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Original Research Paper

Genetics of inheritance of gynoecy, fruit ridge pattern and validation of SNP marker linked to gynoecy in bitter gourd (*Momordica charantia* L.)

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ABSTRACT

An experiment was conducted to study the inheritance of gynoecy and fruit ridge pattern of bitter gourd fruits during 2018–2021. The results revealed that a recessive gene is responsible for expression of gynoecy, and single dominant gene is responsible for expression of discontinuous ridge pattern in both the crosses (IIHRBTGy-491×IIHR Sel-19-1 and IIHRBTGy-491×IIHR Sel-78-4). GTFL-1 SNP marker differentiated between the gynoecious and monoecious lines. The SNP marker showed a clear polymorphism between gynoecious parent (IIHRBTGy-491) and monoecious parents (IIHR Sel-19-1 and IIHR Sel-78-4) giving a 392 bp band in monoecious parents and F_1, but in gynoecious parent, it was not amplified indicating the dominant behaviour of the marker.

Keywords: Bitter gourd, gynoecy, genetics, marker, ridge pattern

INTRODUCTION

Bitter gourd (*Momordica charantia* L.), belongs to the family Cucurbitaceae, order Cucurbitales and tribe Joliffeae. The genus *Momordica* comprises of 60 species widely distributed in Africa, while, in Asia and Australia, only 12 species were found (Schaefer & Renner, 2011). *Momordica charantia* is divided into two botanical varieties *viz*., *M. charantia* var. *charantia*, which produced huge fruits, and M. *charantia* var. *muricata*, which produced small, spherical fruits (Chakravarty, 1990). Based on both historical literature and molecular analyses, eastern India is considered to be the primary centre of bitter gourd diversity (Kole et al., 2020). China is considered as the secondary centre of diversity (Grubben, 1977). Plants with a complete gynoecious flowering habit in bitter gourd have been reported (Ram et al., 2002; Behera et al., 2006; Varalakshmi et al., 2014). Gynoecious line in hybrid production is a boon as it eliminates manual emasculation and pollination and also cost-effective and easier way to exploit hybrid vigour in it. The gynocious sex forms are rare and their early detection is difficult as the sexual differentiation of floral primordia depends on climatic influences and hormonal balance in primordial tissue. Matsumura et al. (2014) reported GTFL-1 SNP loci genetically linked to the putative gynoecious locus at 5.46 cM distance, which was transformed to a traditional DNA marker using invader assay technology.

MATERIALS AND METHODS

The sib-mated seeds of gynoecious bitter gourd germplasm, IIHRBTGy-491 (P_1: small/dark green fruit/continuous ridge) and two monoecious lines namely IIHR Sel-19-1 and IIHR Sel-78-4 (P_2: green, discontinuous ridge, medium-long fruit) used as parents to develop, F_1, F_2 and back cross generations for studying segregating pattern for gynoecy and fruit ridge pattern. Ten plants of each P_1, P_2 and F_1, 20 plants each of B_1 and B_2 populations, whereas, 87 and 77 plants of F_2 population from the respective crosses were evaluated in a randomized complete block design with three replications at ICAR-Indian Institute of Horticultural Research, Bengaluru during 2018-2021.

Segregation ratios of monoecy (M) and gynoecy (G) and ridge pattern were subjected to chi-square test and the goodness of fit to various traditional Mendelian ratios with the assumed phenotypic ratios of F_2 and back cross progenies were determined as recommended by Panse & Sukhatme (1985). The total genomic DNA

Table 1: PCR programme used for GTFL-1 marker amplification

Steps	Temperature (°C)	Time	Cycles
Initial denaturation	95.0	4 min	
Denaturation	94.0	30 sec	35
Annealing	55.0	30 sec	
Primer extension	72.0	1 min	
Final extension	72.0	5 min	
Hold	4.0	-	

was isolated using Doyle & Doyle's modified CTAB (Cetyl trimethyl ammonium bromide) technique (1990). The purified DNA was quantified and the quality of the DNA was assessed using spectrophotometer (Nanodrop 8000, Thermo Scientific). The chi-square ($\chi 2$) calculation was used to determine the goodness of fit of the genotyping of F_2 plants with the expected Mendelian ratio (Panse & Sukhatme, 1985). Amplified products were photographed using a gel documentation system under equal magnification and size of all the alleles was calculated based on the band's position relative to the 100 bp ladder. Primer set used were: 5'-AATTGCCTATAAGAAACCCTGTC and 5'-ATGAGAGCATGGTCATCGCAAG.

RESULTS AND DISCUSSION

Plants with 100 per cent pistillate flowers were defined as gynoecious plant, while all other plants were classified as monoecious. The number of segregants into monoecious and gynoecious were 196 and 64 out of 260 F_2 plants, respectively in the cross IIHRBTGy-491×IIHR Sel-19-1, which was best fitting to 3:1 ratio with a chi-square value 0.12 at probability 0.88 (Table 2). In the test cross with gynoecious parent (B_1 population), out of 60 plants, 29 were monoecious and 31 were gynoecious fitting to 1:1 ratio (x^2 =0.06; P=0.79). In the test cross with monoecious parent, all the plants were monoecious.

The number of segregants in monoecious and gynoecious were 172 and 61, out of 233 F_2 plants, respectively in the cross IIHRBTG-49×IIHR Sel-78-4, fitting to 3:1 ratio with a chi-square value 0.17 and probability 0.67. In the test cross with gynoecious parent (B_1 population), out of 60 plants, 33 were monoecious and 27 were gynoecious fitting to 1:1 ratio (x^2 =0.6; P =0.43) ratio which was similar confirming simple Mendelian segregation with single recessive gene control of gynoecy.

In both the crosses (IIHRBTGy-491×IIHR Sel-19-1 and IIHRBTGy-491×IIHR Sel-78-4), the segregation of F_2 was 3:1 and for backcross population (with gynoecious parent) showed 1:1 ratio, which indicated that gynoecism in the line IIHR BTGy-491 was under the control of a single recessive gene (gy-1). This study confirmed the reports of Behera et al. (2009), Ram et al. (2006), Mishra et al. (2015) and Matsumura et al. (2014). Poole & Grimball (1939) discovered that melon had a similar inheritance pattern for gynoecism. In contrast, Iwamoto & Ishida (2006) reported that gynoecism in bitter gourd was partially dominant.

Table 2 : Phenotypic segregation of gynoecy and monoecy in bitter gourd

Cross	Population	Total number of plants	Observed frequency		Observed ratio	Chi square value	P value (5%)
			Monoecious	Gynoecious			
IIHRBTGy-491×	F_2	260	196	64	3:1	0.12	0.88
IIHR Sel-19-1	B_1	60	29	31	1:1	0.06	0.79
	B_2	60	60	0	-	-	-
IIHRBTGy- 491×	F_2	233	172	61	3:1	0.17	0.67
IIHR Sel-78-4	B_1	60	33	27	1:1	0.6	0.43
	B_2	60	60	0	-	-	-

Table 3 : Phenotypic segregation ratio for ridge pattern in bitter gourd

Cross	Population	Total number of plants	Observed frequency		Observed ratio	Chi square value	P value (5%)
			Discontinuous ridge	Continuous ridge			
IIHRBTGy-491×	F2	260	191	69	3:1	0.32	0.56
IIHR Sel-19-1	B1	60	34	26	1:1	1.06	0.30
	B2	60	60	0	-	-	
IIHRBTGy-491×	F2	233	183	50	3:1	1.55	0.21
IIHR Sel-78-4	B1	60	35	25	1:1	0.66	0.19
	B2	60	60	0	-	-	

Whereas, Cui et al. (2018) identified two closely linked loci for gynoecy (gy1.1 and gy1.2) at the end of linkage group MC01 in gynoecious bitter gourd line 'K44'.

Classical mendelian segregation of ridge pattern in bitter gourd

The number of segregants in fruits with discontinuous and continuous ridge were 191 and 69 out of 260 F_2 plants, respectively in IIHRBTG-49 × IIHR Sel-19-1, which best fitted to 3:1 ratio with a chi-square value 0.32 at probability 0.56 (Table 3). In the test cross with continuous ridge parent (B_1 population) out of 60 plants, 34 showed discontinuous ridge and 26 showed continuous ridge pattern fitting to 1:1 ratio (x^2 =1.06; P=0.30), whereas, in the test cross with discontinuous ridge parent, all the plants showed fruits with discontinuous ridge pattern.

The number of segregants in discontinuous and continuous ridge fruits were 183 and 50 out of 233 F_2 plants, respectively in the cross IIHRBTG-49× IIHR Sel-78-4, which was best fitting to 3:1 ratio with a chi-square value 1.55 and probability 0.21. In the test cross with continuous ridge parent (B_1 population), out of 60 plants, 35 showed discontinuous ridge and 25 showed continuous ridge pattern fitting to 1:1 ratio (x^2 =0.66; P =0.19), whereas, in the test cross with discontinuous ridge parent, all the plants showed fruits with discontinuous ridge pattern.

χ2 analysis for goodness of fit indicated that discontinuous ridge pattern in the two monoecious parents IIHR Sel-19-1 and IIHR Sel-78-4 was under the control of a single dominant gene and continuous ridge pattern of gynoecious IIHR BTGy-491 line was under the control of single recessive gene. Kumari et al. (2015) also observed similar inheritance pattern of tubercles and curviness of bitter gourd fruits, as also confirmed by Rathod et al. (2019).

GTFL-1 marker produced amplification in both monoecious parents and F_1 at 392 bp, whereas, in gynoecious parent it was not amplifying (Fig. 1). For genotyping of the F_2 population, 260 and 230 F_2 DNA samples were carried out with GTFL-1 markers. In the first cross (IIHRBTGy- 491× IIHR Sel-19-1), out of 260 F_2 plants, 184 plants showed monoecious nature with band size of 392 bp and 57 plants showed no banding pattern indicating that were gynoecious. Recombination was also observed wherein 15 phenotypic monoecious plants did not amplify at

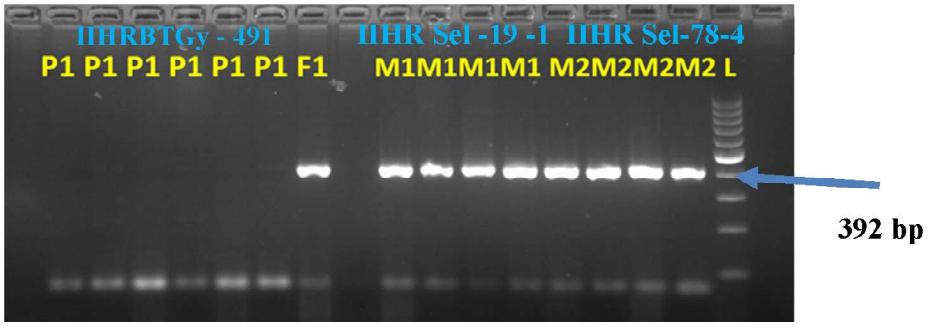

Fig. 1 : Polymorphism between gynoecious parent, IIHRBTGy-491 and two monoecious parents (M1-IIHR Sel 19-1 and M2-IIHR Sel-78-4) with GTFL-1 marker

Table 4 : Genotypic segregation pattern of GTFL-1 marker in both the F_2 populations ($\chi 2$ analysis)

Cross	Observed frequency		Observed ratio	Chi square value	P value (5%)	Number of recom-binants	Recombi-nation-frequency (%)	Co-segregation with trait (%)
	G	M						
IIHRBTGy-491× IIHR Sel-19-1	57	184	3:1	0.23	0.62	19	7.31	92.69
IIHRBTGy-491× IIHR Sel-78-4	49	168	3:1	0.67	0.41	13	5.65	94.35

392 bp and 4 phenotypic gynoecious plants amplified at 392 bp with recombination frequency of 7.31 (Table 4).

In the second cross (IIHRBTGy-491×IIHR Sel-78-4), out of 230 F_2 plants, 168 plants were monoecious in nature with a band size of 392 bp and 49 plants showed no banding pattern indicating that were gynoecious. Recombination was also observed wherein 13 phenotypic monoecious plants did not amplify at 392 bp with a recombination frequency of 5.65. The data were subjected to chi square analysis for observing segregation pattern of molecular marker in the F_2 population.

Based on polymorphic studies, GTFL-1 marker was analysed in F_2 populations in both the crosses. The goodness of fit in segregation ratio of marker locus was tested using $\chi 2_{(n-1)}$ df against expected ratio of 3:1 for dominant marker. The marker was segregated in expected ratio of 3 monoecious and 1 gynoecious with a chi-square value (0.23) and probability (0.62) in the first cross and chi-square value (0.67) and probability (0.41) in the second cross, respectively. The marker co-segregation with the trait showed 92.69% similarity in the cross, IIHRBTGy-491 x IIHR Sel-19-1 and 94.35% similarity in the cross, IIHRBTGy-491 and IIHR Sel-78-4.

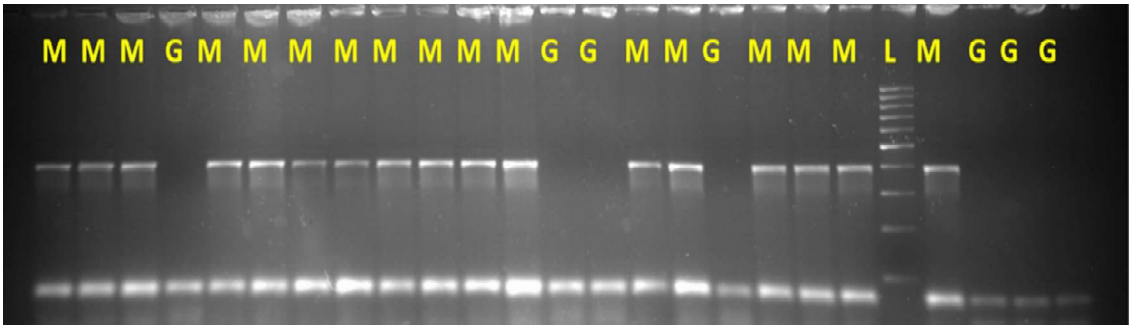

Fig. 2 : Amplification of GTFL-1 in IIHRBTGy-491×IIHR Sel-19-1 F_2 population indicating monoecious (M) and gynoecious (G) banding pattern

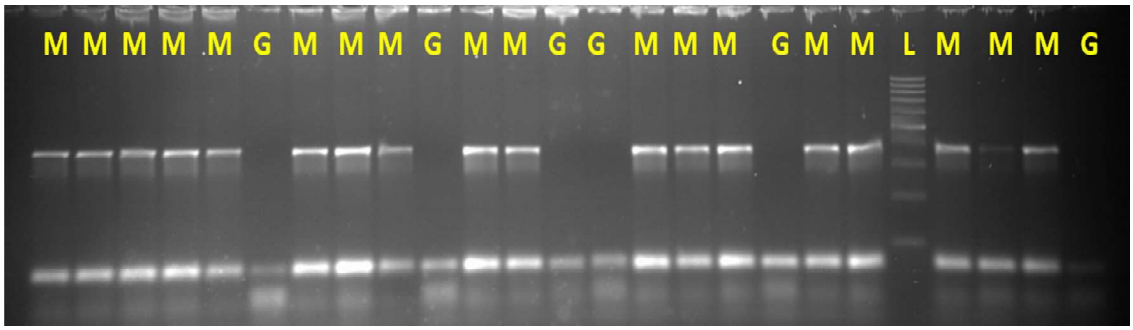

Fig. 3 : Amplification of GTFL-1 in IIHRBTGy-491×IIHR Sel-78-4 F_2 population indicating monoecious (M) and gynoecious (G) banding pattern

The GTFL-1 SNP marker showed clear polymorphism between the parents and also in the F_2 populations (Fig. 2 & 3). This marker was amplified in both monoecious parents and F_1 at 392 bp whereas, in gynoecious parent it was not amplified, indicating the dominant behaviour of the marker. The marker co-segregation with the trait showed 92.69% and 94.35% similarities in both the crosses respectively which is very reliable and can be used for identification of gynoecious lines at an early stage of development for cost effective hybrid seed production. The GTFL-1 marker can further be used for development of gynoecious lines and for fixing the desirable genes in other desirable lines through foreground selection.

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