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Inheritance of fruit colour, fruit ridge pattern and related traits through generation mean analysis in bitter gourd (*Momordica charantia* L.)

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Abstract

Analysis of generation means in P_1, P_2, F_1, F_2, B_1 and B_2 generations of two bitter gourd crosses viz., IIHR Sel-5-8 × IIHR-144-1 and Arka Harit × IIHR-144-1 were performed to determine the nature of gene action governing fruit traits. All the parents and generations differed significantly for the observed traits. For the cross IIHR Sel-5-8 × IIHR-144-1, Chi-square tests in the segregating generations for fruit colour and fruit ridge pattern were fitted well with the Mendelian phenotypic ratio of 3:1. This indicates that green fruit colour of bitter gourd is governed by single dominant gene and is dominant over white fruit colour. Fruit ridge pattern indicated that the discontinuous ridge of bitter gourd is also governed by monogenic dominant gene and is dominant over continuous ridge. All the quantitative traits studied (peduncle length, fruit length and fruit girth) showed non-allelic gene interaction in both the crosses indicating presence of epistatic gene action. Nature of gene action varied with the character and among crosses and both additive and non-additive gene action was involved in expression of the characters having the duplicate epistasis. This indicates use of reciprocal recurrent selection for improvement of these traits and also the possibilities of arriving at better transgressive segregants by selfing the hybrids developed using such selected materials.

Key words: Fruit colour, Inheritance, Generation mean analysis, *Momordica charantia*, Epistasis.

Introduction

Bitter gourd (*Momordica charantia* L.) is an important cucurbitaceous crop grown in India. It is mainly grown for its edible fruits, both in rainy and spring-summer seasons in north India and throughout the year in south India. Bitter gourd is well known for its therapeutic properties and more attention is now being focused on its hypoglycemic properties. It is used for reduction of blood sugar levels in the treatment of type-2 diabetes due to its anti-diabetic properties mainly because of the presence of charantin, saponin, momordicin *etc*. (Behera et al. 2010). India is considered as the primary centre of diversity of bitter gourd and China as the secondary centre of diversity (Grubben 1977). Since immature fruits are sliced during the preparation of various cuisines, exceptional internal fruit quality and uniform green peel colour are desirable. But only a few commercial varieties and hybrids exist in the market. The fruit colour and shape decides the demand in market and consumer preference varies from region to region. Green-fruited types are in high demand in southern China, while white-fruited types are preferred in central China (Dalamu et al. 2012). Dark green to glossy green fruits are preferred in northern India; whereas, in South India consumers prefer dark green long fruits having thick rind with less seeds. Long white fruits are mainly preferred in Kerala state and small dark green fruited types are preferred in eastern parts of the country. Hence, a precise knowledge on gene action responsible for the inheritance fruit colour and fruit related traits are a prerequisite to identify a suitable breeding methodology in bitter gourd improvement programmes. Inheritance of fruit color in the bitter gourd have been studied by various workers and reported that the green color was monogenically dominant over the white color (Dalamu et al. 2012, Hu et al. 2002, Srivastava and Nath 1972) and Liu et al. (2005) reported high heritability of fruit colour controlled by one pair of nuclear genes. Hu et al. (2002) also found the color of some fruits from F1 and backcross populations was lighter than that of the parents. They speculated the light green colors were probably affected by incomplete dominance or modifiers. Immature fruits are green or white and a broad spectrum for color of the green fruit ranges from light green to very dark green.

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There are many pigments in bitter gourd fruits responsible for the colour such as chlorophylls, carotenoids, flavonoids, etc. (Behera et al. 2013, Gross 1991, Rodriguez et al. 1976). The interaction of these pigments is responsible for the color changes at different developmental stages of the fruit. The pigments 'chlorophyll a' and 'chlorophyll b' are the major ones which affect the external color of bitter gourd fruits. The color becomes orange when fruits are mature as chlorophyll concentrations reduce and carotenoids, flavonoids concentrations accumulate (Gross 1991).

Being originated in India, wide variations could be found for almost all characters including quantitative and qualitative traits like fruit colour and fruit ridge. Kim et al. (1990) reported that small fruit was partially dominant over large fruit in population developed from a cross between large fruited (*M. charantia* var. *charantia / maxima*) × small fruited (*M. charantia* var. *muricata / minima*). The fruit length was incompletely dominant and controlled by a minimum of five genes (Zhang et al. 2006). The fruit ridge (discontinuous ridge) of bitter gourd was governed by a single dominant gene (Dalamu et al. 2012). Fruit length, average fruit weight and number of fruits per vine are controlled by additive factors and have direct positive effects on fruit yield (Dey et al. 2005). Hence, this study was carried out to understand the inheritance of qualitative fruit traits based on chi square test and nature and magnitude of gene actions involved in the inheritance of quantitative traits in bitter gourd through generation mean analysis.

Materials and Methods

Three parents (IIHR Sel-5-8, Arka Harit, IIHR-144-1) were selected for the crossing programme which were genetically diverse with respect to fruit characters both qualitatively and quantitatively. IIHR Sel-5-8 is having long creamy white fruits with continuous ridge, Arka Harit is a medium long green fruited type with continuous ridges, whereas IIHR-144-1 (belongs to *M. charantia* var. *muricata*) is a small dark green fruited type with discontinuous (warty) ridges. Populations of two crosses namely, IIHR Sel-5-8 × IIHR-144-1 and Arka Harit × IIHR-144-1 were developed for the genetic analysis. The experiments were conducted in the experimental field of Division of Vegetable Crops, ICAR-IIHR, Bengaluru during December 2016 to March 2018. The parents were planted and crossing was done in December 2016 to April 2017 to produce F_1 seeds. In the following season (from June 2017 to October 2017) F_1 was selfed to produce F_2 seeds and back crossed to parents, i.e. P_1 (IIHR Sel-5-8, Arka Harit) and P_2 (IIHR-144-1) to get B_1 and B_2 respectively. Six generations (P_1, P_2, F_1, F_2, B_1 and B_2) of both crosses were evaluated (during November 2017 to March 2018) separately in randomized complete block design (RCBD) with three replications to study the inheritance pattern *via.*, chi-square test and generation means analysis.

Seeds of the six generations for both families were sown in 98-cell plug-trays using coco-peat as a growing medium, in the net house at Division of Vegetable Crops, ICAR- IIHR, Bengaluru in November 2017. The seedlings were ready for transplanting 15 days after germination (two true- leaf stage) and transplanted in the main field in raised beds, covered by white polythene mulch, at spacing of 150 cm between beds and 50 cm between plants. All the cultural practices were followed to ensure a good crop. Data were recorded on 20 plants of each parent and F_1, 30 plants each in B_1 and B_2 generations, respectively and 100 plants in F_2 per replication for five morphological traits *viz.,* fruit colour, fruit ridge, peduncle length, fruit length and fruit girth at different stages of crop growth. Inheritance on fruit colour and ridge where studied in the cross IIHR Sel-5-8 × IIHR- 144-1, total number of plants (all three replications) falling into different classes, 60 plants of each parent and F_1, 90 plants each in B_1 and B_2 generations, respectively and 300 plants in F_2, were counted and subjected to chi-square analysis for goodness of fit to various classical Mendelian ratios as suggested by Panse and Sukhatme (1985). Generation mean analysis was carried out on three quantitative traits *viz.,* peduncle length, fruit length and fruit girth for both the crosses (IIHR Sel-5-8 × IIHR-144-1 and Arka Harit × IIHR-144-1). For generation mean analysis, six-parameter model for estimation of various genetic components proposed by Hayman (1958) was adopted. Prior to that, the data were tested for the adequacy of the additive-dominance model using the Scaling Test (Mather 1946) and significance of any one of the scales was taken to indicate the presence of non-allelic interactions. The estimates of mean gene effects, such as mean (*m*), additive effect (*d*), dominant effect (*h*), additive × additive (*i*), additive × dominance (*j*) and dominance × dominance (*l*) type for gene interaction were fitted to the data. Statistical analysis was carried out using Generation Mean Analysis (OPSTAT, CCS HAU, Hisar, Haryana).

Results and Discussion

Fruit colour: The fruit colour of all the F_1 plants in both the crosses were dark green indicating that green colour was inherited as a dominant trait (Table 1), while the F_2 populations segregated for fruit colour. Out of total 300 plants in F_2 population of cross IIHR Sel-5-8

Table 1: Single-locus goodness of fit test for fruit colour in the cross IIHR Sel-5-8 × IIHR-144-1 of bitter gourd.

Generation	Total	Green	White	Expected	χ^2	P value
P1	60	-	60	-	-	-
P2	60	60	-	-	-	-
F1	60	60	-	-	-	-
F2	300	227	73	3:1	0.07	0.79
B1	90	48	42	1:1	0.40	0.52
B2	90	90	-	-	-	-

× IIHR-144-1, 227 plants were green fruited and 73 plants had white fruits (Table 1). The observed frequency of F_2 plants fitted well in the expected ratio of 3 green: 1 white as evident from the non-significant ÷2values of 0.07 (P = 0.79). The B_1 population segregated into 48 green and 42 white fruited plants. The observed frequency of B_1 plants fitted in the expected ratio of 1 green: 1 white fruited plant with non-significant ÷2 values of 0.40 (P value 0.52). The B_2, generation had all green fruits. Inheritance pattern of a trait determines the breeding methodologies to be followed for improvement of the trait. In our study, ÷2 analysis for goodness of fit indicated that fruit colour is inherited as single gene with green colour dominant over white. This study confirmed the earlier reports (Dalamu et al. 2012, Miniraj et al. 1993, Srivastava and Nath 1972, Suribabu et al. 1986, Vahab and Peter 1993) which stated green fruit colour is dominant over white fruit colour in bitter gourd.

Fruit ridge: Inheritance pattern of fruit ridge was studied in the same crosses. All F_1 plants showed discontinuous ridge, indicating that this trait was inherited as in a dominant fashion. Out of total 300 plants in F_2 population, 222 segregated as discontinuous ridge and 78 as continuous ridge fitting into the expected ratio of 3:1 (÷^{2} = 0.16; P = 0.68; Table 2). The B_1 population segregated into 49 discontinuous and 41 continuous ridged fruited plants. The observed frequency of B_1 plants fitted well in the expected ratio of 1 discontinuous: 1 continuous ridged fruited plant (÷^{2} = 0.71; P = 0.39; Table 2). Hence, *÷2* analysis for goodness of fit indicated that fruit ridge is inherited as single gene with discontinuous ridged fruits is dominant over continuous ridged fruits. This study also confirmed the earlier report of Dalamu et al. (2012); Srivastava and Nath (1972).

Table 2: Single-locus goodness of fit test for fruit ridge in the cross IIHR Sel-5-8 × IIHR-144-1 bitter gourd.

Generation	Total	Discontinuous	Continuous	Expected	χ^2	P value
P1	60	-	60	-	-	-
P2	60	60	-	-	-	-
F1	60	60	-	-	-	-
F2	300	222	78	3:1	0.16	0.68
B1	90	49	41	1:1	0.71	0.39
B2	90	90	-	-	-	-

Cross 1 (IIHR Sel-5-8 × IIHR-144-1): Significant (P d" 0.05) mean differences were detected between the two parental lines for all traits (Table 3). P_1 (*Momordica charantia* var. *charantia*) consistently possessed higher peduncle length (9.38 ± 0.19 cm), fruit length (12.27 ± 0.19 cm) and fruit girth (13.33 ± 0.29 cm) than P_2 (*Momordica charantia* var. *muricata*). For most traits, F_1 generation means were higher than the mid-parent value. The F_1 generation was intermediate to parental lines for fruit girth. B_1 and B_2 progenies resembled their respective recurrent parent with respect to all traits and F_2 individuals varied drastically for the yield-related characteristics examined. Individual B_1 and F_2 progeny reached or transgressed the phenotypic extremes of either parent for most traits.

Significance of the scaling test (Table 4) strongly suggest that the quantitative traits studied such as peduncle length, fruit length and fruit girth in cross 1 was not under the control of simple additive or dominant gene effects, but interaction (non-allelic interaction) was also seemed to be operative in its inheritance, i.e., phenotypic expression of genes at one locus may be influenced by genes present at other loci. The estimate of mean effects was highly significant, also exhibited the presence of significant additive gene effects. Dominance effects were significant for all three traits.

Among the main gene effects, dominance component was of higher magnitude than additive component for all three traits. The dominance × dominance (l) component of epistasis was highly significant for peduncle length, also the magnitude exceeded the additive (d) and dominance (h) gene effects suggesting its importance. The dominance (h) component of genetic variation was observed to be highly significant and positive in the inheritance of both fruit length and fruit girth suggesting that dominance was in the direction of more fruit length and fruit girth. All three epistatic gene effects, additive × additive (i) additive × dominance (j) and dominance × dominance (l), were highly significant for fruit length whereas, additive × additive (i) and additive × dominance (j) were significant for fruit girth. Opposite signs of *h* and *l* components revealed duplicate epistasis and was present for all the three traits *viz*, peduncle length, fruit length and fruit girth.

The results suggest that both additive and dominant effects were equally important though non-additive portion is marginally higher which indicates the involvement of both additive and dominant genes for the expression of these traits. So, a breeding method which could exploit both kinds of gene action would be appropriate for the improvement of these traits. Duplicate epistasis was also found in this cross for fruit length; hence reciprocal recurrent selection will be the appropriate strategy for the improvement of these traits.

Cross 2 (Arka Harit × IIHR- 144-1): Significant (P d” 0.05) mean differences were observed between the two parental lines for all traits (Table 5). P_1 (*Momordica charantia* var. *charantia*) consistently possessed higher primary peduncle length (5.88 ± 0.15 cm), fruit length (8.42 ± 0.15 cm) and fruit girth (9.76 ± 0.19 cm) than P_2 (*Momordica charantia* var. *muricata*). F_1 generation means were higher than the mid-parent value for all three traits. The F_1 generation was intermediate to parental lines for fruit length and fruit girth. Here also, the B_1 and B_2 progenies resembled their respective recurrent parent with respect to all traits and F_2 individuals varied drastically. Ttransgressive seggregants were observed in individual F_2 and B_1 progeny that reached the phenotypic extremes of either parent for fruit length and fruit girth. Scaling tests (Table 6) were significant for all the three traits studied which suggest that the traits were not under the control of simple additive or dominant gene effects, but interaction (non-allelic interaction) was also seemed to be operative in its inheritance. The estimate of mean effects was highly significant, also exhibited the presence of significant additive (*d*) and dominant (*h*) gene effects.

Among the main gene effects, dominance component was of higher magnitude than additive component for peduncle length, whereas additive component dominates in rest of the two traits *viz*, fruit length and fruit girth. The epistatic components were of matching magnitudes with dominance component for peduncle length suggesting their equal importance, especially the additive x additive and dominance x dominance components of epistasis. For the traits fruit length and fruit girth the additive x dominance component of epistasis were significant and their magnitude is matching towards additive component of main gene effect. It suggests the involvement of both additive and dominant genes for the expression of these traits in this cross also. So, a breeding method which could exploit both kinds of gene action would be appropriate for the improvement of these traits. Mishra et al. (2012) also reported the presence of duplicate type of epistasis with additive × dominance (*j*) and dominance × dominance (*l*) type of gene action for fruit length and fruit diameter in bitter gourd. In similar lines, Dalamu et al. (2012); Sirohi and Choudhury (1980) also reported greater involvement of dominance and dominance × dominance gene action for fruit length, fruit weight in bitter gourd. Celine and Sirohi (1998) found that fruit diameter was controlled by additive and additive × additive gene action.

Gene action studies of genetic parameters governing different traits have been useful in developing breeding strategies for incorporating genes for trait improvement. The results of this study demonstrated that green fruit colour and discontinuous ridges are under the control of dominant genes. The gene effects obtained by generation mean analysis differed with the different genetic backgrounds of the inbred crosses. Involvement

Table 3: Generation mean for different fruit traits in the cross IIHR Sel-5-8 × IIHR-144-1 of bitter gourd.

Character	P_1	P_2	MP	F_1	F_2	B_1	B_2
Peduncle length (cm)	9.38 ± 0.19	4.69 ± 0.09	7.03	4.45 ± 0.13	4.75 ± 0.09	5.92 ± 0.16	3.21 ± 0.10
Fruit length (cm)	12.27 ± 0.19	5.86 ± 0.14	9.06	9.86 ± 0.14	7.09 ± 0.10	9.83 ± 0.16	7.36 ± 0.17
Fruit girth (cm)	13.33 ± 0.29	6.73 ± 0.13	10.03	10.82 ± 0.14	8.17 ± 0.09	10.41 ± 0.14	8.26 ± 0.15

Table 4: Gene effects and standard error for different fruit traits in the cross IIHR Sel-5-8 × IIHR-144-1 of bitter gourd.

Character	A	B	C	D	m	d	h	i	j	l
Peduncle length	1.98* ± 0.39	2.71* ± 0.27	3.95* ± 0.48	0.37 ± 0.26	4.75* ± 0.09	2.71* ± 0.19	-3.33* ± 0.54	-0.74 ± 0.51	0.72 ± 0.44	5.44* ± 0.90
Fruit length	2.48* ± 0.40	1.01* ± 0.39	9.46* ± 0.56	-2.99* ± 0.31	7.09* ± 0.10	2.47* ± 0.23	6.78* ± 0.65	5.98* ± 0.62	-1.46* ± 0.52	-2.48* ± 1.09
Fruit girth	3.34* ± 0.43	1.03* ± 0.36	9.03* ± 0.57	-2.33* ± 0.28	8.17* ± 0.09	2.14* ± 0.21	5.44* ± 0.61	4.66* ± 0.56	-2.32* ± 0.52	-0.29 ± 1.01

Table 5: Generation mean for different fruit traits in the cross Arka Harit × IIHR-144-1 of bitter gourd.

Character	P_1	P_2	MP	F_1	F_2	B_1	B_2
Peduncle length (cm)	5.88 ± 0.15	4.69 ± 0.09	5.28	5.35 ± 0.13	4.46 ± 0.06	5.89 ± 0.09	4.60 ± 0.07
Fruit length (cm)	8.42 ± 0.15	5.86 ± 0.14	7.14	8.59 ± 0.17	7.74 ± 0.10	9.13 ± 0.15	6.24 ± 0.09
Fruit girth (cm)	9.76 ± 0.19	6.73 ± 0.13	8.24	9.45 ± 0.16	8.71 ± 0.11	10.33 ± 0.13	7.55 ± 0.08

Table 6: Gene effects and standard error for different fruit traits in the cross Arka Harit × IIHR-144-1 of bitter gourd.

Character	A	B	C	D	m	d	h	i	j	l
Peduncle length	-0.57* ± 0.27	0.83* ± 0.22	3.42* ± 0.40	-1.58* ± 0.17	4.46* ± 0.06	1.29* ± 0.12	3.22* ± 0.38	3.16* ± 0.35	1.41* ± 0.29	-2.89* ± 0.63
Fruit length	-1.25* ± 0.39	1.97* ± 0.28	0.52 ± 0.58	0.10 ± 0.27	7.74* ± 0.10	2.89* ± 0.18	1.25* ± 0.59	-0.20 ± 0.55	3.23* ± 0.42	0.92 ± 0.93
Fruit girth	-1.46* ± 0.37	1.09* ± 0.27	0.54 ± 0.60	-0.45 ± 0.27	8.71* ± 0.11	2.79* ± 0.16	2.11* ± 0.58	0.91 ± 0.55	2.56* ± 0.39	-1.28 ± 0.88

of duplicate epistasis indicates the selection for high yielding bitter gourd with long fruits becomes complicated. Reciprocal recurrent selection will be the appropriate strategy for the improvement of these traits. Further identification of marker trait associations for such complex traits will help to hasten the bitter gourd improvement for such traits toward desired directions.

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करेला के दो संकरणों जैसे–आई.आई.एच.आर. सेलेक्शन–5–8 x आई.आई.एच.आर.–144–1, अर्का हरित x आई.आई.एच.आर.–144–1 के पी.1, पी.2, एफ1, एफ2, बी.1 एवं बी.2 पीढ़ियों के पीढ़ी माध्य विश्लेषण में फल गुणों को नियंत्रित करने वाले जीन प्रक्रियाओं को सुनिश्चित करने के लिये अध्ययन किया गया। सभी पितषें एवं पीढ़ियों में सार्थक भिन्नता फल के रंग के व फल के धारीदार स्वरूप संकरण संयोज्य आई.आई.एच.आर. सेलेक्शन–5–8 x आई.आई.एच.आर.–144–1 में काई स्क्येर परीक्षण में किया गया जिसमें मेण्डेलियन बाह्य दृश्य प्रारूप अनुपात 3:1 के अनुरूप था। इससे स्पष्ट हुआ कि करेला में फल का रंग एकल प्रभावी जीन से नियंत्रित होता है एवं हरा रंग, सफेद रंग पर प्रभावी है। फल के धारीदार स्वरूप से स्पष्ट हुआ कि धारीदार का अक्रामक स्वरूप भी एकजीनी प्रभावी जीन से नियंत्रित होता है जो क्रमवार धारी पर प्रभावी है। सभी मात्रात्मक गुणों (फलवृंत की लम्बाई, फल की लम्बाई एवं फल व्यास) के अध्ययन से स्पष्ट हुआ कि अयुग्म–जीन परस्पर क्रिया, दोनों संकरणों में इपीस्टाटिक जीन क्रिया की मौजूदगी से है। जीन क्रिया की प्रकृति, गुणों के साथ विविध है एवं संकरणों तथा योज्य व अयोज्य जीन क्रिया गुणों के प्रदर्शन में सम्मिलित है जो डुप्लीकेट इपीस्टासिस को दर्शाता है। पारस्परिक आवर्तक चयन, इन गुणों के उन्नयन के लिए प्रासंगिक है एवं उत्तम उत्क्रामी पृथक्करणों में रोगों के चयन की सम्भावना को स्पष्ट करता है जो संकरों में प्रयुक्त सामग्री को स्व निषेचन कर किया गया है।

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