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Short Communication

Flower and fruit colour variability in Indian spinach (*Basella alba*)

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Flower and fruit colours are complex traits whose expressions are dependent on a number of factors including genetic, environmental and physiological. *Basella alba*, commonly known as 'Malabar spinach'/ 'Indian spinach' are important leafy vegetable blessed with immense nutraceutical values. These are endowed with a special group of plant pigment betalains, giving them a range of colours to the stem, leaf, flowers and fruits. Betalains are being reported as a source of high quality natural colourant with immense nutraceutical values. Betalains are found only in the order Caryophyllales and in some higher fungi with exception to the *Caryophyllaceae* and *Molluginaceae*, which accumulate anthocyanins for their red coloration (Steglich and Strack 1990). Anthocyanin and betalain never co-exist (Stafford 1994). That is why, no plant has yet been reported that produces both betalain and anthocyanin together (Strack et al. 2003). This mutually exclusive nature of betalain and anthocyanin production is a curious phenomenon and it exclusivity remains unexplained. This is in fact surprising that gene homologues for a key enzyme in betalain biosynthesis are present in anthocyanic plants (Christinet et al. 2004) though they are never been found in the same plant (Stafford 1994).

In *Basella alba* var *alba* the stem colour is green while in *Basella alba* var *rubra* stem colour is red/ purple. However, flower colour of both red and green types are with pink/purple tinge and mature fruits are enriched with dark purple fruit juice. During characterization and evaluation of 70 accessions of *Basella* during 2015-2018 at ICAR-IIVR, Varanasi, a range of flower colours comprising purple, pink, white with pink or purple tip were observed (Fig. 1). The fruits were fleshy, stalk less, ovoid or nearly spherical and the colour varies from green, red violet to black during ontogeny. However, the colour of matured fruit is black with bright purple fruit juice in both green and red types. During 2015, a variant was observed in which flower colour was snow white, tender fruits pure green without any red pigmentation and even matured fruits were blackish green with colourless fruit juice. This rare mutant with very low expression of betalains was named as VRB 48-1 and was found to be stable for the last three years. This is actually a variant from the germplasm EC769321, collected from AVRDC, Taiwan. In order to quantify the observations, betalain content was estimated spectrophotometrically (Schwartz et al. 1981) in the unripe and ripe fruits of 4 genotypes (red and green) at ICAR-NBPGR, New Delhi and ICAR-IIVR, Varanasi. The results obtained for total betalains (betanins and vulgaxanthin) shown in Table 1 exhibits maximum pigment content in ripened fruits of VRB-30, (200.93 mg/100 g FW)- a red type, followed by VRB 3 (151.53 mg/100 g FW)-green type. The mature fruits of VRB 48-1 showed the lowest betalain (10.48 mg/100 g FW). Interestingly, the vulgaxanthin content in VRB 48-1 extract which was virtually having no reddish tinge was

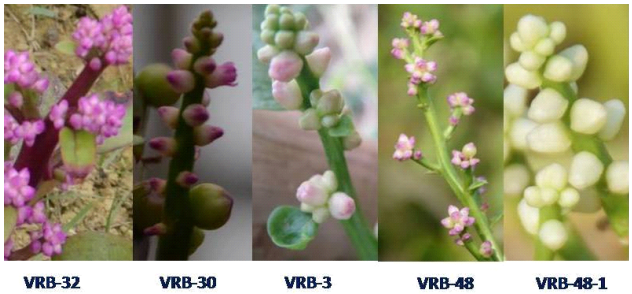

Figure 1: Variability in flower colour of *Basella*

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Table 1. Betalain content in different genotypes of *Basella*

Genotype	Fruit Stage	Colour	Betanin (mg/100 g FW)	Vulgaxanthin (mg/100 g FW)	Total Betalains (mg/100 g FW)
VRB-48-1	Immature	Green	3.98±0.18	7.18±0.14	11.17±0.32
	Mature	Blackish green	4.69±0.09	5.80±0.07	10.49±0.02
VRB-3	Immature	Purplish green	2.35±0.10	4.93±0.03	7.278±0.14
	Mature	Black	127.20±0.88	24.33±0.24	151.53±0.64
VRB-48	Immature	Green with purple tinge	3.77±0.23	5.34±0.26	9.11±0.03
	Mature	Black	115.17±1.05	35.70±0.35	150.87±0.70
VRB-30	Immature	Purple	2.27±0.05	2.56±0.22	4.83±0.17
	Mature	Black	154.82±0.24	46.11±0.09	200.94±0.34

found more than betanin. This could be the reason why even the mature fruits of VRB 48-1 lack red/purple pigmentation. VRB-30 is a red type basella with dark purple pigmentation and thus has the highest content of total betalain amongst the genotypes studied. We also tried to confirm the absence of anthocyanin with pH differentiation method as per AOAC method number 2005.02 (AOAC 2016) and it was clearly evident that there was no anthocyanin in the fruit extract.

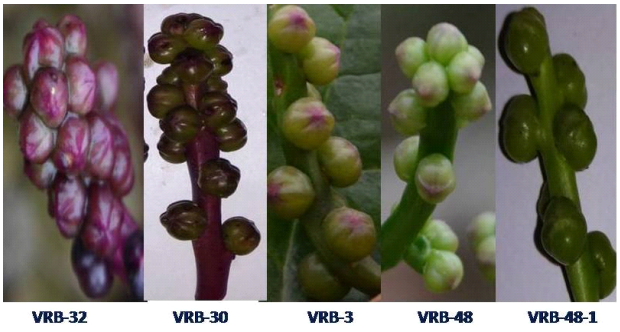

Figure 2: Variability in colour of immature fruits of *Basella*

Generally plants have a wide range of pigments but betalains hold its importance due its unique stability as compared to other naturally occurring pigments like anthocyanins and carotenoids. The colour of betalain does not depend on pH and is more stable than that of anthocyanins (Tanaka et al. 2008). The range of hues available in *Basella* adds beauty as well has great potential to be utilised. Betalains have enormous nutraceutical values and are being used in dye making, bakery and cosmetics. Hence, genotypes with high betalain content may be exploited as a source of betalain for indusrial/pharmaceutical use. Information on the biochemistry and genetics of the plant betalain pathway is scarce. Genetic analysis of the betalain biosynthetic pathway in the ornamental plant *Portulaca grandiflora* indicates that four structural genes would be sufficient to account for the different colour phenotypes observed in petals (Trezzini and Zry¨d 1990). The only betalain biosynthetic enzymes characterised at the molecular level to date are for DOPA 4,5-dioxygenase (DOD) and some of the glycosyl transferase activities. The first plant DOD gene characterised was from *P. grandiflora*, and it defined a novel plant gene family of non-haem dioxygenases (Chritinet et al. 2004, Tanaka et al. 2008). The genotype with colourless fruit juice or with low expression of betalain can never be ignored as it may serve as the key to unravel biosynthetic pathway of betalain synthesis. Besides, it may serve as the basic material for understanding the genetics of betalain in *Basella*, may be used as parent for generation of bi-parental mapping population and further used for mapping of the betalain gene on *Basella* genome. These identified genomic regions can be delineated to further identify the candidate gene(s) associated with the betalain production.

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