# Genetic study of fruit quality traits in watermelon (*Citrullus lanatus*) prebred line derived from *Citrullus lanatus* var. *citroid*

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#### ABSTRACT

Fruit quality is most important for commercially cultivated watermelon [Citrullus lanatus (Thunb.) Matsum and Nakai] cultivars and introgression of resistance gene from wild or related species is a major challenge and imperative to study the inheritance of internal and external fruit quality traits for utilizing in resistance breeding programme. Present study was carried out at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi during summer 2020 to 2022 involving 4 seasons for identifying source of resistance, developing  $F_1$  and other subsequent generations ( $F_2$ ,  $BC_1P_1$  and  $BC_1P_2$ ) and evaluation of parental lines along with breeding population in watermelon. The resistant line DWM-45 was used as a female parent and crossed with commercially popular sweet watermelon variety Arka Manik to produce  $F_1$  and other generation to study the inheritance of internal and external fruit quality traits. Based on the segregation pattern of progeny it is concluded that fruit shape, rind pattern, seed coat colour and colour of flesh at margin near rind and at carpellar region followed monogenic inheritance. Furthermore, our findings revealed a complex genetic control with epistatic interactions for other fruit quality traits such as fruit length, fruit width, fruit weight and total soluble solids. This study provides valuable insights into the genetic basis of various fruit quality traits in watermelon which can have practical applications in breeding programmes, facilitating the development of improved watermelon varieties with desirable fruit characteristics.

**Keywords**: Citrullus lanatus, Epistasis, Inheritance, Monogenic, Prebred line

Watermelon [Citrullus lanatus (Thunb.) Matsum and Nakai] is an important fruit vegetable crop being grown in 7% of the world's vegetable cropping area. India produces about 3.25 million tonnes of watermelon from 119 thousand hectares (FAO 2021). This refreshing fruit is cherished for its juicy sweetness, especially during hot weather. Watermelon fruit is popular due to its lower calorific content and considered as a heart healthy food due to absence of cholesterol and negligible amount of fat and sodium. Understanding the genetic profile of fruit quality traits is essential for developing improved watermelon cultivars with enhanced nutrition and extended storage life. Watermelon bud necrosis disease caused by Watermelon bud necrosis orthotospovirus (WBNV) has emerged as one of the major diseases which limit the watermelon production significantly. The disease incidence of WBNV ranged from 39 to 100% with a yield loss up to 100% has been reported (Kunkalikar et al. 2011). Commercial elite varieties faced many challenges due to biotic and abiotic

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stresses and wild species like Citron (Citrullus lanatus var. citroides) and Citrullus colocynthis closely related to sweet watermelon, offer valuable resistance to different diseases like watermelon bud necrosis virus, Fusarium wilt and powdery mildew (Levi et al. 2011, Wechter et al. 2012, Nagesh et al. 2018, Holkar et al. 2018 and Jamatia et al. 2022). Introgression of desirable traits from wild germplasm to elite cultivars through breeding programs requires systematic sourcing to avoid genetic complications. Therefore, comprehending the inheritance patterns of fruit quality traits is crucial for retaining fruit quality while transferring resistance genes from wild species into cultivated watermelon varieties. By harnessing the potential of wild germplasm, breeders can overcome limitations and develop more resilient and high-quality cultivars for sustainable crop production (Bhatt et al. 2023). Several attempts have been made in the past to understand the genetics of fruit quality traits in cultivated watermelon genotypes (Guner and Wehner 2003, 2004 and Dou et al. 2018). However, no such study to understand the genetics of fruit quality traits in introgression lines derived from Citrullus lanatus var. citroides could be found. Hence, an attempt was made to understand the inheritance of important external and internal fruit quality traits in prebred line derived from Citrullus lanatus var. citroides.

## MATERIALS AND METHODS

The experimental material comprises a pre-bred line DWM-45 developed through selection and continuous selfing of citron germplasm, a wild species closely related to cultivated sweet watermelon and known for its resistance genes against various watermelon diseases. Present study was carried out at the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi during summer 2020 to 2022 across 4 seasons. In the first season of summer 2020, screening against WBNV was done and DWM-45 was identified and validated as new source of resistance. The resistant line (DWM-45) was crossed with commercially popular variety and highly susceptible to BND, Arka Manik to generate F<sub>1</sub> during summer of 2021 and in next season i.e. during rainy (kharif) season of 2021,  $F_1$ s were selfed and backcrossed with  $P_1$  (Parent 1) and  $P_2$ (Parent 2) to generate F<sub>2</sub>, BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub> (backcross) populations respectively. To study the inheritance of internal and external fruit quality traits parental lines and F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub> generations were evaluated in the main field during summer of 2022. DWM-45, the female parent, exhibits fruits with greenish-white solid rind, spherical shape, bitter and creamish-white leathery flesh, TSS ranging from 2.1 to 3.3 °B, and yellowish-white seed coat. In contrast, Arka Manik, the pollen parent, produces oval fruits with dark green solid stripes, light green inter-stripe colour, sweet red flesh with TSS ranging from 10 to 12 °B, and dotted black seed coat. Fruits were harvested at 90 to 95 days after sowing (DAS), when they reached horticultural maturity. Fruit quality traits like seed coat colour, fruit stripe and inter-stripe colour, rind pattern were assessed visually. Fruits were cut transversely to assess the flesh colour at 3 different regions (1) at the centre, (2) at the carpellar region, and (3) at the margin of rind. TSS of fruit were measured by using hand refractometer (Erma Inc., Japan). Length and width of all fruits were measured to determine the fruit shape using fruit shape index formula, length (cm)/width (cm). Fruit shape classification was done as suggested by Dou et al. (2018) with some modifications. The classes of fruit shape index <0.9, 0.9-1.10, 1.11-1.30 and >1.30 were classified as flat, spherical, oval and oblong, respectively.

A test for goodness of fit was performed using chisquare analysis of the expected mono/dihybrid segregation of classical Mendelian ratios (Panse and Sukhatme 1985) as:

$$\chi 2 = \Sigma \frac{(Observed\ number - Expected\ number)^2}{Expected\ number}$$

The traits such as fruit length, fruit width, fruit weight and TSS were subjected to generation mean analysis. Scaling test was done to determine presence or absence of non-allelic gene interactions and their types as described by Mather (1949) and Hayman and Mather (1955). A six-parameter model as given by Hayman (1958) was followed for the estimation of various genetic components.

## RESULTS AND DISCUSSION

This study investigated the genetics of various fruit

quality traits in segregating generations of watermelon (DWM-45 × Arka Manik). With respect to fruit shape, plants of P<sub>1</sub> (DWM-45) had spherical fruits, P<sub>2</sub> (Arka Manik) had oval fruits, and their F<sub>1</sub> generation had all spherical fruits. The F<sub>2</sub> generation (Table 1) exhibited a 3:1 ratio of spherical to oval fruits, supporting monogenic segregation ( $\chi 2$  = 0.41, P = 0.52). Further backcrossing confirmed the single dominant gene control, with BC<sub>1</sub>P<sub>1</sub> showing a 1:0 ratio (spherical: oval) ( $\chi 2 = 0$ , P = 1), and BC<sub>1</sub>P<sub>2</sub> displaying a 1:1 ratio (spherical: oval) ( $\chi 2 = 0.07$ , P = 0.78). The results on inheritance pattern of fruit shape in this study were similar to previous reports on single gene inheritance (Tanaka et al. 1995, Guner and Whener 2004 and Dou et al. 2018), however, Maragal et al. (2019) in the cross between BIL 53 and IIHR-140-152 did not observe Mendelian pattern of inheritance for fruit shape in watermelon.

All plants in  $F_1$  generation had fruits with dark green elongated stripes like  $P_2$  (Arka Manik) (Fig 1). In the  $F_2$  progeny, a 3:1 segregation occurred for dark green and medium green stripes ( $\chi 2 = 0.084$ , P = 0.77). Backcross progenies (BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub>) showed 1:1 and 1:0 segregation for stripe colours with  $\chi 2$  values of 0.13 (P = 0.71) and 0 (P = 1) respectively (Table 1). In the  $F_2$  population, inter-stripe colour segregated as 142 light green and 56 greenish white, fitting an expected 3:1 ratio ( $\chi 2 = 1.13$ , P = 0.28). Both fruit stripe and interstrip colour traits were monogenetically inherited and showed independent inheritance, consistent with Maragal *et al.* (2019) findings.

In the  $F_1$  generation, all plants had fruits with tiger skin rind pattern (i.e., presence of green stripes on rind).  $F_2$  generation showed a 3:1 segregation ratio ( $\chi 2 = 1.51$ , P = 0.22). This suggests that a single dominant gene responsible for inheritance of rind pattern. In  $BC_1P_1$ , half had stripes ( $\chi 2 = 1.23$ , P = 0.26), while  $BC_1P_2$  had all striped fruits ( $\chi 2 = 0$ , P = 1). Similar findings were reported by Gusmini and Whener (2006) and Maragal *et al.* (2019) confirming the monogenic inheritance of the rind pattern in watermelon. However, Kumar and Wehner (2011) reported involvement of dominant duplicate gene action for solid dark green rind pattern versus light green rind.

Segregants displayed varying fruit flesh colours in distinct regions (center, margin, and carpel) (Fig 2). Therefore, we examined flesh colour inheritance by recording flesh colour at specific region of flesh and observed independent segregation. The F<sub>1</sub> population showed white flesh colour dominance over red at flesh margin. In the F<sub>2</sub> plants, there was a 3:1 segregation ratio for white: red. The backcross populations BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub> displayed 1:0 and 1:1 segregation ratios, respectively. These results confirm a single dominant gene's control over flesh colour at the margin ( $\chi$ 2= 0.06, P = 0.98 for F<sub>2</sub>;  $\chi$ 2= 0, P = 1 for BC<sub>1</sub>P<sub>1</sub>;  $\chi$ 2= 0.30, P = 0.53 for BC<sub>1</sub>P<sub>2</sub>). The flesh colour of fruits at the carpel region of watermelon was observed in F<sub>2</sub>, BC<sub>1</sub>P<sub>1</sub>, and BC<sub>1</sub>P<sub>2</sub> populations. In F<sub>2</sub>, the segregation ratio of white to red flesh matched the expected 3:1 ratio (152:46). Backcross populations, BC<sub>1</sub>P<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub>, exhibited ratios of 1:0 and 1:1, respectively, confirming the control of a single

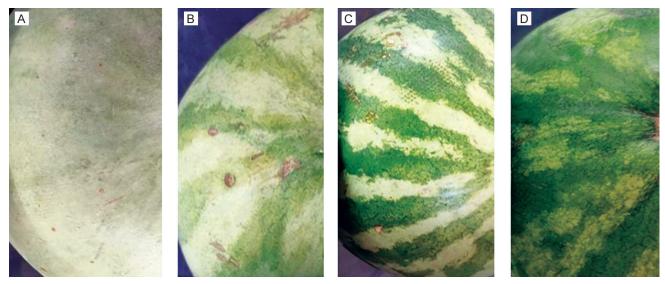


Fig 1 Different rind colour and pattern observed in segregating progenies of cross DWM-45 × Arka Manik.
A, Greenish white rind without stripes; B, Greenish white rind with light green stripes Dark green stripes; C, Dark green stripes;
D, Green rind with dark green stripes.

dominant gene with white colour being dominant over red. Different genes controlled the flesh colours at each region, aligning with previous research (Gusmini and Whener 2006 and Maragal *et al.* 2019).

In the cross between DWM-45 ( $P_1$ ) having yellowish white seed coat and Arka Manik ( $P_2$ ) with black seed coat, the  $F_2$  population showed a segregation ratio of 145:53 (dotted black: yellowish white) in accordance with the 3:1 ratio. The chi-square value was 0.32 with a P-value of 0.57. This indicated that a single dominant gene governed the seed coat colour. These findings aligned with previous studies (Maragal *et al.* 2019) reporting the dominance of black seed colour over others.

In case of fruit quality traits namely, fruit length (cm), fruit width (cm), fruit weight (kg), and Total Soluble Solids ( $^{\circ}$ B), we did not observe simple mendelian segregation in F<sub>2</sub> and backcross populations. Therefore, six generation mean analysis and scaling test were performed to know the

gene action involve in the expression of these traits. In six generation mean analysis, hybrid outperformed parents in all quantitative traits i.e., fruit length (cm), fruit width (cm) and fruit weight (kg) except TSS (°B). Segregating progenies had lower TSS than  $P_2$ . The  $F_1$  and  $F_2$  generations had higher trait means than  $P_1$  and  $BC_1P_1$ . The  $BC_1P_2$  generation performed better than  $P_1$ ,  $F_2$ , and  $BC_1P_1$  generations in fruit length, fruit width, and fruit weight (Table 2).

For fruit length (cm), fruit width (cm), fruit weight (kg), and Total Soluble Solids ( ${}^{\circ}$ B), an epistatic digenic interaction was found to be a suitable fit, since the scaling tests were significant (Table 3). Subsequent analysis of individual traits revealed specific genetic components influencing their expression. Fruit length was found to be influenced by dominant (h) gene action in positive direction and by additive (d) gene action in negative direction. In case of fruit length, h and l components had opposite signs, which indicate involvement of duplicate gene action. For fruit

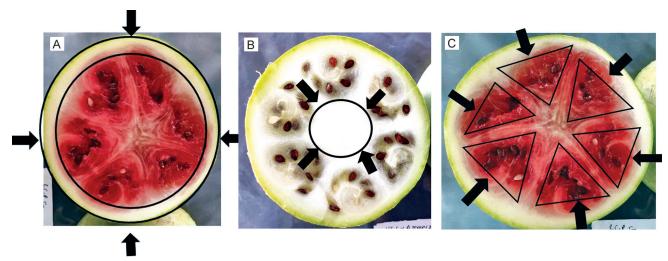


Fig 2 Intermediate flesh colour observed in progenies of DWM-45 × Arka Manik. (A) Flesh colour at margin; (B) flesh colour at centre; (C) flesh colour inside the carpel.

Table 1 Chi-square analysis for simple mendelian inheritance of different fruit quality traits in watermelon

Trait	Population	Best fit ratio	χ2 value	P value (P<0.05)	Gene action	
Fruit shape	F <sub>2</sub>	3:1 (155 Spherical: 47 oval)	0.41	0.52	Single dominant gene Spherical>oval	
	$BC_1P_1$	1:0 (51 Spherical: 0 oval)	0	1		
	$BC_1P_2$	1:1 (27 Spherical: 25 oval)	0.07	0.78		
Stripe colour	$F_2$	3:1 (104 Dark green: 37 medium green)	0.084	0.77	Single dominant gene, Dar green>medium green	
	$BC_1P_1$	1:1 (16 Dark green: 14 medium green)	0.13	0.71		
	$BC_1P_2$	1:0 (52 Dark green: 0 medium green)	0	1		
Inter stripe colour	$F_2$	3:1 (142 Light green: 56 greenish white)	1.13	0.28	Single dominant gene, Light	
	$BC_1P_1$	1:1 (24 Light green: 28 greenish white)	0.38	0.58	green>greenish white	
	$BC_1P_2$	1:0 (52 Light green: 0 greenish white)	0	1		
Rind pattern	$F_2$	3:1 (141 Striped rind: 57 solid rind)	1.51	0.22	Single dominant gene,	
	$BC_1P_1$	1:1 (33 Dark green: 22 medium green)	1.23	0.26	Striped rind>solid rind	
	$BC_1P_2$	1:0 (52 Dark green: 0 medium green)	0	1	pattern	
Flesh colour at margin	$F_2$	3:1 (150 White: 48 red)	0.06	0.80	Single dominant gene	
	$BC_1P_1$	1:0 (52 White: 0 red)	0	1	White>red	
	$BC_1P_2$	1:1 (28 White: 24 red)	0.30	0.53		
Flesh colour at carpellar region	$F_2$	3: 1 (152 White: 46 red)	0.65	0.42	Single dominant gene	
	$BC_1P_1$	1:0 (52 White: red)	0.30	0.58	White>red	
	$BC_1P_2$	1:1 (29 White: 23 red)	0.69	0.40		
Seed coat colour	$F_2$	3:1 (145 Dotted black: 53 yellowish white)	0.32	0.57	Single dominant gen	
	$BC_1P_1$	1:1 (28 Dotted black: 24 yellowish white)	0.30	0.58	Dotted black>yellowish	
	$BC_1P_2$	1:0 (52 Dotted black: 0 yellowish white)	0	1	white	

F<sub>1</sub>, first filial generation; F<sub>2</sub>, second filial generation; BC<sub>1</sub>P<sub>1</sub>, backcross with DWM-45; BC<sub>1</sub>P<sub>2</sub>, backcross with Arka Manik.

width additive (d), additive  $\times$  additive (i), and additive  $\times$ dominance (j) components were significant with negative sign, indicating a complex interplay of genes governing fruit width. Fruit weight was significantly influenced by the additive (d) and additive  $\times$  dominance (j) components in negative direction. In case of fruit width and fruit weight same sign of dominant (h) and dominant  $\times$  dominance (l)components indicated involvement of complementary gene action for these traits. The analysis of TSS revealed the significance of j and l components, with the h component showing significance in the opposite direction of l, suggesting the involvement of duplicate gene action for TSS in the cross between DWM-45 and Arka Manik (Table 3). Similarly, Maragal et al. (2019) reported the involvement of duplicate gene action for TSS in watermelon. These results align with previous studies by Jatothu et al. (2013) who also observed the involvement of polygenes and non-allelic gene action in controlling quantitative traits.

The study provides valuable insights into the genetic control of various fruit traits in watermelon. Fruit shape is controlled by a single dominant gene, fruit stripe and interstripe colour are monogenically inherited with independent inheritance. The presence of stripes on the fruit was dominant over the absence of stripes. Similarly, flesh colour at different regions segregates independently with monogenic inheritance. Seed coat colour was governed by a single dominant gene, with black being dominant over yellowish white. Fruit quality traits such as fruit length, width, weight, and Total Soluble Solids (TSS) were not governed by simple Mendelian segregation but involve complex gene interactions, including epistasis and duplicate gene action.

This study has proven to be invaluable in assessing gene action and understanding the genetics of different fruit quality traits in watermelon. By gaining deeper insights into these mechanisms, we can effectively enhance and optimize

Table 2 Means and standard errors of six generations of watermelon fruit quality traits in the cross DWM-45 × Arka Manik

Trait	DWM-45 (P <sub>1</sub> )	Arka Manik (P <sub>2</sub> )	$F_1$	$F_2$	$BC_1P_1$	$BC_1P_2$
Fruit length (cm)	$9.51 \pm 0.27$	$17.54 \pm 0.49$	$17.79 \pm 0.67$	$14.04 \pm 0.72$	$10.83 \pm 0.26$	$17.11 \pm 0.56$
Fruit width (cm)	$9.57\pm0.20$	$15.73 \pm 1.0$	$17.70\pm0.51$	$13.71 \pm 0.73$	$10.86\pm0.10$	$14.65 \pm 0.20$
Fruit weight (kg)	$0.81\pm0.01$	$2.43 \pm 0.11$	$2.66\pm0.13$	$1.52 \pm 0.035$	$1.24 \pm 0.21$	$2.19\pm0.34$
TSS (°B)	$2.87 \pm 0.12$	$10.66 \pm 0.50$	$4.62 \pm 0.48$	$3.96 \pm 0.57$	$3.44 \pm 0.13$	$5.05 \pm 0.83$

F<sub>1</sub>, first filial generation; F<sub>2</sub>, second filial generation; BC<sub>1</sub>P<sub>1</sub>, backcross with DWM-45; BC<sub>1</sub>P<sub>2</sub>, backcross with Arka Manik.

Table 3 Estimates of gene effects based on scaling test, type of epistasis of watermelon fruit quality traits in the cross DWM-45 × Arka Manik

Parameter	Fruit length (cm)	Fruit width (cm)	Fruit weight (kg)	TSS (°B)
A	-1.15 (0.72)	1.95 (0.71)	2.46** (0.13)	0.53 (0.19)
В	-1.88* (0.84)	-2.08* (0.83)	-1.064 (0.19)	5.10** (0.21)
C	-1.10 (1.25)	-1.85 (1.24)	-0.57 (0.31)	3.92** (0.31)
D	-1.09 (0.47)	2.04* (0.48)	1.18 (0.13)	-0.01 (0.16)
m	80.94** (0.16)	77.52** (0.16)	26.85** (0.05)	65.08** (0.05)
d	-6.97** (0.35)	-7.17** (0.35)	-6.42** (0.08)	-7.70** (0.11)
h	2.39** (1.09)	0.65 (1.09)	0.62 (0.28)	-0.40 (0.34)
i	1.09 (0.95)	-2.04* (0.96)	-1.18 (0.25)	0.01 (0.32)
j	-0.83 (0.45)	-3.50** (0.44)	-3.12** (0.08)	3.76** (0.13)
1	-1.84 (1.88)	0.86 (1.88)	0.95 (0.45)	2.15* (0.56)
Type of epistasis	Duplicate	Complementary	Complementary	Duplicate

Standard errors in parenthesis; Total Soluble Solids (TSS); mean (m), additive (d); dominance (h); additive  $\times$  additive (i); additive  $\times$  dominance (j) and dominance  $\times$  dominance (l); Significant at \*P<0.05, \*\*P<0.01.

breeding for desirable fruit quality traits in watermelon. Along with this, the application of molecular mapping to analyse various fruit quality traits and subsequently employing marker-assisted selection will be very effective to fasten the quality breeding in watermelon. Furthermore, findings of this study will be helpful to use DWM-45 as a resistance source to incorporate resistance in elite cultivar without affecting its quality traits.

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