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Screening of eggplant genotypes with respect to anthocyanin content

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ABSTRACT

Seventeen eggplant lines were analysed for total anthocyanin content from the peel at the edible maturity stage of the fruits. For this, the trial crop was grown in the kharif season of 2018-2019 in RBD design with three replications. The purple-fruited lines had higher anthocyanin content than the green and white-fruited lines. Significant differences were observed among the lines for anthocyanin content irrespective of the degree of purple skin colour. Pusa Shyamla had the highest anthocyanin content (139.04 mg/100g) followed by Pusa Purple Round (51.33 mg/100g). The HPLC analysis identified cyanidin-3-O-glucoside, delphinidin-3-O-glucoside and delphinidin-3-rutinoside anthocyanins in brinjal lines. The highest delphinidin-3-O-glucoside was found in purple fruited variety Pusa Upkar (7.44 mg/kg FW) and delphinidin 3-rutinoside in purple fruited line BR-40-7 (18.83 mg/kg FW). The lines rich in anthocyanin content were identified in the present study and can be used in the breeding programme in transferring the genes in high-yielding eggplant varieties.

Keywords: *Solanum melongena*, Delphinidin-3-O-glucoside, Delphinidin 3-rutinoside, HPLC.

INTRODUCTION

Eggplant (*Solanum melongena* L.; 2n = 2x = 24) is one of the most extensively grown Solanaceous crops in the country. In Hindi, it's called "*Baingan*," while in English, is called "Aubergine or Brinjal." Being originated in India, it has a wide range of diversity in terms of several traits. India's productivity (17.37 MT/ha) is very poor compared to other countries, even though the world's second-largest producer after China (National Horticulture Board, 11). Eggplant is one of the most significant vegetable crops recognized for its nutritional advantages due to the presence of minerals, fibres and bioactive compounds such as phenolics, anthocyanin (Nasunin), flavonoids (Nandi *et al.,* 10; Santhiya *et al*., 15). Furthermore, eggplant has been discovered to have important medicinal qualities and used as medicines from ancient times. The most prevalent phenolic compound is chlorogenic acid (CGA), which has anti-oxidant, anti-carcinogenic, anti-inflammatory, anti-obesity, and anti-diabetic (type 2) effects (Nandi *et al*., 10). Anthocyanins are the most prevalent pigments in plants and have been associated with a variety of health advantages, including protection against cancer and neurological disorders. In general, the acceptance of purple fruited brinjal is more probably due to its skin colour. Besides, the different parts of the plants like leaf lamina, veins, stem and flower contain anthocyanin pigments. For its high anthocyanin content, it is considered one of the top ten vegetables in terms of capacity to absorb oxygen radicals (Todaro *et al.,* 16).The anthocyanin content of fruit skin in eggplant is higher than that of other Solanaceous crops including potato, tomato and pepper (De Pascual-Teresa and Sanchez-Ballesta, 5).

Anthocyanin content of red- and purple-fleshed potato ranged from 7 to 35 mg, and 6 to 17 mg, respectively (Brown, 3). Mes *et al.* (9) found differences in anthocyanin content in tomato fruit with *Aft* gene. They observed total anthocyanin content of 72.32 mg/100 g fresh weight (FW) of fruit skin in one year and it ranged from 17.68 to 36.35 mg/100 g FW in another year. The monomeric anthocyanin content as determined by a pH differential method (Giusti and Wrolstad, 6), exhibited a greater anthocyanin content of 450.1 mg/kg fresh weight for eggplant compared to violet pepper yielding 321.5 mg/kg fresh weight (Sadilova *et al*., 14).

The study by Nandi *et al.* (10) reported the total anthocyanin content in eggplant fruit varied from 0.00 to 945.34 mg 100 g^{-1} FW. Bhanushree *et al.* (2) reported a wide range of variation for total anthocyanin content in eggplant fruit ranging from 0.010 to 120.23 mg 100g^{-1} FW. The difference in anthocyanin content varied due to the use of different genotypes in these studies. Azuma *et al.* (1) studied eggplant accession and related species and reported delphinidin-3-rutinoside as major compound. In a study conducted by Li *et al.* (8) found delphinidin-3-glucoside and delphinidin-3-rutinoside in samples from eggplant peel (cv. Black Beauty).

The colour of the fruit is a complex trait with varying degrees of pigmentation depending to environmental effects, fruit growth stage and other

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genetic factors. The goal of this study was to find eggplant lines with high anthocyanin content which can be utilized in the breeding programme and genes can be identified to transfer into high yielding lines.

MATERIALS AND METHODS

The present study was carried out in the research field of Division of Vegetable Science, ICAR-IARI, New Delhi, India. A total of 17 eggplant lines comprising advanced breeding lines, land races and released varieties were used and the details are given in Table 1. These lines were assessed for the presence of anthocyanin pigmentation in different parts of the plants and the intensity were compared by using RHS colour chart as per guidelines of PPV&FRA (12) (Table 2). The presence of pigmentation of flower, leaf and fruit are given in Figure 1.

Fresh fruits of each line were harvested at edible maturity stage. Total anthocyanin content in fruit peel was estimated by standard protocol given by Giusti and Wrolstad (6). The procedure is briefly described as, the peel of the 3 fruits in each replication were scraped using sharp knife and 5 g peel were blended with 25 ml ethanolic HCL with help of pestle and mortar. The extract was transferred in a 50 ml conical flask. The solution was stored overnight at 4°C. On the next day the solution was filtered in Whatman no 1 paper. The residue was again filtered with ethanolic HCL. The volume was finally made up to 100 ml. The solution was stored in dark for an hour and the reading was taken at 535 nm wave length using a double beam spectrophotometer (Model UV-1900i, Shimadzu Scientific Instruments, Inc. U.S.A). Total anthocyanin content was finally estimated and expressed as mg/100 g fresh weight.

$$\text{Total anthocyanin (mg/100 g fruit weight)} = \frac{\text{OD value} \times \text{dilution} \times \text{total volume made up} \times 100}{\text{Weight of sample taken} \times e \times \text{total fruit weight}}$$

(Where e =98.2 absorbance of a solution containing 0.1 mg/ml anthocyanin)

For HPLC analysis, anthocyanins pigment was extracted from 16 lines except DBWL-22-1-11 following standard protocol of Revilla *et al.* (13) using high-speed homogenizer (Ika) and a rotating evaporator (Laborota, Heidolph, Germany). Fruit tissues were mixed with 97% ethanol (97 ml + 3 ml concentrated HCl. The ethanolic extract was filtered and the remaining material was extracted again with the same solvent until it was colourless. Filtrates were mixed and concentrated on a rotavapor at 40°C under vacuum to produce a viscous substance. The extract was kept at -40°C until it was characterised.

The anthocyanins were separated in reverse phase HPLC system using C_{18} column (Hypersil;

Table 1. Eggplant genotypes/varieties/lines, their characteristics and source used for screening for anthocyanin pigmentation.

Sl. No.	Eggplant genotypes/ varieties/lines	Characteristics (shape, colour)	Source
1	Pusa Purple Round	Round dark purple	IARI, New Delhi
2	BR-40-7	Round dark purple	Haryana
3	Pusa Shyamla	Long dark purple	IARI, New Delhi
4	Pusa Bindu	Round dark purple	IARI, New Delhi
5	Pusa Uttam	Oval round medium purple	IARI, New Delhi
6	NDB-25	Long dark purple	NDUAT, Faizabad, Uttar Pradesh
7	DBL-187	Long dark purple	IARI, New Delhi
8	Pusa Upkar	Oval round medium purple	IARI, New Delhi
9	Pusa Kranti	Long dark purple	IARI, New Delhi
10	Swarna Mani	Big round purple	ICARRCER, Jharkhand
11	BR-112	Round purple	Haryana
12	Pusa Hara Baigan 1	Long green	IARI, New Delhi
13	DBGR-131	Big round green	IARI, New Delhi
14	Pusa Safed Baigan 1	Small round white	IARI, New Delhi
15	DBL-186	Long purple	IARI, New Delhi
16	Pusa Vaibhav	Round purple	IARI, New Delhi
17	DBWL-22-1-11	Long white	Meghalaya

Table 2. Morphological traits recorded for anthocyanins pigmentation and intensity.

Plant parts	Anthocyanin pigmentation
Stem	Anthocyanin colouration
	The intensity of anthocyanin colouration
Leaf	Blade colour
	The intensity of the colour of the blade
	Colour of vein
	The intensity of the colour of the vein
Flower	Flower colour
Fruit	Colour of skin at commercial harvesting
	The intensity of the colour of skin
	Colour of calyx
	The intensity of the colour of calyx

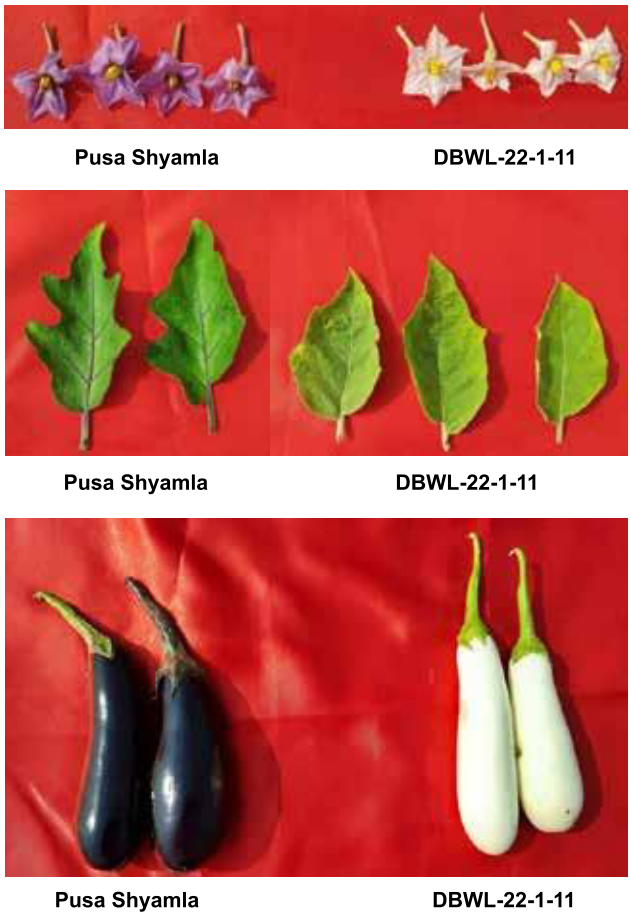

Fig. 1. Anthocyanin pigmentation in flower, leaf vein and fruit in Pusa Shyamla and DBWL-22-1-11.

250 mm × 4.6 mm × 5 µ; Thermo Fischer Scientific, USA). Total anthocyanin was also calculated as cyanidin-3-glucoside equivalent after considering all the peaks together.

All the HPLC quality solvents (acetonitrile, ethanol, formic acid, and trifluoroacetic acid reagents) were procured from Merck, and Sigma Aldrich, India. The 3-O-glucoside standards of cyanidin (>99 % purity), delphinidin (>98 % purity), and 3-rutinoside of delphinidin (>98 % purity) (HPLC grade) were obtained from Sigma Aldrich, India. The retention times and UV-Vis spectra of standards compounds were used for the profiling of anthocyanin by comparison. The result was further confirmed by spiking the samples with standards. Anthocyanidins were characterized after acid hydrolysis followed by mass spectrometric analysis. Unknown chromatographic peaks were tentatively identified via their MS/MS data and by literature data.

The parameter was statistically analyzed online through http://www.psbvb.in/rbd.html site for analysis of variance (ANOVA). Difference in means were estimated using Fisher's least significant difference at 5% level of significance.

RESULTS AND DISCUSSION

The visual observations on pigmentation in different parts of the plants were taken as per Table 2. The anthocyanin pigmentation varied between different lines. The stem was pigmented in all the lines except DBWL-22-1-11 and Pusa Safed Baingan 1, where the stem was green. The intensity of stem anthocyanin pigmentation was strong in Pusa Bindu and Swarna Mani, medium in Pusa Shaymla, Pusa Uttam, NDB-25, DBL-186, Pusa Upkar and weak in DBL-187, Pusa Hara Baingan 1. The leaf blade colour was purple in Pusa Bindu, Swarna Mani, light purple in Pusa Shyamla, NDB-25, green in DBL-186, DBL-187, Pusa Hara Baingan 1, DBR-40-7. The colour of vein was purple in all the lines except DBL-186, DBWL 22-1-11 where the veins were green in colour (Fig. 1). Pusa Bindu had anthocyanin pigmentation in all parts of the plant. The flower colour was purple in all the lines except DBWL 22-1-11 which had white flowers. The colours of fruits were purple in thirteen genotypes. The fruits were green in two genotypes and it was white in two genotypes. But the intensity of colour of fruit skin varied with lines where it was dark in 8 genotypes, medium in 5 genotypes. The presence of pigments is due to genetic architecture of the lines. The results of total anthocyanins content in peel measured through spectrophotometric method (Giusti and Wrolstad, 6) is given in Table 3. Significant differences were observed for anthocyanin content among the lines. The extracted anthocyanin from dark purple and white fruited lines was shown in Fig. 2. Among the lines, high total anthocyanins were found in dark purple/purple fruited lines followed by the green fruited lines and lowest were recorded in white fruited lines. Among the lines, Pusa Shyamla had highest anthocyanin content (139.04 mg/100 g) followed by Pusa Purple Round (51.33 mg/100 g), BR-40-7 (45.50 mg/100 g), Pusa Bindu (42.92 mg/100 g), Pusa Uttam (42.01 mg/100 g). Pusa Safed Baingan-1 had the lowest anthocyanin content (0.24 mg/100 g). Anthocyanin is an important pigment responsible for the purple colouration of fruits. Our study corroborates the findings of Bhanushree *et al.* (2); Nandi *et al.* (10) and Koley *et al.* (7) as they found higher anthocyanin in purple fruited genotypes. The difference in anthocyanin content varied due to use of different genotypes used in these studies. Even in purple fruited lines, the anthocyanin concentration varies among the lines. It was not always true that dark purple fruited lines had high anthocyanin content except for Pusa Shyamla which does not signify positive correlation between fruit colour and

Table 3. Total anthocyanins content and concentration of Delphinidin 3-rutinoside, Delphinidin 3-glucoside and Cyanidin 3-glucosidein different eggplant lines.

Eggplant lines	Total anthocyanins content (mg/100 g fruit weight)	Delphinidin 3-rutinoside (mg/kg)	Delphinidin 3-glucoside (mg/kg)	Cyanidin 3-glucoside (mg/kg)
Pusa Purple Round	51.33	5.20	3.42	2.07
BR-40-7	45.50	18.83	3.97	2.10
Pusa Shyamla	139.04	1.60	0.31	0.98
Pusa Bindu	42.92	3.57	0.29	0.13
Pusa Uttam	42.01	7.97	5.99	1.00
NDB-25	37.82	15.88	4.14	2.05
DBL-187	37.23	18.20	3.12	2.55
Pusa Upkar	36.70	14.01	7.44	2.18
Pusa Kranti	34.01	1.65	4.93	4.43
Swarna Mani	21.46	3.96	0.50	1.39
BR-112	14.15	4.14	0.89	2.14
Pusa Hara Baigan 1	0.76	4.67	3.07	0.99
DBGR-131	0.32	1.16	0.88	0.30
Pusa Safed Baigan 1	0.24	4.28	0.36	0.61
DBL-186	37.27	13.09	2.07	1.64
Pusa Vaibhav	25.84	0.95	3.54	1.65
DBWL-22-1-11	0.079	-	-	-
CD at 0.05	0.937407	2.09	1.58	0.70
CV (%)	0.017146	0.17	0.32	0.25

Fig. 2. The extracted anthocyanin from DBWL-22-1-11 and Pusa Shyamla.

anthocyanin concentration. Probably, the dark colour of the fruit and the quantity in anthocyanin depends on genetic architecture and relationship with other biochemical compounds. But the actual reason needs to be investigated in follow up studies. This study is confirmed by the previous report of Koley *et al.* (7) where dark purple fruited line Kashi Taru, PR-5, CHBR-2 had low anthocyanin content as compared to light purple fruited line Pant Rituraj and Punjab Barsati.

The anthocyanins from peel were identified based on retention time and by comparison with the available standards and available literature. The detailed HPLC study at 280 nm and 520 nm revealed that the extract contains anthocyanins as its major constituents but also having other phenolic compounds (Fig. 3). HPLC profile of the purified extract of the lines revealed that there was one major peak followed by minor. The major peak for cyanidin-3-glucoside (C3-G) which eluted at a retention time of 17.226 minutes in LC gave a PDA spectrum with maxima at 517.6 nm (Fig. 4), the major peak for delphinidin-3-rutinoside which eluted at a retention time of 13.782 minutes in LC gave a PDA spectrum with maxima at 527.4 nm (Fig. 5). The major peak for delphinidin-3-glucoside which eluted at a retention time of 16.000 minutes in LC gave a PDA spectrum with maxima at 526.1 nm (Fig. 6). It is a major peak in the anthocyanin mixture of fruit peel. Preliminary observation from the Rt value and its UV spectrum was considered to be cyanidin-3-glucoside (C3-G) (confirmed by LC run of commercial standard). Total anthocyanin was also calculated as cyanidin-3-glucoside (C3-G) equivalent after considering all the peaks together. Our results contradict the findings of Condurache *et al*. (4) as they reported delphinidin 3-O-rutinoside as the major one.

The concentration of delphinidin 3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside

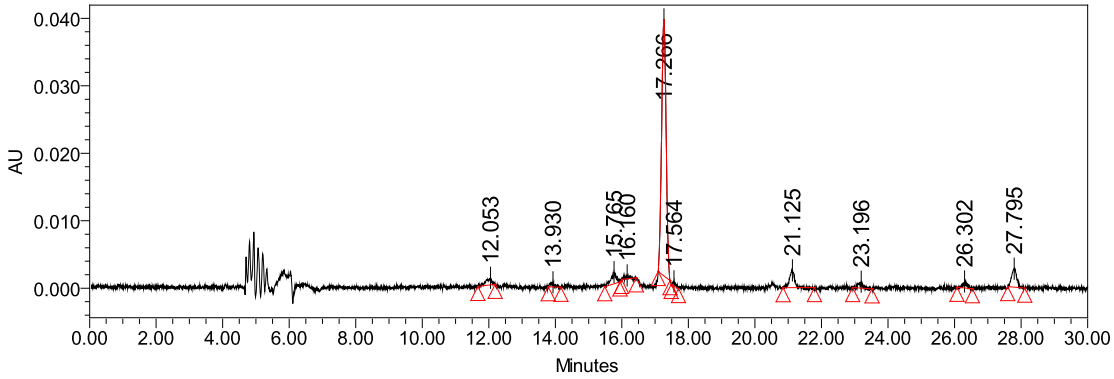

Fig. 3. HPLC chromatogram of anthocyanin extracted from Pusa Shyamla.

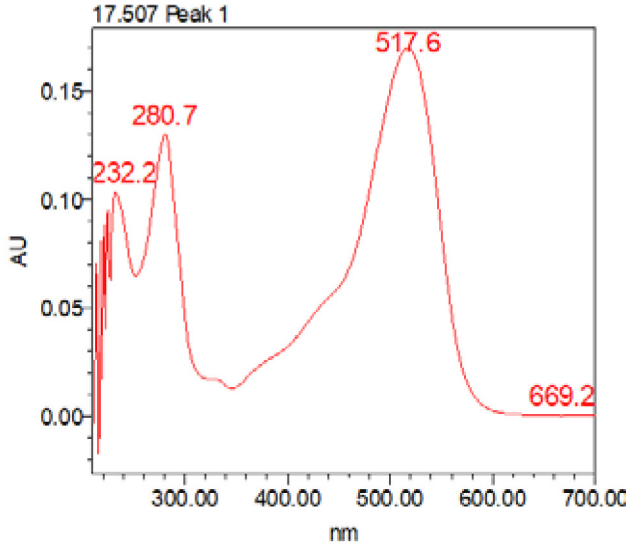

Fig. 4. Standard curve of Cyanidin 3-glucoside (C3G).

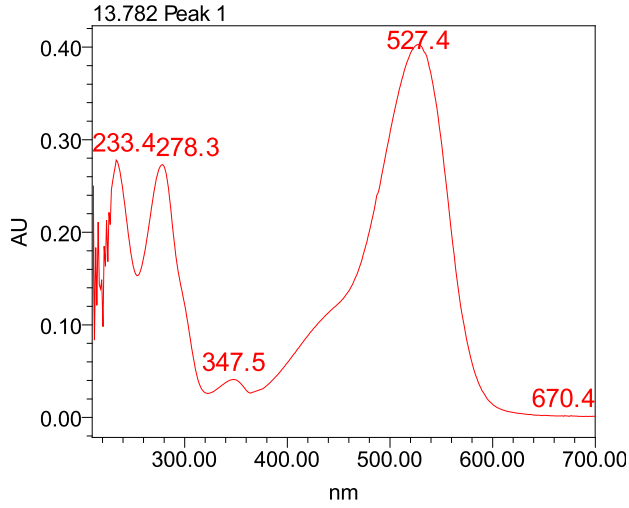

Fig. 5. Standard curve of Delphinidin 3-rutinoside (D3R).

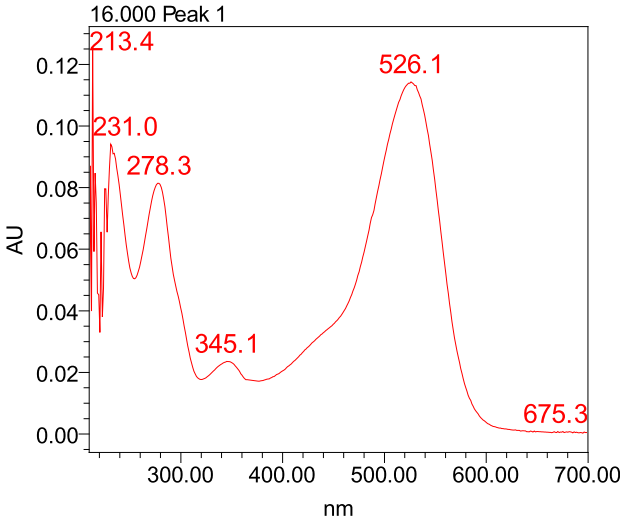

Fig. 6. Standard curve of Delphinidin 3-glucoside (D3G).

through HPLC is represented in Table 3. The concentration of delphinidin-3-rutinoside was highest in line BR-40-7 (18.83 mg/kg), followed by DBL-186 (18.20 mg/kg) and the lowest in Pusa Vaibhav (0.95 mg/kg). The cyanidin-3-O-glucoside was highest in Pusa Kranti (4.43 mg/kg), followed by DBL-187 (2.55 mg/kg) and lowest in DBGR-131 (0.30 mg/kg). The delphinidin -3-O-glucoside was found to be highest in Pusa Upkar (7.44 mg/kg), followed by Pusa Uttam (5.99 mg/kg) and lowest in Pusa Shyamla (0.31 mg/kg).

Anthocyanin profile of eggplant lines showed a high predominance of two anthocyanins, namely delphinidin-3-glucoside and cyanidin-3-glucoside. The lines evaluated in the present study viz. Pusa Shyamla, Pusa Purple Round and BR-40-7 can be used to study the genetics of anthocyanin pigmentation for use in breeding programme. The identified lines rich in anthocyanin can be used as parental line in eggplant varietal development programme.

AUTHORS' CONTRIBUTION

Conceptualization (PS, BST, AK); Designing of the experiments (PS, YAL, BST, AK); Contribution of experimental materials (PS, YAL, BST, AK); Execution of field/lab experiments and data collection (HSM, YAL); Analysis of data and interpretation (HSM, AK); Preparation of the manuscript (PS, HSM, AK).

DECLARATION

The authors declare that they have no conflict of interest.

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