussion

NaN_3-induced common bean mutants exhibited a wide variety of seed types (Fig. 1). The variety Hwachia had cream seed coat colour with red mottles (Table. 1). Six mutants (SA-01, SA-04, SA-05, SA-06, SA-13-1 and SA-20) appeared to have seed coat colour and mottles similar to Hwachia. Mutants SA-08 and SA-17 had pink seed coat colour with red stripes. Mutants SA-08-2 and SA-12-2 had purple seed coat colour with cream stripes, while mutants SA-11-2, SA-13-2 and SA-34-2 had red seed coat colour with cream mottles. Mutants SA-09 and SA-11-2 had cream seed colour but with purple stripes. SA-16 and SA-24 were the only two mutants that having white seed coat colour with reddish-brown mottles, while SA-08-1 tended to have cream colour but with purple stripes. SA-13-1 and SA-16 were the only two mutants having kidney type seed (Table 1). These changes in seed colour might have resulted from NaN_3-induced mutations in colour or in intensifying genes (Sena et al., 1991).

NaN_3 treatment also induced significant changes in seed size (Table 1). The 100 seeds weights of tested accessions varied from a low of 28.60 to 61.79 g. The Hwachia had relatively medium seed size (100 seeds weight 44.45 g) (Table 1). However, two mutants SA-08-1 and SA-08-2 had smaller seeds (100 seeds weight 31.83 and 28.60 g, respectively), while SA-04 had large seeds (61.79 g) when comparing a variety Hwachia (Table 1). As for seed coat, it represented about 7.86% (SA-01) to 10.27% (SA-20) of the whole seed weight (Table 1).

Table 1
Seed types and dry weights (g 100 $seeds^{-1}$) of whole seed and seed coat of common bean mutants and their wild type cultivar Hwachia.

Accession	Seed type[a]	Whole seed	Seed coat
Hwachia	2M	44.45 ± 6.46	5.45 ± 0.46
SA-01	2M	53.82 ± 3.06	4.23 ± 0.55
SA-04	2M	61.79 ± 3.41	5.02 ± 0.19
SA-05	2M	36.93 ± 2.56	3.29 ± 0.08
SA-06	2M	46.30 ± 3.96	4.52 ± 0.15
SA-08-1	2S	31.83 ± 0.44	2.84 ± 0.16
SA-08-2	7S	28.60 ± 3.44	2.82 ± 0.36
SA-09	2S	48.32 ± 5.15	4.45 ± 0.40
SA-11-1	5S	50.11 ± 1.63	4.22 ± 0.05
SA-11-2	6M	50.52 ± 1.59	4.25 ± 0.29
SA-12-1	2S	48.70 ± 1.64	4.86 ± 0.06
SA-12-2	7S	48.94 ± 2.49	4.68 ± 0.19
SA-13-1	2MK	53.11 ± 2.38	4.49 ± 0.26
SA-13-2	6M	53.26 ± 0.17	4.46 ± 0.06
SA-16	1MK	47.56 ± 2.27	4.59 ± 0.07
SA-20	2M	43.25 ± 1.28	4.44 ± 0.21
SA-24	1M	46.21 ± 1.63	4.36 ± 0.10
SA-34-1	5S	43.69 ± 2.06	4.29 ± 0.21
SA-34-2	6M	48.46 ± 4.44	4.51 ± 0.27
$LSD_{0.05}$		5.07	0.43

Values are means ± SD, $n = 4$.
[a] Seed class designation according to Durán et al. (2005), where number indicates colour (1 = white, 2 = cream, 5 = pink, 6 = red, 7 = purple) and letters placed next to the number indicate pattern or shape (*M* = mottled, *S* = striped and *K* = kidney).

The seed coats of common bean seeds are concentrated with a considerable level of phenolics that can diminish protein and mineral bioavailability (Aparicio-Fernández et al., 2005; Luthria & Pastor-Corrales, 2006; Ranilla, Genovese, & Lajolo, 2007). However, these phenolics are also the most important anti-oxidative plant components (Xu, Yuan, & Chang, 2007). The results of total phenolics analysis for the seed coats of Hwachia and its' NaN_3-induced mutants are given in Table 2. The Hwachia variety had 29.94 mg gallic acid g^{-1} of total phenolics on seed coat dry weight base. The levels of total phenolics for 18 NaN_3-induced mutants varied from a low of 25.96 g to 38.60 mg gallic acid g^{-1} DW. However, 17 of 18 mutants had a higher level of total phenolics than the Hwachia variety. Only mutant SA-16 accumulated less total phenolics than Hwachia in its seed coat part. Several studies have indicated that the common bean variety, with dark-coloured seed coat, generally contained more total phenolics, than the variety with light-coloured seed coat (Rocha-Guzmán et al., 2007; Xu et al., 2007). In the present study, four mutants (SA-08-2, SA-24, SA-34-1 and SA-34-2) had total phenolics level >38 mg gallic acid g^{-1} (i.e., 27% higher than wild type cultivar Hwachia), each with different seed coat colour and stripes. The highest total phenolics level was found on mutant SA-20 that having beige seed coat colour with reddish-brown stripes (Table 2). Thus, no sound relationship between the seed coat colour and the total phenolics contents is detectable for the tested mutants.

Both anthocyanins and proanthocyanidins are reported to be beneficial to human due to their significant anti-oxidative capacities (Castañeda-Ovando et al., 2009; Dixon et al., 2005; McGhie & Walton, 2007). The levels of total anthocyanins and total proanthocyanindins accumulated in seed coats were shown in Table 2. The levels of total anthocyanins for 18 NaN_3-induced mutants, expressed on per unit dry weight of seed coat base, varied from a low of 0.19 to 1.29 mg g^{-1}. The mutant SA-13-2 had highest concentration of total anthocyanins (1.29 mg g^{-1}), and then followed

Table 2
The levels of total phenolics (mg gallic acid g^{-1} seed coat dry weight), total anthocyanins (mg g^{-1} seed coat DW) and total proanthocyanidins (mg g^{-1} seed coat DW) in seed coats of common bean mutants and their wild type cultivar Hwachia.

Accession	Total phenolics	Total anthocyanins	Total proanthocyanidins
Hwachia	29.94 ± 2.79	0.31 ± 0.01	12.94 ± 0.49
SA-01	30.74 ± 2.10	0.34 ± 0.02	13.06 ± 0.94
SA-04	32.17 ± 2.18	0.40 ± 0.05	13.61 ± 0.75
SA-05	31.70 ± 0.48	0.39 ± 0.07	13.71 ± 1.18
SA-06	34.76 ± 1.74	0.45 ± 0.03	14.24 ± 2.52
SA-08-1	38.23 ± 0.31	0.20 ± 0.03	15.41 ± 2.04
SA-08-2	38.14 ± 0.03	0.40 ± 0.04	13.61 ± 1.68
SA-09	36.62 ± 1.28	0.44 ± 0.11	13.85 ± 0.33
SA-11-1	37.32 ± 0.98	0.19 ± 0.01	18.63 ± 1.54
SA-11-2	38.34 ± 0.50	1.11 ± 0.07	17.44 ± 1.58
SA-12-1	33.21 ± 1.36	0.44 ± 0.06	12.65 ± 1.59
SA-12-2	36.80 ± 0.70	0.93 ± 0.07	12.83 ± 0.47
SA-13-1	37.34 ± 0.92	0.36 ± 0.03	11.49 ± 1.96
SA-13-2	37.62 ± 0.67	1.29 ± 0.11	15.21 ± 2.23
SA-16	25.96 ± 3.40	0.17 ± 0.03	13.23 ± 0.78
SA-20	38.60 ± 0.21	0.28 ± 0.02	16.85 ± 2.62
SA-24	35.99 ± 2.76	0.45 ± 0.04	14.47 ± 2.28
SA-34-1	38.45 ± 0.57	0.24 ± 0.01	14.10 ± 1.84
SA-34-2	38.07 ± 0.84	1.05 ± 0.03	17.40 ± 1.36
$LSD_{0.05}$	2.38	0.09	2.71

Values are means ± SD, $n = 4$.

Table 3
The levels of several monomeric anthocyanidins (μg g^{-1} seed coat dry weight) (delphinidin, cyanidin and petunidin) in seed coats of common bean mutants and their wild type cultivar Hwachia.

Accession	Delphinidin	Cyanidin	Pelargonidin
Hwachia	0	429.0 ± 38.6	114.7 ± 20.0
SA-01	0	448.4 ± 53.2	104.0 ± 8.7
SA-04	0	426.9 ± 19.8	127.6 ± 13.2
SA-05	0	603.6 ± 1.0	116.9 ± 9.5
SA-06	0	611.2 ± 22.2	235.7 ± 4.9
SA-08-1	298.3 ± 6.3	214.8 ± 7.0	23.8 ± 1.7
SA-08-2	513.3 ± 80.4	331.4 ± 43.5	50.2 ± 1.5
SA-09	400.1 ± 27.8	334.0 ± 32.4	67.4 ± 6.3
SA-11-1	0	835.9 ± 18.5	297.7 ± 37.3
SA-11-2	0	1132.3 ± 174.8	730.2 ± 87.2
SA-12-1	181.2 ± 14.7	180.4 ± 3.2	59.3 ± 1.6
SA-12-2	276.3 ± 16.4	311.3 ± 21.1	134.3 ± 20.1
SA-13-1	0	280.0 ± 28.4	86.2 ± 3.4
SA-13-2	0	812.4 ± 37.2	327.0 ± 8.2
SA-16	0	444.5 ± 62.3	122.5 ± 17.1
SA-20	0	1059.5 ± 61.8	280.7 ± 5.0
SA-24	0	454.8 ± 20.5	167.5 ± 8.3
SA-34-1	0	755.6 ± 47.4	253.8 ± 17.7
SA-34-2	0	991.8 ± 16.8	662.3 ± 37.4
$LSD_{0.05}$	23.6	62.1	30.2

Values are means ± SD, $n = 4$.

by mutant SA-11-2 (1.11 mg g^{-1}), mutant SA-34-2 (1.05 mg g^{-1}) and mutant SA-12-2 (0.93 mg g^{-1}). These values were higher than the results of red seed coat or light brown with red stripe seed coat varieties, but were lower than black seed coat varieties reported by Ranilla et al. (2007). Overall, 13 out of 18 mutants tended to have higher level of total anthocyanins than wild type variety Hwachia (0.31 mg g^{-1}). These results suggest that the expressions of some candidate genes encoding the key enzymes, such as anthocyanidin synthase and leucoanthocyanidin reductase, for flavonoid biosynthesis might be enhanced by NaN_3-induced mutation (Blair et al., 2007).

Only limited data are available to evaluate the levels of proanthocyanidins in common bean seed (Aparicio-Fernández et al., 2005). In the present study, there were considerably higher levels of total proanthocyanidins in comparison with total anthocyanins, found in the seed coats of Hwachia and its' mutant (Table 2). Similar findings were also reported by Aparicio-Fernández et al. (2005). The levels of total proanthocyanidins for the tested accessions, expressed on per unit dry weight of seed coat base, varied from a low of 11.49 to 18.63 mg g^{-1}. These values were higher than the data reported by Xu et al. (2007). The variety Hwachia had 12.94 mg g^{-1} proanthocyanidins. Mutant SA-11-1 had the highest level of total proanthocyanidins, and then followed by SA-11-2 and SA-34-2. Only three mutants showed a slightly lower level of total proanthocyanidins than wild type cultivar.

There is a huge variety of anthocyanidins spread in nature (Castañeda-Ovando et al., 2009), but only five individual anthocyanins, namely pelargonidin, cyanidin, malvidin, petunidin, and delphinidin are present in common bean seed. Takeoka et al. (1997) reported that the delphinidin was the major constitute accumulated in the seed coat of black colour common bean. On the other hand, pelargonidin was the major anthocyanidin in red kidney bean (Yoshida et al., 1996). In this study, the profiles of monomeric anthocyanidins were determined by using HPLC and the results showed that only cyanidin and pelargonidin were detectable in Hwachia and 13 mutants, with more cyanidin accumulated than pelargonidin in seed coats (Table 3). On the other hand, three monomeric anthocyanidins including delphinine, cyanidin and pelargonidin were detectable in other five mutants (SA-08-1, SA-08-2, SA-09, SA-12-1 and SA-12-2), with delphinidin having the highest level (Table 4). These results suggest that the NaN_3-induced mutation also affects the synthetic pathways of individual anthocyanidins in common bean seed coat.

Several techniques including DPPH· radicals scavenging, ferric reducing antioxidant power and lipid peroxidation inhibition ability have been adopted to measure antioxidant activity in seeds of selected legumes, but each with different specificity and sensitivity (Xu et al., 2007). Thus, to assess and compare the antioxidant potential among the tested mutants by using a single technique is rather difficult. A combination of several tests would provide a more reliable assessment of the antioxidant activity profiles of mutants. The DPPH· radicals scavenging activities in seed coats of tested mutants are present in Table 4. The scavenging activity values ranged from 78.68 g (mutant SA-12-1) to 97.36 mg trolox equivalent per g seed coat dry weight (mutant SA-08-1). Only three mutants (SA-01, SA-04 and SA-12-2) had the DPPH· radicals scavenging activity slightly lower (but statistically insignificant at $p < 0.05$) than the wild type cultivar Hwachia (83.21 mg trolox equivalent per g dry weight). Nine mutants had DPPH· radicals scavenging activities in seed coats over the level of 90 mg trolox equivalent per g seed coat DW.

A large variation in ferric reducing antioxidant power (FRAP), ranging from 681 g to 1103 mg trolox g^{-1} g dry weight was also observed (Table 4). The seed of Hwachia had the FRAP of 82.5 mg trolox g^{-1} g seed coat dry weight. Nearly all the mutants (except SA-01) had greater FRAP than Hwachia. The ranking in reducing powers among the mutants generally paralleled that of DPPH· scavenging activities. The highest FRAP was recorded for SA-08-1, which was almost 51% higher than that of Hwachia.

The variations of lipid peroxidation inhibition (LPI) were considerably larger than that of DPPH· radicals scavenging ability and FRAP (Table 4). Again, there is only one mutant (SA-01) having the LPI ability lower than Hwachia. The greatest inhibition ability was recorded for SA-08-1 (82.98 mg trolox g^{-1} g seed coat dry weight), which had 4-fold increase in inhibition ability as comparing to Hwachia (19.83 mg trolox g^{-1} g seed coat dry weight). Only three mutants (SA-01, SA-04 and SA-24) had smaller LPI ability than cultivar Hwachia (Table 4).

Significant differences ($p < 0.05$) in $ABTS^-$ radical-scavenging activity were also found among mutants and the wild type cultivar (Table 4). The measured $ABTS^-$ radical-scavenging activities of 19 common bean accessions ranged from 264.7 g to 385.9 mg trolox

Table 4
The activities (mg trolox equivalent g^{-1} dry weight of seed coat) of DPPH· scavenging, ferric reducing antioxidant powers (FRAP), lipid peroxidation inhibition (LPI) and $ABTS^{-}$ radical-scavenging activity in seed coats of common bean mutants and their wild type cultivar Hwachia.

Accession	DPPH· scavenging	FRAP	LPI	$ABTS^{-}$ scavenging
Hwachia	83.21 ± 2.98	732 ± 61.9	19.83 ± 2.13	273.4 ± 6.6
SA-01	79.32 ± 7.55	681 ± 84.1	16.94 ± 4.18	264.7 ± 22.3
SA-04	81.77 ± 9.96	792 ± 87.0	19.11 ± 3.32	288.2 ± 27.0
SA-05	86.23 ± 1.52	805 ± 36.4	30.40 ± 6.04	293.8 ± 17.3
SA-06	92.39 ± 6.48	890 ± 20.1	39.41 ± 3.62	385.9 ± 27.7
SA-08-1	97.36 ± 0.15	1103 ± 72.3	82.98 ± 12.21	238.5 ± 13.3
SA-08-2	96.85 ± 0.23	1073 ± 16.2	62.65 ± 20.99	328.1 ± 8.8
SA-09	92.49 ± 4.21	965 ± 14.5	62.23 ± 15.72	292.2 ± 19.2
SA-11-1	95.09 ± 3.22	1029 ± 43.6	35.72 ± 3.54	358.0 ± 8.0
SA-11-2	96.93 ± 0.06	1049 ± 54.6	40.66 ± 5.88	334.9 ± 20.1
SA-12-1	78.68 ± 4.58	784 ± 31.5	29.36 ± 4.59	284.7 ± 7.5
SA-12-2	82.75 ± 4.21	844 ± 10.1	49.28 ± 14.00	324.0 ± 16.4
SA-13-1	96.52 ± 0.36	885 ± 74.4	26.42 ± 14.6	280.7 ± 32.7
SA-13-2	96.61 ± 0.23	910 ± 99.4	26.96 ± 5.88	321.4 ± 10.3
SA-16	85.75 ± 1.08	868 ± 25.4	37.7 ± 4.34	366.4 ± 15.9
SA-20	92.08 ± 0.29	1090 ± 118.3	35.83 ± 8.08	345.8 ± 11.3
SA-24	85.47 ± 4.97	856 ± 60.7	15.00 ± 10.88	316.0 ± 32.2
SA-34-1	88.95 ± 0.48	1020 ± 46.2	40.16 ± 14.08	345.7 ± 21.1
SA-34-2	88.29 ± 0.59	975 ± 118.1	26.36 ± 7.83	342.4 ± 17.2
$LSD_{0.05}$	6.60	108	15.55	31.9

Values are means ± SD, $n = 4$.

Table 5
The correlation coefficients, across all mutants and wild type cultivar Hwachia, for the tested antioxidant activities and the levels of antioxidants.

Correlated seed traits	Antioxidant		
	Total phenolics	Total anthocyanins	Total proanthocyanidins
DPPH· scavenging activity vs. antioxidants	0.992**	0.770**	0.970**
Ferric reducing antioxidant power vs. antioxidants	0.904**	0.660**	0.849**
Lipid peroxidation inhibition ability vs. antioxidants	0.856**	0.598**	0.838**
$ABTS^{-}$ radical-scavenging activity vs. antioxidants	0.977**	0.775**	0.969**

** Significant at $p < 0.01$.

g^{-1} g seed coat dry weight. The mutant SA-06 exhibited the highest $ABTS^{-}$ radical-scavenging activity, and then followed by SA-16 and SA-11-1. SA-01 was the only mutant exhibiting the slightly lower $ABTS^{-}$ radical-scavenging activity (but statistically insignificant at $p < 0.05$) than Hwachia.

The compounds responsible for the tested antioxidant activities are most likely to be the total phenolics, anthocyanins and proanthocyanidins (Marles, Ray, & Gruber, 2003; McGhie & Walton, 2007; Xie & Dixon, 2005). Results of the correlation analyses between the tested antioxidant activities and the levels of antioxidants, across all accessions, re-confirm these findings (Table 5). However, the greatest correlation coefficients were found for the measured antioxidant activities and total phenolics, and then followed by the measured antioxidant activities and total proanthocyanidins, as well as the measured antioxidant activities and total anthocyanins, even though all the correlation coefficients were significant at $p < 0.01$ (Table 5). These results are consistent with other reports that total phenolics confer a high anti-oxidative activity in coloured legumes (Kim et al., 2006; Kong, Chia, Goh, Chia, & Brouillard, 2003). Thus, the level of total phenolics can be used to predict the capacity of the antioxidant activity exhibited by the tested common bean mutants.

In conclusion, the results obtained indicate the great influence of NaN_3-induced mutation on seed shape and seed size of tested common bean mutants. The NaN_3-induced mutation also occurs in the levels of total phenolic, anthocyanins and proanthocyaindins and various kinds of antioxidant activities. NaN_3-induced mutation also affects the profile of monomeric anthocyanidins, with two (cyanidin and pelargonidin) or three (delphinine, cyanidin and pelargonidin) monomeric anthocyanins were detectable in the seed coat depending on the tested mutants. The majority of mutants tend to have greater antioxidant activities (DPPH· radicals scavenging, FRAP, LPI ability and $ABTS^{-}$ radical-scavenging activity) than the wild type cultivar Hwachia. The enhanced anti-oxidative abilities are related to the higher levels of total phenolics, anthocyanins and proanthocyanidins accumulated in their seed coat. It appears that several mutants, which are enriched with both total anthocyanins and total proanthocyanidins (SA-11-2, SA-13-2 and SA-34-2) or enriched only with total proanthocyanidins (SA-11-1 and SA-20) may be useful in food and other applications.

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