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Genetic analysis and identification of molecular marker linked to the gene for fruit skin colour in eggplant (*Solanum melongena* L.)

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Abstract

Eggplant or brinjal is one of the most important Solanaceous crop cultivated widely throughout the country. The dark purple coloured fruit is preferred by consumer due to high anthocyanin content. The degree of pigmentation is unstable, possibly due to influence of environment, growth stage of fruit, etc. The present investigation was carried out to know the genetics of fruit colour and also to identify SSR marker linked to the trait. Cross was successfully attempted between Pusa Safed Baingan 1 (white coloured fruit) × Pusa Uttam (dark purple coloured fruit) to develop F_1. A single F_1 plant was selfed to develop 168 F_2 plants and also backcross (36 BC_1P1, 33 BC_1P_2) progenies developed. The skin colour of parents, F_1, backcross and F_2 plants was evaluated at edible maturity stage and compared with RHS colour chart. Bulk segregant analysis (BSA) was carried out to identify SSR marker linked to the gene for fruit skin colour. Segregation of fruit colour was analyzed by Chi square (ë²) test for goodness of fit. The fruit of F_1 plants was intermediate revealed incomplete dominance. Out of 168 F_2 plants, 125 were purple coloured, 31 green and 12 white which clearly segregated into 12:3:1 (P:G:W) ratio suggesting dominant epistasis with ë² value of 0.28 (*P*=0.80-0.90). The BC_1P_1 (Pusa Safed Baingan 1 backcrossed with F_1) showed 15 purple coloured, 11 green coloured and 10 white coloured which segregated in 2:1:1 ratio. Among the 18 parental polymophic SSR markers, only one marker (emg21I17$_{165/200}$) was found to be polymorphic in BSA. This marker is segregated in 1:2:1 ratio suggesting co-segregation and linked with the gene for fruit skin colour. The result will be very useful in designing breeding strategies for developing dark purple coloured variety in eggplant and also the identified SSR marker will be useful in marker assisted breeding.

Keywords: Eggplant, fruit skin colour, SSR marker, MAS

Introduction

Eggplant or brinjal (*Solanum melongena* L.; 2n = 2x = 24), an important member of Solanaceae family, is cultivated globally and accompanied with divergent shapes and colors of skin. It is herbaceous plant grown as annual or biennial with erect, semi-spreading or spreading habits. It is mainly self-pollinated, but due to the presence of heterostyly and tip pore anther dehiscence, cross pollination occur and known as often cross pollinated crop. Wide variation is observed for shape, size and skin colour in different parts of India (Prasad et al. 2015, Chattopadhyay et al. 2009). It is used in ancient medicine due to presence of various desirable phenolic compounds. The main phenolic compound is chlorogenic acid (CGA) which has antioxidants, anti-carcinogenic, anti-inflammatory, anti-obesity, anti-diabetic (type 2) effects (Plazas et al. 2013). The purple skin colour in eggplant is due to polyphenolic anthocyanin and present in the vacuoles of cell in the fruit epicarp (skin) (Helmja et al. 2007; de Pascual Teresa and Sanchez-Ballesta 2008). The most common anthocyanin is nasunin which helps in neutralizing free radicals (Chaudhary and Mukhopadhyay, 2012), fighting cancer (Salem et al. 2013), and also has anti-aging activity (Mai et al. 2012). Therefore, brinjal stands among the top ten vegetables for oxygen radical absorbance capacity (Hanson et al. 2006).

Fruit color is a component that was affected mostly during eggplant domestication. It is very important characteristic for consumer preference as well as

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breeders point of view which display wide range of variations. As eggplant has gained an important component in human daily diet and is being given concern in research, the breeding of brinjal varieties with high purple pigmentation is an effective method to increase the daily intake of these antioxidants. In the recent past, the genetics of fruit skin colour have been reported and many QTLs, genes have been detected or cloned in various crop species (Huang and Hsieh 2015 and Dou et al. 2018). In eggplant the information on genetics of fruit colour is contradictory wherein presence and absence of is under monogenic dominant control (Daunay et al. 2004). Some QTLs for colour development in eggplant fruit have been mapped (Doganlar et al. 2002, Nunome et al. 2001). But, understanding the genetics of skin colour in eggplant is lagging behind than other Solanaceae crops (Paran and Van der Knaap 2007). Molecular markers are powerful tool for tagging and mapping of useful genes in different crop species (Michelmore et al. 1991). The known genetics of skin colour and identification of molecular markers linked to the gene of fruit skin colour is a useful strategy for breeding eggplant varieties with high anthocyanin content. Therefore, the study was undertaken to know the genetics of fruit skin colour and association with SSR markers.

Materials and Methods

Plant materials: The cross was attempted between Pusa Safed Baingan 1 (white skin colur) × Pusa Uttam (dark purple skin colour). The F_1 fruit was light purple (intermediate) in colour. Both the parents were backcrossed with F_1 to develop BC_1P_1 [(Pusa Safed Baingan 1 × Pusa Uttam) × Pusa Safed Baingan 1)] and BC_1P_2 population [(Pusa Safed Baingan 1 × Pusa Uttam) × Pusa Uttam)]. A single F_1 plant was selfed to develop F_2 progeny. The line Pusa Safed Baingan 1 was derived from an indigenous material collected from West Garo Hills, Meghalaya, India. Pusa Uttam was progeny selection of cross GR x 91-2. In the *Kharif* season of 2017, both the parental lines (20 plants each), BC_1P_1 (36 plants), BC_1P_2 (33 plants) and F_2 (168 plants) were transplanted in July at the research farm of the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi.

Phenotypic observation of fruit skin colour: Observations were recorded from five randomly selected plants from parents, each from BC_1P_1, BC_1P_2 and F_2 generations at edible maturity stage for fruit skin colour. The colour of skin were visually observed and compared with RHS colour chart (6th Edition) and scoring was done as White: 1, Greenish white: 2, Whitish green: 3, Very pale purple: 4; Pale purple: 5, Very light purple: 6, Light purple: 7, Purple: 8, Dark purple: 9 (Fig. 1). Segregation of fruit colour analyzed by Chi-square (÷2) test for goodness of fit (Panse and Sukhatme, 1967).

Fig. 1: Scoring of F_2 fruits according to colour where, 1: Dark purple; 2: Pale purple; 3: Light purple; 4: Very light purple; 5: Pale purple; 6: Very pale purple; 7: Whitish green; 8: Greenish white; 9: White

DNA extraction and genotyping of F_2 plants: For the markers analysis only parents and 168 F_2 plants were taken into consideration. DNA was extracted from both the parents and all F_2 plants using CTAB method with modification (Murray and Thompson, 1988). A total of 241 SSR markers were selected from linkage group of Nunome et al. (2009) and used for parental polymorphism between Pusa Safed baingan 1 and Pusa Uttam. DNA bulk was prepared by pooling DNA of ten white fruited plants (B1) and ten dark purple fruited plants (B2) to identify the molecular markers which are putatively linked to the gene for fruit skin colour as per Michelmore et al. (1991). Standard protocol for PCR was followed. The white and dark purple bulks along with parents were screened with polymorphic SSR markers found during parental polymorphism survey. In the gel, different band sizes were present in both the parent and scoring was done accordingly.

Results and Discussion

Genetic analysis of fruit skin colour: The phenotyping of fruit skin colour of parents, F_1, BC_1P_1, BC_1P_2 and F_2 plants is presented in Table 1. Among the parents all the fruits of Pusa Safed Baingan 1 were white in colour whereas fruits of Pusa Uttam were dark purple in colour (Fig. 2). The colour of F_1 fruit was light purple in colour which was intermediate in expression of the parents and this result indicates co-dominance nature of this trait. Similar study was observed by Nunome et al. (2001). A total of 36 BC_1P_1, 33 BC_1P_2 and 168 F_2 plants were phenotyped according to the colour score as

Table 1: Segregation of fruit colour in BC_1P_1, BC_1P_2 and F_2 and inheritance study

Parents/ Progenies	Phenotype of fruit skin colour									Observed plants			Expected ratio	Chi square (χ2)	probability
	DP	P	LP	VLP	PP	VPP	WG	GW	W	P	G	W			
Pusa Safed Baingan 1									20						
Pusa Uttam	20														
F_1			40												
F_2	18	29	28	18	14	18	17	14	12	125	31	12	12:3:1	0.280	0.80-0.90
BC1P1	0	1	2	1	11	0	9	2	10	15	11	10	2:1:1	1.056	0.50-0.70
BC1P2	7	15	9	2	0	0	0	0	0	33	0	0	-	-	-

DP: Dark purple; P: Purple; LP: Light purple; VLP: Very light purple; PP: Pale purple; VPP: very pale purple; WG: Whitish green; GW: Greenish white; W: White

described in Fig 1. Out of 168 F_2 plants, 125 were purple coloured (dark purple 18, purple 29, light purple 28, very light purple 18, pale purple 14, very pale purple 18), 31 were green coloured (whitish green 17 and greenish white 14) and 12 were white. The segregation of purple: green: white followed 12:3:1 ratio with chi square (÷2) value of 0.28 with probability value of 0.80-0.90 suggesting that the fruit skin colour is governed by dominant epistasis gene action (Table 1). This result is also supported by discrete distribution of fruit colour (data not shown). Our study contradicts the previous report where anthocyanin presence (v/s its absence) is under monogenic dominant control (gene provisionally symbolized A) (Daunay et al. 2004). The observation in BC_1P_1 (Pusa Safed Baingan 1 was backcrossed with F_1) showed 15 purple skinned fruit, 11 green coloured fruit and 10 white coloured fruit which segregated in 2:1:1 ratio with chi square value of 1.056 (P=0.5-0.7). Among BC_1P_2 (Pusa Uttam backcrossed with F_1) plants all the fruits were purple in colour (dark purple 7, purple 15, light purple 9, very light purple 2) and no green or white fruit observed. Our study clearly depicts that purple is dominant over green and white. As in the parental

Fig. 2: Variability for fruit skin colour in parents, F_1, BC_1P_1, BC_1P_2 and F_2 population; P_1: Pusa Safed Baingan 1; P_2: Pusa Uttam; BC_1P_1: [(Pusa Safed Baingan 1 × Pusa Uttam) × Pusa Safed Baingan 1]; BC_1P_2 : [(Pusa Safed Baingan 1 × Pusa Uttam) × Pusa Uttam]

Table 2: Amplification result of the SSR marker emg21I17$_{165/200}$ in 168 F_2 plants

Fruit colour	No of plants	No of plants with target band	No of plants without target band	No of plants without amplification product
Deep purple	18	14	0	4
Purple	29	23	5	1
Light purple	28	23	5	0
Very light purple	18	10	7	1
Pale purple	14	13	1	0
Very pale purple	18	14	4	0
Whitish green	17	10	7	0
Greenish white	14	5	9	0
White	12	8	0	4

lines green colour fruit was not there but in F_2 and BC_1P_1 progenies green colour fruit observed which suggest that there is another gene giving green colour fruit and denoted as *G*. The purple colour gene is denoted as *P*. Both *P* and *G* in recessive form give white fruit coloured fruit (*ppgg*). The green colour fruit is produced when P in recessive and G is in homozygous or heterozygous dominant form *(ppG_)*. The gene dosage effect cannot be over-ruled which give variation in purple/green pigmentation in F_2 and backcross progenies. This is the first study where we clearly demonstrate and symbolize the gene responsible for fruit skin colour in eggplant.

SSR marker linkage analysis: During parental polymorphism survey with SSR markers, 18 were found to be polymorphic. These polymorphic markers were run in BSA (white pool and dark purple pool DNA) along with the parents. Out of 18 markers, only one SSR marker (emg21I17$_{165/200}$) was found to be polymorphic in bulk segregant analysis and selected for genotyping of 168 F_2 plants. The results of single plant analysis and the segregation of marker are presented in Table 2 and Fig. 3. The SSR marker emg21I17$_{165/200}$ amplified a fragment of 165 bp size in Pusa Safed Baingan 1 and 200 bp size in Pusa Uttam. The specific band of 200 bp was present in the purple pool and dark purple parent (Pusa Uttam), each 10 plants of purple pool and absent in white pool, white parent (Pusa Safed Baingan 1), each 10 plants of white pool. Among 125 purple coloured plants, 22 plants could not amplify the target band specific to Pusa Uttam parent. In 31 green coloured plants, 16 plants were unable to amplify target band. Among12 white fruited plants 8 plants were abled to amplify target band specific to Pusa Safed Baingan 1 whereas 4 plants could not able to amplify any band. The representative gel photograph of F_2 genotyping is presented in Fig. 3. Total 38 plants were not able to amplify target band. The marker was segregated in 1: 2: 1 ratio (165 bp in 37 plants with allele resembles to Pusa Safed Baingan 1 *i.e.* P_1, 62 plants with heterozygous band and 200 bp in 33 plants with allele resembles to Pusa Uttam *i.e.* P_2). This study clearly showed that the SSR marker is co-segregating with the gene of interest. Yi et al. (2009) identified six AFLP markers to be

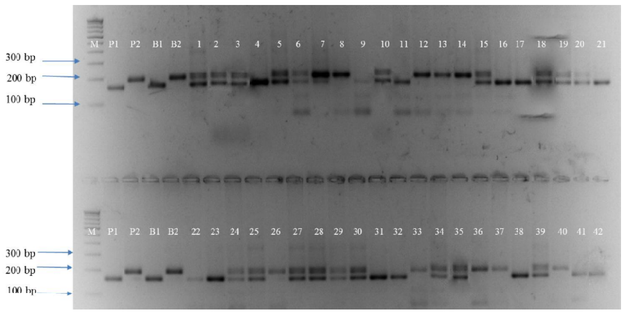

Fig. 3: Genotyping of 168 F_2 plants with SSR emg21I17 marker segregating for fruit colour; M: 100 bp ladder, P1: Pusa Safed Baingan 1; P2: Pusa Uttam; B1: White fruited bulk; B2: Purple fruited bulk; Lane 1-42: 42 F2 plants

associated with peel color of brinjal through bulked line analysis (BLA). Anthocyanin accumulation was found to be determined by a major locus on linkage group 10 which explained as much as 93% (*fap10.1* and *pa10.1*) of the phenotypic variation (Daunay et al. 2004). Nunome et al. (2003) found association of fruit colour with some markers in linkage group 7. They also reported that anthocyanin presence and accumulation are controlled by several different genetic factors in eggplant. In our study we could not find QTLs due to availability of only one polymorphic marker in BSA. The markers developed in this study may be utilized in markers assisted breeding of eggplant improvement. The population will be useful in studying genetics of various traits and trangressive segregants may be identified to develop varieties in future.

सारांश

सोलेनेसी कुल की फसलों में बैंगन पूरे देश में व्यापक रूप से खेती की जाने वाली महत्वपूर्ण फसल है। गहरें बैंगनी रंग के फलों को उपभोक्ताओं द्वारा अधिक पसंद किया जाता है और यह एन्थोसायनिन सामग्री से भरपूर होता है। रंजकता की डिग्री अस्थिर है, जो संभवतः पर्यावरण के प्रभाव, फलों के विकास के चरण आदि के कारण होता है। फल के रंग आनुवंशिकी को ज्ञात करने के लिए और लक्षण से जुड़े एसएसआर मार्कर की पहचान करने के लिए वर्तमान परीक्षण किया गया। संकरों (एफ$_1$) को विकसित करने के लिए संकरणों में पूसा सफेद बैंगन–1 (सफेद रंग का फल) x पूसा उत्तम (गहरे बैंगनी रंग का फल) का प्रयोग किया गया। एक एकल एफ$_1$ पौध से 168 एफ$_2$ पौधों को विकसित किया गया और प्रतीप संकरण (36 बीसी$_1$ पी$_1$, 33 बीसी$_1$ पी$_2$) पूर्वजों को विकसित किया गया। मातृ–पितृ, एफ$_1$, प्रतीप संकरण और एफ$_2$ पौधों की त्वचा का रंग खाद्य परिपक्वता स्तर पर मूल्यांकन किया गया और आरएचएस रंग चार्ट के साथ तुलना की गई। फलों की त्वचा के रंग लिए जीन से जुड़े एसएसआर मार्कर की पहचान करने के लिए थोक अलग–थलग विश्लेषण (बीएसए) किया गया। आवेश की अच्छाई के लिये रूपरेखा तैयार कर परीक्षण द्वारा फलों के रंग के अलगाव का विश्लेषण किया गया। एफ$_1$ पौधों का फल मध्यवर्ती था जिसमें पता चला कि अधूरा प्रभुत्व मौजूद है। कुल 168 एफ$_2$ पौधें में से, 125 बैंगनी रंग के, 31 हरे और 12 सफेद थे जो स्पष्ट रूप से 12:3:1 अनुपात में 0.25 (पीत्र 0.80–0.90) के काई मूल्य के साथ प्रमुख एपिस्टासिस का सुझाव देते हैं। बीसी$_1$पी$_1$ (पूसा सफेद बैंगन–1 को एफ$_1$ के साथ प्रतीप संकरण किया गया था) में 15 बैंगनी रंग का फल, 11 हरे रंग का फल और 10 सफेद रंग का फल पाया गया जिसे 2:1:1 के अनुपात में पाया गया। 18 पैतृक पॉलीमोफिल एसएसआर मार्करों में से केवल एक मार्कर (ईएमजी 21/17165/200) बीएसए में बहुरूपी पाया गया। इस मार्कर को 1:2:1 अनुपात में अलग–अलग सह–अलगाव का सुझाव दिया जाता है और फलों की त्वचार के रंग के लिए जीन के साथ जोड़ा जाता है। यह परिणाम बैंगन में गहरे बैंगनी रंग की विविधता विकसित करने के लिए प्रजनन रणनीतियाँ की रूपरेखा तैयार करने में बहुत उपयोगी होगा और साथ ही चिन्हित एसएसआर मार्कर, मार्कर असिस्टेड प्रजनन में उपयोगी होगी।

References

Chattopadhyay DK and Webster DC (2009) Thermal stability and flame retardancy of polyurethanes. Prog Polym Sci 34(10): 1068-1133.

Chaudhary B and Mukhopadhyay K (2012) Induction of anthocyanin pigment in callus cultures of *S melongena* L. in response to plant growth regulators and light. IOSR J Pham 2:76-80.

Daunay MC, Aubert S, Frary A, Doganlar S, Lester RN, Barendse G, van der Weerden, G, Hennart JW, Haanstra J, Dauphin F and Jullian E (2004) Eggplant (*Solanum melongena*) fruit colour: pigments, measurements and genetics. Proc XIIth EUCARPIA meeting on genetics and breeding of Capsicum and eggplant, Netherlands, 17-19 May 2004, pp 108-116.

De Pascual-Teresa S and Sanchez-Ballesta MT (2008) Anthocyanins: from plant to health. Phytochem Rev 7(2): 281-299.

Doganlar S, Frary A, Daunay M, Lester R, Tanksley S (2002) Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in eggplant. Genetics 161: 1713–1726.

Dou J, Lu X, Ali A, Zhao S, Zhang L and He N (2018) Genetic mapping reveals a marker for yellow skin in watermelon (*Citrullus lanatus* L.). PLoS One 13(9): 0200617.

Hanson PM, Yang RY, Tsou SC, Ledesma D, Engle L and Lee TC (2006) Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics, and ascorbic acid. J Food Compos Anal 19(6): 594-600.

Helmja K, Vaher M, Gorbatšova J and Kaljurand M (2007) Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. Proc Estonian Acad Sci Chem 56: 172-186.

Huang HY and Hsieh CH (2015) Genetic research on fruit color traits of the bitter gourd (*Momordica charantia* L.). Hort J 86(2): 238-243.

Mai Y and Eisenberg A (2012) Self-assembly of block copolymers. Chem Soc Rev 41(18): 5969-5985.

Michelmore RW, Paran I and Kesseli RV (1991) Identification of markers closely linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Nat Acad Sci USA 88: 9828–9832.

Murray MG and Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8(19): 4321-4326.

Nunome T, Ishiguro K, Yoshida T and Hirai M (2001) Mapping of fruit shape and colour development traits in eggplant (*Solanum melongena* L.) based on RADP and AFLP markers. Breed Sci 51: 19-26.

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H and Ohyama A, Fukuoka H (2009) Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). Theor Appl Genet 119: 143-1153.

Nunome T, Suwabe K, Iketani H and Hirai M (2003) Identification and characterization of microsatellites in eggplant. Plant Breed 122: 256-262.

Panse VC and Sukhatme PV (1967) Statistical Methods for Agricultural Workers, Indian Council of Agricultural Research, New Delhi, 152-162.

Paran I and Van der Knaap E (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. J Exp Bot 58: 3841-3852.

Plazas M, Andújar I, Vilanova S, Hurtado M, Gramazio P, Herraiz FJ and Prohens J (2013) Breeding for chlorogenic acid content in eggplant: interest and prospects. Not Bot Horti Agro 41(1): 26.

Prasad V, Dwivedi VK, Deshpande AA and Singh BK (2015) Genetic combining ability for yield and other economic traits in brinjal (*Solanum melongena* L.). Veg Sci 42(2): 25-29.

Salem MZM, Zeidler A, Böhm M and Srba J (2013) Norway spruce (*Picea abies* [L.] Karst.) as a bioresource: Evaluation of solid wood, particleboard, and MDF technological properties and formaldehyde emission. BioResources 8(1): 1199-1221.

Yi L, Sun BJ, Sun GW, Liu HC, Li ZL, Li ZX, Guo-ping W and Chen RY (2009) AFLP and SCAR markers associated with peel colour in eggplant (*Solanum melongena* L.). Agri Sci China 8(12): 1466-1474.