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RESEARCH ARTICLE

Horticulture

Genetic control of fruit length, external colour and number of fruits per vine in bitter gourd studied using *Charantia* × *Muricata* crosses

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Abstract: The present study was undertaken to ascertain the genetic control of external fruit colour, fruit length, and the number of fruits per vine in bitter gourd using reciprocal crosses of *Momordica charantia* var. *muricata* and *M. charantia* var. *charantia* and to identify suitable lines of *M. charantia* var. *muricata* to be used in bitter gourd improvement programmes. *Muricata* accession collected from Girandurukotte (GK), Sri Lanka, was selected as a suitable accession for crop improvement based on higher gynoecy and consistency of characteristics. Crossing success between *Charantia* and *Muricata* varieties was 100 % indicating cross-compatibility between the two types. External fruit colour, fruit length and the number of fruits per vine of bitter gourd were found to be quantitative traits, potentially controlled by many genes, each with a small effect. The number of fruits per vine was found to show cytoplasmic inheritance, as the F_1 generation recorded fruit numbers per vine similar to those of C*harantia* varieties, when C*harantia* varieties were used as the female parent of the reciprocal crosses made between *Charantia* and *Muricata* varieties. However, the suspected cytoplasmic effect was not useful, as it influenced to reduce the number of fruits per vine. In addition, the number of fruits per vine showed several transgressive segregants in both extremes of the F_2 populations. In bitter gourd improvement programmes, GK *Muricata accession* may be used to improve cultivated *Charantia* varieties with respect to fruit characteristics such as external fruit colour, fruit length and the number of fruits per vine through a breeding program with the directional selection made towards obtaining the desired characters.

Keywords: Bitter gourd, *Charantia* and *Muricata* varieties, fruit length, fruit colour, genetic control, number of fruits per vine.

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is one of the most important vegetables which belongs to the family Cucurbitaceae. Based on fruit size, shape, colour, and surface texture, bitter gourd is classified into two botanical varieties, *viz.*, *M. charantia var. charantia* and *M. charantia var. muricata* (wild progenitor). *Momordica charantia* var. *charantia* has large fusiform fruits, while *M. charantia* var. *muricata* develops small and round fruits with tubercles having tapered ends (Chakravarty, 1990).

Widely cultivated bitter gourd *M. charantia var. charantia* bears 8–10 fruits per vine and has desirable fruit characteristics such as large fruits preferred by farmers and consumers. Though limited in the extent of commercial cultivation and not popular, the small-fruited edible bitter gourd *M. charantia var. muricata* bears a large number of fruits (15–25) per vine and carries field-level resistance to leaf curl viruses.

The fruit length, number of fruits per vine, and fruit colour are important fruit and yield characteristics for

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improving bitter gourd for high yield and consumer preference. Fruit length and the number of fruits per vine are controlled by additive factors and have direct positive effects on fruit yield (Sharma & Bhutani, 2001). Rao *et al.* (2021) also reported that fruit length and the number of fruits per vine are controlled by quantitative trait loci (QTL). Zhang *et al.* (2008) reported that fruit length is incompletely dominant and is controlled by a minimum of five genes. According to Kumari *et al*. (2015), fruit length in the bitter gourd is quantitatively inherited and is controlled by four genes. Fruit colour of bitter gourd (green vs. white) is highly heritable and is controlled by two genes, in which green is dominant over white (Liu *et al*., 2005). A recent finding reported that the expression of chlorophyll has complex interactions and thus the fruit colour of bitter gourd is a quantitative trait (Huang & Hsieh, 2017).

The utilization of potentially important wild bitter gourd varieties in varietal improvement programs has been reported (Behera *et al*., 2008). Thus, hybridization between *M. charantia* var. *muricata* and *M. charantia* var. *charantia* appears to be an effective approach in bitter gourd improvement programmes for fruit length, the number of fruits per vine, and external fruit colour. For this purpose, further studies on the genetic control of fruit characteristics of bitter gourd using crosses between *M. charantia* var. *muricata* and *M. charantia* var. *charantia* will be important. Thus, the objectives of the present study were to identify the best out of five *M. charantia* var. *muricata* accessions collected from different regions in Sri Lanka, and to ascertain the genetic control of important fruit characteristics, *viz.*, fruit length, the number of fruits per vine, and external fruit colour, using direct and reciprocal crosses between *M. charantia* var. *muricata* and *M. charantia* var. *charantia.*

MATERIALS AND METHODS

Identification of promising *Muricata* accessions

The small-fruited bitter gourd accessions (*M. charantia* var. *muricata*) were collected from five different regions in Sri Lanka, *viz.*, Giradurukotte (GK), Polonnaruwa (PN), Dehiatthakandiya (DK), and Hambanthota (HT), representing the Low Country Dry zone, and Muruthalawa (MT) representing the Mid Country Wet zone of Sri Lanka. The accessions were compared in an experiment conducted in a protected house at the Horticultural Crops Research and Development Institute (HORDI), Gannoruwa, Sri Lanka. Thirty plants from each accession were potted with one plant per pot and kept in the protected house in a Randomized Complete Block Design (RCBD), with three replications against the sunlight intensity gradient observed in the protected house. Each accession had 10 pots per replicate.

Morphological characteristics important for bitter gourd crop improvements such as mature vine length, days to flower initiation, number of fruits per vine, and external fruit colour were evaluated to select the best accessions. Green and yellowish-white are the two main fruit colours so that Matale Green and Thinnaweli White varieties were used as the green and yellowish-white fruit colour standards, respectively. Accessions were evaluated over three consecutive seasons (2016 Dry, 2016/17 Wet, and 2017 Dry seasons) to observe stability and uniformity of characteristics within accessions. Data were analysed using ANOVA and mean separation was performed using Duncan's Multiple Range Test at $p \leq 0.05$ using SAS software version 9.1. Before the analyses, each data set was tested for normality, error homogeneity, the correlation between means and variances, and additivity of main effects to ensure the non-violation of the assumptions of ANOVA. None of the data sets was found to be violating any of the assumptions so that the use of ANOVA on the original (non-transformed) data could be justified.

Genetic control study

The *Muricata* accession from Giradurukotte was used to make distant crosses with recommended bitter gourd *Charantia* varieties 'Matale Green' and 'Thinnaweli white'. Seeds of all possible crosses of *Muricata* × Matale Green and *Muricata* × Thinnaweli White and their reciprocal crosses (altogether four crosses) were planted to raise the F_1 generations. All the F_1 seeds from each cross (four F_2 populations) were used to establish an F_2 population in the field and were maintained according to the recommended cultural practices by the Department of Agriculture (DOA).

Fruit length, number of fruits per vine, and the external fruit colour were recorded in all F_1 and F_2 populations. From each F_2 population, a sample of 150 to 220 individual plants was used to study the genetic control of each of the three characteristics measured in the F_2 populations using segregation patterns by plotting frequency distributions in terms of histograms.

RESULTS AND DISCUSSION

Identification of promising *Muricata* accessions

More than 90% within line uniformity was ascertained on observational basis in all the accessions in all three seasons for external fruit colour, length of matured vine, and the number of fruits per vine. The length of the matured vine, days to flower initiation, number of fruits per vine, and external fruit pericarp colour of *Muricata* accessions over three consecutive seasons of 2016 Dry, 2016/17 Wet, and 2017 Dry, are presented in Tables 1 and 2.

The cross, accession × season interaction was found to be not significant ($p \leq 0.05$) for vine length, days to flower initiation, and the number of fruits per vine so that the means of those characteristics over seasons could be compared among accessions. The differences in vine length, days to flower initiation, and the number of fruits per vine observed among selected accessions were consistent over seasons; so that differences between accessions could mainly be attributed to genetic effects. Thus, accessions were genetically different and making selection among them appeared effective. The GK accession was selected as the comparatively better-performing accession for the crossing programme for introgression of important traits, as it recorded the highest number of fruits per vine, the longest mature vine length, the lowest number of days to initiate female flowers, and green external fruit colour over all three seasons (Table 1).

Table 1: Mature vine length and days to flower initiation of site based *Muricata* accessions over three consecutive seasons of 2016 Dry, 2016/17 Wet and 2017 Dry.

Site based Accession	Vine length (m)[#]				Days to flower initiation[#]								Number. of fruits/vine[#]				External fruit colour[# £]		
					Male				Female										
	S1	S2	S3	Mean*	S1	S2	S3	Mean*	S1	S2	S3	Mean*	S1	S2	S3	Avg.*	S1	S2	S3
Girandurukotte	2.5	2.8	2.7	2.66a	33	32	33	32.70a	45	40	44	43.0c	18	22	29	23.0a	G	G	G
Muruthalawa	1.4	1.5	1.4	1.43c	35	33	34	3.00a	50	50	49	49.7a	11	10	8	9.6c	LG	LG	LG
Dehiattakandiya	1.5	1.6	1.5	1.53c	30	32	32	1.30a	45	45	44	44.7b	15	13	13	13.3b	LG	LG	LG
Polonnaruwa	1.2	1.4	1.4	1.33c	32	33	32	2.33a	45	44	45	44.7b	10	12	10	10.0c	G	G	G
Hambantota	1.8	2.0	1.9	1.90b	33	33	32	2.70a	50	48	50	49.3a	12	12	15	13.0b	G	G	G

[#] S1- 2016 Dry Season, S2- 2016/17 Wet Season, S3- 2017 Dry Season

* Means with the same letter within a column are not significantly different at 0.05 probability level

Table 2: External fruit colour, fruit length and number and mid-parent value of fruits/vine of GK *Muricata* accession, Matale green and Thinnaweli white parental lines and their F_1 cross combinations

Population	External fruit colour	Fruit length* (cm)	Fruits/vine* (number)	Mid-parent value
GK Muricata accession	Green	3.5c	20.0a	
Matale Green	Green	32.0a	12.0bc	
Thinneweli White	Yellowish White	37.0a	8.0d	
GK Muricata acc. × Matale green (F_1)	Green	6.0b	17.0a	16
Matale green × GK *Muricata* acc.(F_1)	Green	6.5b	12.0bc	16
GK Muricata acc. × Thinneweli white (F_1)	Light green	8.0b	14.0b	14
Thinneweli white × GK *Muricata acc.* (F_1)	Light green	8.2b	9.0cd	14
CV %		14.0	12.5	

*Values with the same letter within a column are not significantly different at 5% probability level.

Determination of the genetic control of traits

In each cross, more than 95% of female flowers set fruits following crossing, and this revealed the genetic compatibility and close relationship between the GK *Muricata* accession and *Charantia*. Thus, the use of GK *Muricata* accession as promising germplasm for bitter gourd improvement would be possible. This is in agreement with Rathod *et al*. (2019), who reported a high percentage of fruit set (> 85%) with abundant seeds per fruit and seedling survivability of the cross between *M. Charantia var. Charantia* and *M. Charantia var. Muricata*. They also suggested that *Charantia* and *Muricata* are genetically close to each other and compatible. Bai & Beevy (2012) and Bharathi *et al*. (2012) explained the closeness between these two botanical varietal groups based on conventional crossing methods.

External fruit colour

The external fruit colour of parental lines of GK *Muricata* accession Matale Green and Thinnaweli White and their F_1 cross combinations of GK *Muricata* accession × Matale green, Matale green × GK *Muricata accession,* GK *Muricata* accession × Thinneweli white and Thinneweli white × GK *Muricata accession* are presented in Table 3. Both the F_1 cross combinations involving the cross between Thinnaweli White with yellowish-white fruit colour and GK *Muricata* accession with green fruit colour and the reciprocal cross showed light green fruit colour, so that incomplete dominance could be operating. This was in agreement with Lion *et al*. (2002) who also speculated that the light green colour in F_1 is due to incomplete dominance or modifiers.

The external fruit colour of both the F_2 populations derived from the cross between GK *Muricata* accession and Thinnaweli White and the reciprocal cross showed a continuous variation approaching normal distribution from green to yellowish-white (Figure 1). Thus, the external fruit colour appeared to be a quantitative characteristic controlled by many genes, each with a possible small additive effect. This is in agreement with Huang & Hsieh (2017), who reported that the external fruit colour of bitter gourd is controlled by many genes as expression of chlorophyll has complex interactions. Liu *et al.* (2005) and Lion *et al*. (2002) reported that fruit colour (green vs. white) in bitter guard is controlled by two genes, where green is dominant over white. Therefore, the desired fruit colour may be achieved through directional selection.

As no F_2 segregation was observed for external fruit colour of the cross between the GK *Muricata* accession and Matale Green, while all parents and F_1 plants had the same green external fruit colour, both the parents, GK *Muricata* accession and Matale Green, may carry the same gene/genes alleles for green fruit colour.

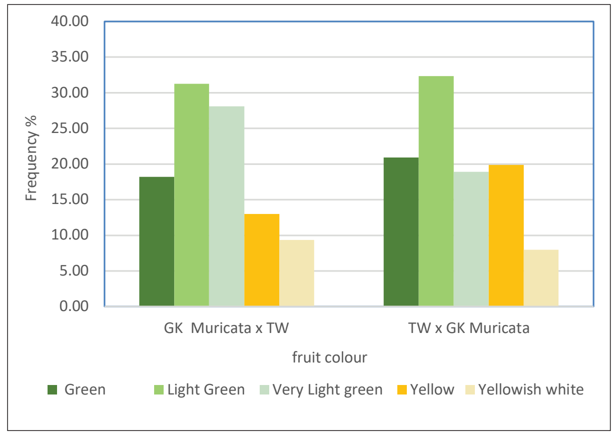


Figure 1: Percentage distribution of external fruit colour in two F_2 populations derived from the cross of GK *Muricata* accession × Thinnaweli White, and the reciprocal cross.

Fruit length

The fruit length of the GK *Muricata* accession, Matale Green and Thinnaweli White parental lines, and their F_1 cross combinations are presented in Table 2. All four F_1 cross combinations recorded significantly ($p \leq 0.05$) shorter fruit length than Matale Green and Thinnaweli White, however, significantly ($p \leq 0.05$) longer fruit length than that of the GK *Muricata* accession. The fruit length of F_1 was very close to that of GK *Muricata* accession and showed incomplete dominance for short fruit length. This is in agreement with Kim *et al*. (1990) and Zhang *et al.* (2008), who reported that the fruit length of bitter gourd showed incomplete dominance and Kumari *et al*. (2015) who reported that short fruit length is partially dominant over long fruit length. Furthermore, Behera *et al*. (2008) reported that the fruit length of bitter gourd is controlled by a single dominant gene or few genes, as *Muricata* parental characteristics are expressed in the fruit length of $F_{1.}$

Fruit length in four F_2 populations derived from the crosses of GK *Muricata* accession × Matale Green and its reciprocal cross and cross of GK *Muricata* accession × Thinnaweli White and its reciprocal cross showed continuous variations from short (0–5 cm) to long (35–40 cm) fruits (Figure 2). Thus, the current study is in agreement with the previous studies (Sharma & Bhutani, 2001; Zhang *et al*., 2008; Kumari *et al*., 2015) verifying that fruit length of bitter gourd may be a quantitative characteristic controlled by many genes, each with an effect varying in size. For genetic improvement of the trait, directional selection may be used for the desired direction as the trait is quantitative. Kumari *et al*. (2015) reported that selection for fruit length is effective as it has a high broad-sense heritability.

In all F_2 populations, longer fruit lengths than that of the parents with the longest fruit length were observed, and they are suspected to be transgressive segregants which may be effectively utilized for bitter gourd improvement.

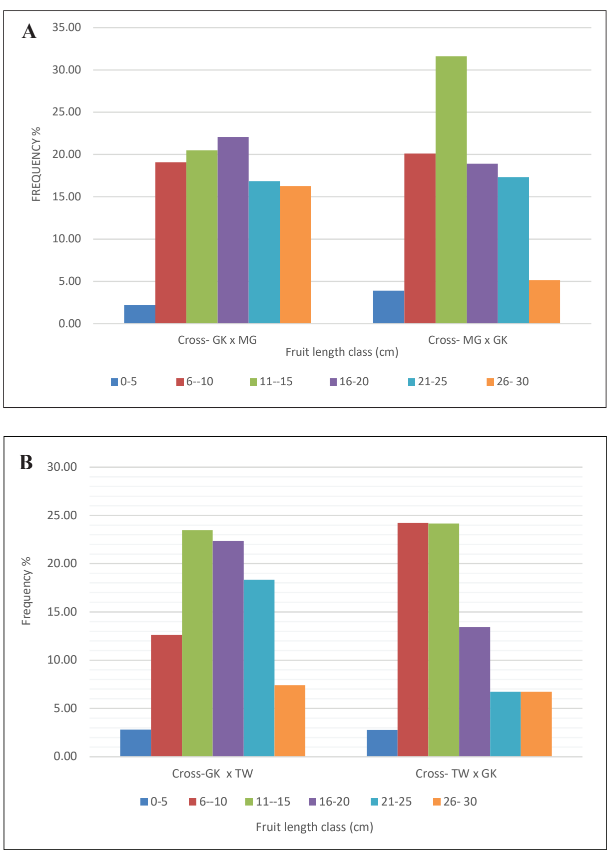


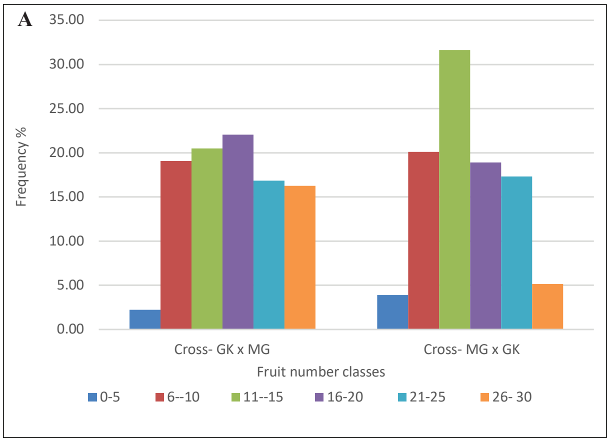
Figure 2: Histogram of fruit length in four F_2 populations derived from the crosses of (A) Girandurukotte *Muricata* accession (GK) × Matale Green, its reciprocal (Matale green × GK), (B) Girandurukotte *Muricata* accession × Thinnaweli White, and its reciprocal (Thinnaweli white × GK).

Number of fruits per vine

The number of fruits per vine of GK *Muricata* accession Matale Green, and Thinnaweli White parental lines and their F_1 cross combinations are presented in Table 3. The behavior of F_1 progenies of GK *Muricata* accession × Thinnaweli White and GK *Muricata* accession × Matale Green cross combinations indicated that the number of fruits per vine may be controlled by many genes with additive effects. However, the F_1 progenies of the reciprocal crosses of the above-indicated crosses, *viz*., Thinnaweli White × GK *Muricata* accession and Matale Green × GK *Muricata* accession recorded nine fruits pre vine and 12 fruits per vine, respectively which were not significantly ($p \leq 0.05$) different from that of the respective female parents, while much lower than the mid-parent value (Table 3). Thus, the number of fruits per vine in bitter gourd may be controlled by both nuclear gene effects as well as a cytoplasmic effect depending on the female parent used in the cross combination.

GK *Muricata* accession had no cytoplasmic effect but both Matale Green and Thinnaweli White (*Charantia* varieties) may have a cytoplasmic effect on the number of fruits per vine. However, this cytoplasmic effect may not be useful as its influence was to reduce the number of fruits per vine. As no previous studies are available on such a cytoplasmic effect on the number of fruits per vine in bitter gourd, further studies are needed to confirm this.

The number of fruits per vine of all four F_2 populations derived from the crosses of GK *Muricata accession* × Matale Green and its reciprocal cross and cross of GK *Muricata* accession × Thinnaweli White and its reciprocal cross segregated in the same pattern showing a continuous variation from the lowest fruit number (0–5) to the highest fruit number (26–30) per vine (Figure 3). Based on the segregation pattern of the respective F_2 populations, the number of fruits per vine in bitter gourd appeared to be a quantitative characteristic that is mainly controlled by many genes with additive effects. This is in agreement with Sharma & Bhutani (2001) who reported that the number of fruits per vine is a quantitative trait controlled by additive factors, and Rao *et al*. (2021) who reported that the number of fruits per vine is controlled by QTL. The cytoplasmic effect may have been nullified in all F_2 populations where female parents were Matale Green and Thinnaweli White. All four F_2 distributions had segregants with values much lower and higher than the lowest and highest parents, respectively and these may be transgressive segregants appearing in both extremes (Figure 3).



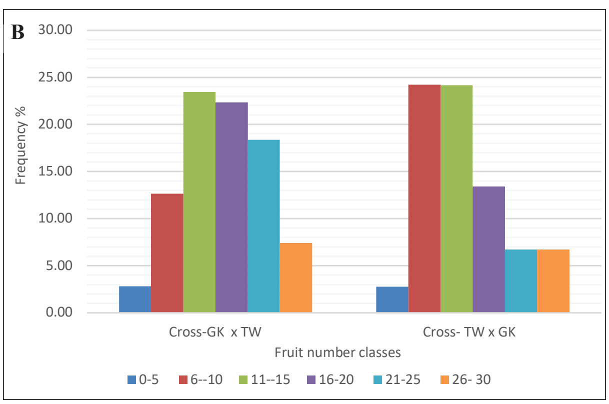


Figure 3: Distribution of the number of fruits per vine in four F_2 populations derived from the crosses (A) *Muricata* (GK) × Matale Green, and its reciprocal cross (Matale Green × GK), (B) *Muricata* × Thinnaweli White and its reciprocal cross (Thinnaweli White × GK).

Although the highest number of fruits per vine recorded among parents was 20, F_2 segregants suspected to be transgressive with the number of fruits per vine as high as 26-30 were recorded, so that potential for improving the number of fruits per vine in bitter gourd through directional selection appears very high. This may be comparatively more efficient in the F_2 population derived from the cross between GK *Muricata* accession × Matale Green as the number of plants with 26-30 fruits per vine was the highest (about 27) in that population.

CONCLUSIONS

The bitter gourd accession of GK *Muricata* collected from the Giradurukotte region in Sri Lanka was identified as a rich genetic source for bitter gourd improvement due to its consistency in characters and higher gynoecy. External fruit colour, fruit length, and the number of fruits per vine of bitter gourd appeared to be quantitative characteristics controlled by many genes, each with a potential small effect. GK *Muricata* accession may be used to improve presently cultivated varieties Matale Green and Thinnaweli White, with respect to the above fruit characteristics, through a breeding programme with the directional selection made towards the desired direction.

The cytoplasmic effect of Matale Green and Thinnaweli White varieties on the number of fruits per vine is considered to be not useful as its influence was to reduce the number of fruits per vine. The appearance of transgressive segregants in fruit length and the number of fruits per vine in respective F_2 populations were about 3% and 10%, respectively, and they are of interest for further improvement.

Conflict of interest statement

The authors declare no conflict of interest regarding the publication of this article.

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