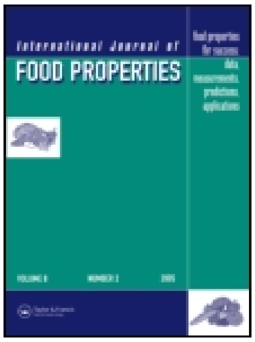

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ASSESSMENT OF ANTIOXIDANT CONSTITUENTS AND ANTI-OXIDATIVE PROPERTIES OF VEGETABLE SOYBEAN

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Vegetable soybean is fast becoming a popular nutritious snack outside South-Eastern countries where its consumption is in vogue since ages. Reports have appeared in the literature concerning the nutritional value of green seeds; however, the information on its antioxidative properties is lacking. In the present investigation, 16 vegetable-type soybeans were evaluated for antioxidant constituents and antioxidative properties at picking stage. Vitamin C, total phenolic content, and total isoflavone were significantly different among different genotypes with ranges of 34.8–88.7 mg/100 g seed, 0.68–1.39 mg gallic acid equivalent/g, and 8.6–33.2 mg/100 g, respectively. 'Dada-cha 2000' had the highest total isoflavone followed by 'ASG328 Sricanan'. Genotypic differences were also observed for antioxidative properties viz. ferric reducing antioxidative power, free radical-scavenging activities, and β-carotene bleaching inhibition. 'Dada-cha 2000' showed the highest ferric reducing antioxidative power value (10.9 µmoles/g), while 'Kegone' showed the lowest (3.95 µmoles/g). 'Dada-cha ma-me', 'Dada-cha 2000', 'ASG328 Sricanan', and 'ASG328 Kohine' exhibited the highest free radical-scavenging activities. 'AGS439' and 'Dada-cha ma-me' exhibited the highest values for β-carotene bleaching inhibition followed by 'ASG328 Sricanan'. Correlation studies revealed significant positive correlation of ferric reducing antioxidative power and free radical-scavenging activities with individual forms of isoflavones and total isoflavone content.

Keywords: ***Vegetable soybean, Ferric reducing antioxidative power, Free radical-scavenging activity, Total phenolic content, Isoflavones, Vitamin C.***

INTRODUCTION

Vegetable soybean is a specialty soybean, which differs from the regular soybean in taste. For human consumption, the pods from the vegetable-type genotypes are picked when the soybean plant reaches between reproductive stages of R_6 and R_7. At this stage, the pod cavity is completely filled and the seeds reach maximum size but pod shell and seeds enclosed in it have not yet started turning yellow. Vegetable soybean has been known to people in many South-East Asian countries by different nomenclature viz. *edamame* in Japan, *mao dou* in China, and *poot kong* in Korea, for hundreds of years; however, in major soybean growing countries viz. United States, Brazil, Argentina, and India, vegetable soybean is a relatively new concept. In these countries, the traditional soy products

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of the South-East countries viz. soy milk, *tofu, natto, miso*, which are processed from mature seeds, could not become popular due to their off-flavour, time-consuming preparation, and exotic appeal to the local masses. On the contrary, immature seeds of vegetable soybean stand easy acceptability because they are sweet in taste, free from off-flavour, quick-to-cook, and more importantly, they are similar to other beans, such as chickpea, green pea, and French bean, in appearance. For consumption, whole pods of vegetable soybean are boiled with a pinch of salt and the seeds are popped inside the mouth. In the backdrop of spurt in oxidative stress related diseases viz. diabetes, atherosclerosis, cancer, across the globe in the last decade, the emphasis for inclusion of soybean in a regular diet has been advocated by the health experts based upon the various reports showing the presence of various antioxidants *viz.* isoflavones, anthocyanin, present in soybean seeds and the products processed from them.[1] Soy isoflavones have been shown to give protective effects against atherosclerosis and type-2 diabetes.[2,3] Antioxidants and antioxidative properties in mature seeds of grain-type soybean and soy-based foodstuffs have been recently investigated.[1,4] Recently, Kumar et al.[5] investigated antioxidants and antioxidative properties of a grain-type soybean cultivar 'JS335' at different reproductive stages and reported higher total phenolic content, ferric reducing antioxidative power, and free radical-scavenging activity in immature soybean compared to the mature seed. Vegetable-type soybean genotypes have been evaluated for nutritional components.[6,7] However, investigations concerning their antioxidant potential and the various antioxidants are lacking. In the present study, 16 prominent vegetable-type genotypes, procured from Asian Vegetable Research and Development Centre, Taiwan, were raised in the field and assessed for their antioxidant potential following ferric reducing antioxidative power (FRAP) assay, DPPH free radical-scavenging activity, and β-carotene scavenging activity. In addition, concentration of antioxidant constituents like vitamin C, total phenols, and isoflavones in vegetable-type genotypes was determined with an aim to study their relationship with antioxidative properties in the green seeds.

MATERIAL AND METHODS

Sixteen vegetable-type accessions of soybean, procured from Asian Vegetable Research and Development Centre, Taiwan, were raised in the fields of the National Research Centre for Soybean, Indore (22° N), India. Three replications of each genotype were planted in the field in the three-row plot in randomized complete block design. Each plot was 3 m long with spacing of 0.45 m between the rows. Green pods of each vegetable-type genotype were picked on the arrival of R_6–R_7 stage (when pods were completely filled with seeds but still green).

Moisture Content

To determine moisture content, 20 green seeds (in triplicate) were kept in a hot air oven at 75°C till their weight became constant. Moisture content of the green seeds was determined by weight differences. Green seeds shelled from the green pods of all the vegetable genotypes were stored at −40°C until they were analyzed for antioxidative properties and antioxidant constituents. Green seeds of all the genotypes were freeze dried using a lyophilizer and the finely ground flour was used for biochemical analyses.

Vitamin C Content

Vitamin C was determined following Plaza et al.[8] In brief, freshly green seeds (1 g) were ground in 4% oxalic followed by the centrifugation at 10,000 rpm for 10 min. The supernatant so obtained was suitably diluted and titrated against 2,6 dichlophenol indophenol dye.

Isoflavones

Concentration of individual and total isoflavones in green seeds of these genotypes was determined in the form of aglycones by following the method given by Vyn et al.[9] In brief, 0.125 g of finely ground flour from each genotype was digested with 1 ml of conc. HCl and extracted with 5 ml of 80% ethyl alcohol at 80–90°C for 2 h with occasional shaking followed by centrifugation at 10,000 rpm for 10 min. The resulting supernatant was filtered through a syringe membrane filter (0.22 μm, 13 mm dia.) before loading into an HPLC system. The HPLC conditions were maintained as described earlier.[4] Then 20 μl of the filtrate was injected into a Shimadzu high performance liquid chromatograph (LC10AT *vp*) equipped with a UV detector (SPD 10 AT Vp) and oven (CTO-10) housing a C18 silica column (Phemomenex 5 micron with a dimension of 250 × 4.6 mm), preceded by a guard column (Phenomenex 4.0 × 3.0 mm) using a Shimadzu auto injector. The column oven was maintained at 40°C. Isoflavones were resolved by employing the binary gradient mode with solvent A (10% ACN) and solvent B (38% ACN) at a flow rate of 0.8 ml/min for 25 min. The solvent system was run as follows (% solvent A/solvent B): 0 min (0/100), 5 min (10/90), 20 min (0/100), and 25 min (0/100). The resolution of the isoflavones was monitored at 260 nm by CSW version 1.7. Retention times for daidzein, glycitien, and genistein were 6.19, 6.59, and 9.74 min, respectively, as shown in Fig. 1. The concentration of individual isoflavone was calculated by comparing the peak area of samples with that of respective standards (procured from Sigma Aldrich, India).

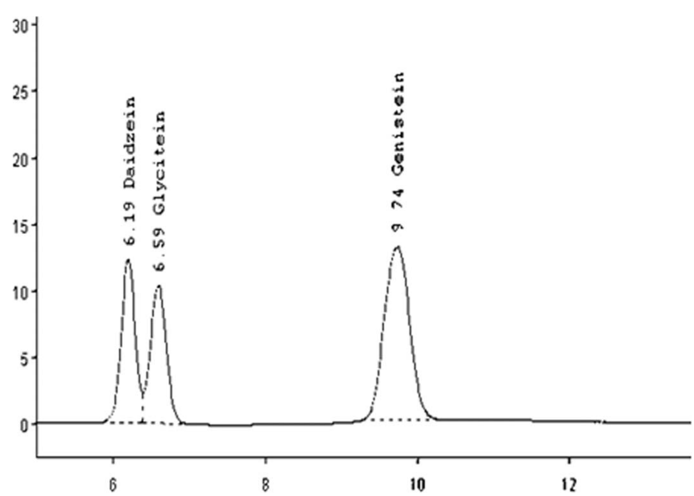

Figure 1 Separation of daidzein, glycitein, and genistein through HPLC.

Extraction of Antioxidants

Freeze-dried green seeds were ground into fine flour (100-mesh). Soy flour (1.0 g) was extracted with 70% aqueous acetone (15 ml) at 25°C in the dark overnight. The mixture was centrifuged at 3000 rpm for 10 min. The residues were re-extracted with 5 ml of the 70% acetone. The extracts were combined and stored at 4°C in a dark place for further analysis.

Determination of Total Phenol Content (TPC)

The TPC of the soybean extracts was determined using Folin-Ciocalteu reagent following the method of Singleton and Rossi.[10]

Antioxidant Power Assay

Total antioxidant capacity of the soybean extract was determined using the ferric reducing antioxidant power as described by Benzie and Strain.[11] DPPH free radical scavenging activity of the extract was evaluated using absolute ethanolic solution of DPPH following Mellors and Tappel.[12] Beta-carotene bleaching assay was carried out by following the method as given by Miller.[13] Beta-carotene (2 mg) was added in chloroform (10 ml) taken in a round-bottom flask. Subsequently, linoleic acid (40 mg) and Tween 20 (400 mg) were added and the contents of the flask were mixed. Chloroform of the mixture so obtained above was evaporated under vacuum at 40°C, followed by the addition of 100 ml of distilled water and mixing the contents vigorously. A volume (4 ml) of the prepared emulsion was transferred in test tubes and 1 ml of sample extract was mixed. An analytical control was also prepared simultaneously, containing 4 ml of emulsion and 1 ml of 70% acetone. The absorbance was measured at 470 nm against blank at the start of the reaction (0 min) followed by intervals of 15 min for 1 h. All the tubes were kept in a water bath maintained at 50°C during the course of the reaction. The rate of β-carotene bleaching (R) was calculated as: $R = \ln(A_{t=0}/A_{t=t}) \times 1/t$; where $A_{t=0}$ is the initial absorbance at time 0, $A_{t=t}$ is the absorbance at 15, 30, 45, and 60 min, and t is the time in min. Beta-carotene bleaching for each sample was determined by computing the average of R values at 15, 30, 45, and 60 min. The antioxidative activity was calculated as:

$$\%\ \text{Antioxidative activity} = 100 \times \left(R_{control} - R_{sample}\right)/R_{control},$$

where $R_{control}$ and R_{sample} are average bleaching rates of emulsion without and with extract, respectively.

Statistical Analysis

Samples prepared in triplicate were used for carrying out the above biochemical analyses, and values obtained from triplicate samples were used for correlation study. All the statistical analyses were carried out using SPSS (version 18.0).

RESULTS AND DISCUSSION

Vitamin C

Moisture content of the green seeds ranged from 66.76 to 78 with an average of 72.18 g/100 g seed for 16 vegetable-type genotypes (data not given). Data presented in Table 1 shows the significant genotypic variation for vitamin C content. On dry weight basis, it varied 3-fold ranging from 34.8 ('Dada-cha 2000') to 88.7 ('AGS439') with a mean value of 55.0 mg/100 g seed. To compare our results with the literature, data for vitamin C content of 16 vegetable-type genotypes were converted on fresh weight and a range of 10.3–21.3 with mean value 15.1 mg/100 g was observed. Song et al.[14] reported vitamin C content to the magnitude of 17 mg/100 g for a Korean vegetable soybean variety, which is near to the vitamin C content of most of the genotypes in the present study. Moriyama and Oba[15] reported a vitamin C value of 23.4 mg/100 g for the immature green seeds of soybean, which is significantly higher than that of all the vegetable-type genotypes investigated in the present study. The same authors reported vitamin C content for some other legumes, such as kidney bean (22.3 mg/100 g), broad bean (17.1 mg/100 g), green pea (20.4 mg/100 g), and peanut (27.8 mg/100 g), which are significantly higher than the average vitamin C content in the present study.

Isoflavones

Concentration of individual and total isoflavones in immature seed is given in Table 1. Significant differences were observed for individual and total isoflavones content

Table 1 Antioxidant constituents of immature seeds of 16 vegetable-type soybean genotypes.

Genotype	Vitamin (mg/100 g)	Daidzein (mg/100 g)	Glycitein (mg/100 g)	Genistein (mg/100 g)	Total ISF (mg/100 g)	Total phenol (mg GAE/g)
AGS432	66.7^{e}	7.30^{c}	0.6^{a}	6.4^{e}	14.3^{c}	1.25^{bc}
AGS433	54.8^{d}	7.7^{c}	2.3^{c}	2.7^{a}	12.7^{b}	1.30^{c}
AGS434	51.2^{c}	5.6^{bc}	1.4^{bc}	2.6^{a}	9.7^{a}	1.18^{bc}
AGS435	56.3^{d}	5.3^{bc}	1.1^{b}	3.0^{bc}	9.5^{a}	1.22^{bc}
AGS436	58.1^{d}	4.3^{ab}	2.2^{d}	3.2^{bc}	9.7^{a}	1.39^{c}
AGS437	56.8^{d}	3.8^{a}	5.3^{f}	7.6^{f}	16.7^{d}	1.33^{c}
AGS438	41.4^{b}	7.6^{c}	4.6^{e}	1.9^{a}	14.2^{c}	1.29^{c}
AGS439	88.7^{h}	6.6^{c}	1.2^{bc}	2.6^{ab}	10.5^{b}	1.17^{bc}
AGS440	38.2^{ab}	4.8^{ab}	1.1^{ab}	2.8^{ab}	8.6^{a}	1.22^{bc}
Dada-cha 2000	34.8^{a}	17.2^{g}	8.5^{h}	7.5^{f}	33.2^{g}	1.36^{c}
Dada-cha ma-me	44.9^{b}	15.9^{f}	5.2^{ef}	5.9^{e}	27^{f}	1.08^{b}
GC98017-7-196-1-2-BNG	50.6^{c}	5.2^{b}	0.9^{ab}	3.7^{cd}	9.8^{a}	1.18^{b}
GC99010-35-1-2-2-BNG	68.1^{f}	7.8^{c}	1.6^{c}	3.0^{b}	12.3^{b}	1.30^{c}
Kegone	52.8^{cd}	6.8^{c}	1.7^{c}	4.4^{d}	12.9^{bc}	0.068^{a}
ASG328 Kohine	39.0^{ab}	10.8^{d}	5.4^{f}	6.2^{e}	22.4^{e}	1.01^{b}
ASG328 Sricanan	77.9^{g}	14.0^{e}	8.0^{g}	6.0^{e}	27.9^{f}	1.05^{b}
Mean	55.0	8.2	3.2	4.3	15.7	1.19

Values given are mean of triplicate samples. Values with different superscripts in the same column are significantly different from each other at $P < 0.05$.

among genotypes. Daidzein ranged from 3.8 (‘AGS437’) to 17.2 (‘Dada-cha 2000’) with a mean value of 8.2 mg/100 g seed. For glycitein content, ‘AGS432’ and ‘Dada-cha 2000’ exhibited the lowest (0.6 mg/100 g seed) and highest value (8.5 mg/100 g seed), respectively. Genistein ranged from 1.9 (‘AGS438’) to 7.6 (‘AGS437’) with a mean value of 4.3 mg/100 g seed. Total isoflavone content of immature seeds ranged from 8.6 (‘AGS440’) to 33.2 (‘Dada-cha 2000’) with a mean value of 15.7 mg/100 g seed. Mebrahtu et al.[16] analyzed 31 vegetable-type genotypes for isoflavones content at the immature seed stage and reported mean values to the magnitude of 7.1, 2.9, 3.3, and 13.4 mg/100 g seed for daidzein, glycitein, genistein, and total isoflavones content, respectively, which are close to the corresponding mean values observed in the present study.

Total Phenol Content

Total phenol content (TPC) of green seeds ranged from 0.68 (‘Kegone’) to 1.39 (‘AGS436’) with a mean value of 1.19 mg gallic acid equivalent (GAE)/g seed (Table 1). Average total phenol content in the present study is less than the value (1.94 mg GAE/g seed) reported recently for soybean cultivar ‘JS335’ at R_6 stage in a separate study from our laboratory.[5] Turkmen et al.[17] reported TPC to the magnitude of 1.83 mg GAE/g for green pea and 3.55 mg GAE/g for green beans, which are significantly higher than the average value observed in our study. Furthermore, Ismail et al.[18] reported total phenolic content to the magnitude of 0.49, 0.63, 0.93, and 1.14 mg GAE/g for French bean, winged bean, string bean, and snow pea, respectively. All of the 16 vegetable-type soybean genotypes in our study exhibited higher total phenolic content than the French bean and winged bean reported in the study of Ismail et al.[18]

Ferric Reducing Anti-Oxidative Power (FRAP)

Immature seeds of the vegetable-type soybean genotypes exhibited 2.7-fold variation for FRAP value, which ranged from 3.95 (‘Kegone’) to 10.94 μmoles/g (‘Dada-cha 2000’) (Table 2). It is notable that ‘Kegone’, which showed the lowest value for FRAP, also exhibited the lowest value for TPC. The average FRAP value (6.95 μmoles/g) in the present study was 8-fold higher compared to the value (0.84 μmoles/g) reported for immature seeds of 35 pea varieties by Nilsson et al.[19] Furthermore, Strangeland et al.[20] reported FRAP value of 2.1 and 7.1 μmoles/g for immature seeds of French bean and brown beans, respectively, which shows that average antioxidative activity value of vegetable soybeans in the present study was as high as that of brown beans and about 3.5-fold that of French bean.

Free Radical-Scavenging Activity (FRSA) Using DPPH

Table 2 exhibits significant genotypic variations for FRSA, ranging from 10.03 (‘Kegone’) to 24.65% (‘Dada-cha ma-me’) with a mean value of 17.4%. ‘Kegone’, the genotype with the lowest values for TPC as well as FRAP, was also found to have the lowest value for FRSA. Barring four genotypes viz. ‘Dada-cha 2000’, ‘Dada-cha ma-me’, ‘ASG328 Kohine’, and ‘ASG328 Sricanan’, all the vegetable genotypes in the present study exhibited lower FRSA than that reported for green pea (21.3%) by Turkmen et al.[17] The same authors observed FRSA to the magnitude of 43.8% for green beans, which is about 2.0- to 4.0-fold higher compared to that of values observed for vegetable genotypes in our study. Furthermore, the average FRSA of immature vegetable-type soybean genotypes

Table 2 Antioxidant properties of immature seeds of 16 vegetable-type soybean genotypes.

Genotype	FRAP (µmoles/g)	FRSA (% reduction)	β-Carotene bleaching inhibition assay (%)
AGS432	5.19^{ab}	15.92^{cd}	77.20^{e}
AGS433	5.63^{ab}	19.27^{a}	47.91^{b}
AGS434	8.70^{c}	12.35^{ab}	66.66^{d}
AGS435	4.29^{a}	11.66^{ab}	50.00^{b}
AGS436	4.08^{a}	15.56^{cd}	39.58^{a}
AGS437	6.00^{b}	14.92^{b}	42.10^{ab}
AGS438	9.56^{cd}	14.82^{b}	52.63^{bc}
AGS439	5.22^{ab}	12.90^{ab}	94.11^{f}
AGS440	7.13^{b}	18.08^{bc}	56.14^{c}
Dada-cha 2000	10.94^{d}	23.12^{e}	72.72^{de}
Dada-cha ma-me	10.02^{cd}	24.65^{e}	90.80^{f}
GC98017-7-196-1-2-BNG	6.34^{b}	18.73^{c}	79.41^{e}
GC99010-35-1-2-2-BNG	5.16^{ab}	17.84^{bc}	68.42^{de}
Kegone	3.95^{a}	10.03^{a}	68.42^{de}
ASG328 Kohine	9.65^{cd}	23.76^{e}	74.55^{e}
ASG328 Sricanan	9.30^{cd}	24.31^{e}	76.36^{e}
Mean	6.95	17.37	66.06

Values given are mean of triplicate samples. Values with different superscripts in the same column are significantly different from each other at $P < 0.05$.

(17.4%) in the present study was higher than the corresponding values for winged bean (14.4) and French bean (16.3%); however, it was found to be about one-fourth of the values reported for string bean (77.7%) and snow pea (61.2%) in an earlier investigation.[18]

Beta-Carotene Bleaching Assay

Inhibition of β-carotene bleaching is also one of the major indices of antioxidative activity. Data presented in Table 2 shows significant genotypic variation ($p < 0.05$) for inhibition of bleaching of β-carotene dye. It ranged from 39.58 (‘AGS436’) to 94.11 (‘AGS439’) with an average of 66.06%. We did not find any report in the literature with the investigation of β-carotene bleaching activity in immature seeds of vegetable or conventional grain-type soybean. Although, we could compare our results with the study of Ismail et al.,[18] which assessed vegetable beans viz. winged bean, French bean, string bean, and snow bean for inhibition of β-carotene bleaching activity. The authors reported inhibition of β-carotene bleaching to the magnitude of 74.9, 72.2, 85.2, and 55.0%, for winged bean, French bean, string bean, and snow bean, respectively. Inhibition of β-carotene bleaching for some of the vegetable-type soybean genotypes (‘AGS432’, ‘AGS439’, ‘Dada-cha 2000’, ‘Dada-cha ma-me’, ‘GC98017-7-196-1-2-BNG’, ‘AGS328 Kohine’, ‘AGS 328 Sricanan’) in our study were higher than that of French bean and winged bean reported in the study of Ismail et al.[18]

Correlation Study

In view of lack of information concerning the relationship among antioxidative properties and antioxidant constituents of green immature seeds of vegetable soybean, we

Table 3 Significant correlations between antioxidants and antioxidative properties of vegetable-type soybean genotypes.

Parameter	FRSA	Daidzein	Glycitein	Genistein	Total isoflavones
FRAP	0.683**	0.734**	0.744***		0.736***
FRSA		0.770***	0.669**	0.499*	0.766***
β-Carotene bleaching inhibition		0.526*			
Daidzein			0.769***	0.534*	0.932***
Glycitein				0.651**	0.914***
Genistein					0.757***

[a]Values superscripted with*, **, and *** are positively significant at p value <0.01, <0.005, and <0.001, respectively.

computed the correlation coefficients among these parameters (Table 3). Total isoflavone content was found to have highly significant positive correlation with FRSA ($r = 0.766^{***}$) as well as FRAP ($r = 0.736^{***}$). The study of Akitha Devi et al.,[21] which showed positive correlation between DPPH free radical scavenging activity and total isoflavones concentration of mature seeds of grain-type soybean cultivars and the soy products, supports the significant interrelationship observed between these characters in vegetable-type soybean in our results. Among individual forms of isoflavones, daidzein exhibited a stronger positive correlation with FRSA ($r = 0.770^{***}$) than FRAP ($r = 0.734^{**}$). Glycitein also exhibited significant positive correlation with FRAP ($r = 0.744^{***}$) and FRSA ($r = 0.669^{**}$) but its relationship with the former antioxidative property was stronger than the latter. Genistein was found to have significant positive correlation with FRSA; though it was weak ($r = 0.499^{*}$). Beta-carotene bleaching activity exhibited significant correlation with only daidzein content ($r = 0.526^{*}$).

CONCLUSION

Vegetable soybeans have been in use for ages in South-East Asia, and lately as a healthy snack becoming increasingly popular in other parts of the world. The present study is the first ever assessment of the major antioxidant constituents and antioxidative properties of vegetable soybean genotypes. Vitamin C concentration of immature vegetable-type soybean was found to be very close to other vegetable legumes; however, a high level of isoflavones observed in the present study makes it distinct from popular green beans investigated in the literature. A higher magnitude of TPC and most of the antioxidative properties observed in the present study for the vegetable soybean than that of reported values for widely used green beans viz. French and winged bean highlights the importance of vegetable soybean as an alternative green bean for antioxidative value. Correlation studies suggested that vegetable-type soybean genotypes with comparatively high daidzein and glycitein content may be sought for harnessing maximum antioxidative value of the green seeds to avail protection against oxidative-stress related diseases. The study also revealed that genotypes ‘Dada-cha 2000’, ‘ASG328 Kohine’, and ‘ASG328 Sricanan’, which exhibited comparatively high levels of antioxidative properties, are the promising vegetable-type genotypes for direct consumption. Genotypic variation for different antioxidative properties observed in our results suggests that there lies sufficient scope for selection and development of suitable vegetable-type soybean genotypes for enhanced nutraceutical value.

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