



Crossability studies among *Momordica charantia* var. *charantia* and *Momordica charantia* var. *muricata*

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ABSTRACT

Inter and intra-specific hybridization is a powerful breeding method for crop improvement and it has potential to broaden the genetic base of the cultivated species. *M. charantia* var. *muricata* is considered as a wild progenitor of bitter gourd (*M. charantia*) and it has important novel genes for biotic and abiotic stress tolerance. In the present experiment, conducted during the 2016–17 and 2017–18, intra-specific hybrids were produced among *M. charantia* var. *charantia* and *M. charantia* var. *muricata* and these were characterized for plant morphology, pollen-pistil compatibility, cytology and molecular relation among the parents and hybrids. In both direct and reciprocal crosses, the crossability percent was nearly 90% with high percent of pollen viability. The morphological traits of the parents were highly diverse and the hybrids showed intermediate pattern of character expression. The pollen-pistil behaviour and compatibility reaction were studied for the first time and observed higher per cent of compatibility in direct cross (*M. charantia* var. *charantia* × *M. charantia* var. *muricata*; 90.98%) and comparatively lower in reciprocal cross (*M. charantia* var. *muricata* × *M. charantia* var. *charantia*; 84.43%). Cytological and molecular marker (SSR) techniques were used for hybridity confirmation.

Key words: Bitter melon, Intra-specific hybridization, Pollen-pistil compatibility, Cytology, SSR marker, Hybridity test.

Bitter gourd (*Momordica charantia* L.; $2n = 2x = 22$) an important Cucurbitaceous vegetable, is widely grown in tropical and subtropical regions of the world. It has occupied a special position among the cucurbitaceous crops because of its hypoglycemic action and nutraceutical values. The monoecious sex form is predominant in bitter gourd, which leads to cross-pollination.

The genus *Momordica* consists of 59 species (Schaefer and Renner, 2010), widely distributed in the tropical African continent and in South East Asia. About 12 species are widely distributed in Asia and of which 9 dioecious and 3 monoecious (Bharathi and John 2013). *Momordica* is a monophyletic genus and originated in Tropical Africa (Schaefer and Renner 2010). Indian bitter gourd genotypes comprises two varietal groups, *M. charantia* var. *charantia*, bears large fusiform fruit with numerous tubercles and *M. charantia* var. *muricata* (wild) which is small and disc shaped fruiting type with numerous tubercles on its surface (Chakravarty 1990). These two botanical varieties (*M.*

charantia var. *charantia* and *M. charantia* var. *muricata*) exhibit remarkable diversity for their morphological traits.

During the process of crop domestication and development of modern cultivars, many important genes have been eliminated, altered the nutritional content and reduced the plant defence mechanism compared to their progenitor weedy species. Reconstitution of desirable gene (/s) from weedy species to cultivated form is the only way to broaden genetic base that concedes crops to adopt changing climate through the transfer of specific adaptive alleles. Interspecific and intraspecific hybridization is the mainly used to transfer desirable traits/genes from wild/semi-wild species to cultivated species. *M. charantia* var. *muricata* is considered as a progenitor species of *M. charantia* var. *charantia* (Degner 1947, Walters and Decker-Walters 1988). It could possibly contribute to broaden the narrow genetic base of bitter gourd through intraspecific hybridization. In order to understand the relationship between these two botanical species and their complete crossability spectrum the present experiment was carried out.

MATERIALS AND METHODS

The present study was conducted during the 2016-17 and 2017-18 at ICAR-Indian Agricultural Research Institute, New Delhi. The breeding material comprised of one of each long fruited genotype DBGS-2 (*M. charantia* var.

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charantia) and semi wild type with small-fruited genotype DBGS-34 (*M. charantia* var. *muricata*). The crossing was made in both direction, when DBGS-2 used as female the resultant progeny was designated as Hybrid-1 and when it was used as male parent then the hybrid was designated as Hybrid-2. About 50 female buds were crossed in both directions. Crossability was analysed based on crossability indices, pollen viability, pollen germination on artificial media, pollen-pistil interaction using aniline blue fluorescent methods. The per cent of fruit set, average number of seeds per fruit and seed germination percentage was calculated using formulae given below.

$$\text{Fruit set (\%)} = \frac{\text{Number of fruit set}}{\text{Total number of flower crossed}} \times 100$$

$$\text{Average number of seeds per fruit (\%)} = \frac{\text{Number of fruit set}}{\text{Total number of seed obtained}} \times 100$$

$$\text{Seed germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Pollen viability of the parents and their hybrids were tested using 1% aceto-carmin stain. The pollen viability was observed through *in vitro* pollen germination as suggested by Kielkowska and Havey (2012). For pollen-pistil interaction study, 12, 24 and 48 h after self and cross pollinated, buds were selected. Germination of pollen grains on stigma was examined using five of each self and cross pollinated buds. Pistils were stained in 0.05% aniline blue (prepared in 50mM potassium phosphate buffer) and kept under dark for 12 h for adequate staining. Dissected pistils were mounted on a glass slide in the same staining solution under a coverslip. The pollen germination was visualized on the stigmatic surface under UV light (390–420 nm) using confocal fluorescent microscope (Leica SP5, Leica Biosystem, Germany) and image was taken. The per cent pollen germination was calculated using the given formula.

$$\text{Pollen germination (\%)} = \frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains adhered}} \times 100$$

Six qualitative and 14 quantitative traits from five individual plants were taken from *Momordica charantia* var. *charantia*, *Momordica charantia* var. *muricata* and their hybrids. Morphological similarity/difference patterns analyzed using analysis of variance (ANOVA).

Mitotic and meiotic observations were recorded in population using male flower bud and root tips respectively. For mitotic studies, seeds of parents and hybrids were soaked overnight and germinated on seed germinator paper. The root tips of 1-2 cm long penetrated to germinator paper were excised between 10.00-11.00 am and fixed in 3:1 ratio of ethanol and glacial acetic acid fixative solution for 24 hr. After washing, root tips were hydrolysed in 1N HCL at 60°C for 12 min then washed with water followed by Feulgen staining for 30 min and then squashed in 1.5% Aceto-orcin solution. Similar protocol was followed for meiosis study and images were captured.

For hybridity test, leaf DNA was extracted using modified CTAB method (Saghai-Marooof *et al.* 1984). Simple Sequence Repeat (SSR) markers were used to confirm the hybridity. The amplified PCR products were separated on 4% agarose gel with ethidium bromide stain using electrophoresis. A total 534 SSR markers were screened for parental polymorphism, out of which 10 markers showed parental polymorphism and were used for hybridity conformity.

RESULTS AND DISCUSSION

The results of the crossability behaviour of *M. charantia* var. *charantia* (DBGS-2) and semi-wild variety *M. charantia* var. *muricata* (DBGS-34) are presented in Table 1. The cross *M. charantia* var. *charantia* (♀) × *M. charantia* var. *muricata* (♂), recorded highest fruit set (96%) with more number seeds/fruit. In reciprocal cross (*M. charantia* var. *muricata* (♀) × *M. charantia* var. *charantia* (♂), female parent was capable of producing more number of fruits

Table 1 Crossability behaviour, pollen viability and In vitro pollen germination of *M. charantia* var. *charantia*, *M. charantia* var. *muricata*

Cross combination	No. of flower pollinated	No. of fruit obtained	Per cent fruit set	Average No. of seeds/fruit	Germination (%)	Total No. of seedlings	Total no. of plants	Seedling survival (%)
MCC (♀) × MCM (♂)	50	48	96	22	97.5	20	20	100
MCM (♀) × MCC (♂)	200	174	87.5	12	89.5	20	17	85
<i>Pollen viability (%)</i>				<i>Pollen germination (%)</i>				
<i>M. charantia</i> var. <i>charantia</i>		94.25 ± 0.85a				73.5 ± 2.90a		
<i>M. charantia</i> var. <i>muricata</i>		96.13 ± 0.71a				75.25 ± 2.21a		
Hybrid-1		95.00 ± 0.58a				69.50 ± 3.01a		
Hybrid-2		96.25 ± 0.25a				73.51 ± 2.40a		
F value		1.69NS				0.99NS		

* MCC, *M. charantia* var. *charantia*; MCM, *M. charantia* var. *muricata*. * NS, Non-significant at 1, 5 and 0.1% probability levels, respectively.

with smaller size and hence 200 female flowers buds were pollinated and recorded 85.57% fruit set with average of 12 seeds/fruit. The crossability study revealed that both the botanic varieties were readily crossable and produced 100% viable hybrids when the *M. charantia* var. *charantia* was used as maternal parent. However, the reciprocal cross shows reduced fruit set percentage, it may be due to semi-wild nature of *M. charantia* var. *muricata* and some amount of incompatibility reaction between cultivated and wild varietal group. Earlier reports sustained the fact of reduced fruit set in interspecific and intraspecific hybridisation among *Momordica* spp. (Bharathi *et al.* 2012). The high per cent of fruit set (>85%) with abundant seeds/fruit and seedling survivability suggested that *M. charantia* var. *charantia* and *M. charantia* var. *muricata* are genetically close to each other. The close relationship between semi-wild and cultivated varieties of *Momordica* was also suggested (Bai and Beevy 2012, Bharathi *et al.* 2012).

Bai and Beevy (2012) and Bharathi *et al.* (2012) explained the closeness between these two botanical varietal groups based on conventional crossing methods. However, no studies mentioned about the percent closeness between these two species and why there was reduced percent of crossability when *M. charantia* var. *muricata* was used as female parent and *M. charantia* var. *charantia* was used as pollen parent. Hence, for the first time an attempt was made to identify the cause for reduced per cent fruit set and closeness between these two varietal groups. In crosses, pollen viability and pollen germination test was carried out and no significance difference was observed for pollen viability and per pollen germination in both the species and their hybrids (Table 1), thus it is reconfirmed that there is close relationship between *M. charantia* var. *charantia* and *M. charantia* var. *muricata*. The results showed that *M. charantia* var. *muricata* (syn *M. charantia* var. *abbreviate*) is the progenitor of cultivated bitter melon (Degner 1947, Walters and Decker 1988).

Pollen grains germinated rapidly on the stigmatic surface and pollen tube penetrated through the transmission tissue of the style and entered into the ovule (Fig 1). When *M. charantia* var. *charantia* was selfed, pollen germination was 95.79% which was higher than *M. charantia* var. *muricata* (93.96%) followed by Hybrid *M. charantia* var. *charantia* × *M. charantia* var. *muricata* (90.98 %) and the lowest pollen germination on stigma was noticed in the cross *M. charantia* var. *muricata* × *M. charantia* var. *charantia* (84.43%) (Table 2). The pollen tube growth was substantially reduced in the style after pollen tube penetrated on the stigmatic surface of *M. charantia* var. *charantia* (83.15%), *M. charantia* var. *muricata* (84.72%), Hybrid-1 (83.06%) and Hybrid-2 (74.64%). In *M. charantia* var. *charantia*, the per cent pollen tube reached to ovule was 58.50% followed by *M. charantia* var. *muricata* (57.48%) and Hybrid-1 (54.76%) and lowest was noticed in Hybrid-2 (48.15%). The crossability studies indicated close relationship between these two varietal groups, but pollen-pistil interaction indicates the reduction in pollen tube which reached to ovules.

Table 2 Pollen-pistil behavior of *M. charantia* var. *charantia* and *M. charantia* var. *muricata* during crossability

HAP	Adhered	Germinated	Pollen tube in style	Pollen tube reach to Ovule
<i>M. charantia</i> var. <i>charantia</i> (Self)				
12	304.67	291.67 (95.74)	236.00 (80.94)	176.00 (60.26)
24	324	310.33 (94.18)	245.67 (79.39)	182.67 (58.83)
48	287	282.00 (98.26)	251.33 (89.13)	159.00 (56.40)
Total	915.67	884	733	517.67
Mean		(97.79)	(83.15)	(58.50)
<i>Hybrid-1</i> (<i>M. charantia</i> var. <i>charantia</i> × <i>M. charantia</i> var. <i>muricata</i>)				
12	299.67	274.67 (91.66)	212.00 (77.26)	158.00 (57.58)
24	313	283.00 (91.52)	239.67 (83.70)	160.00 (55.88)
48	305.67	274.33 (89.76)	242.00 (88.22)	139.33 (50.83)
Total	918.33	832.00	693.67	457.33
Mean		(90.98)	(83.06)	(54.76)
<i>M. charantia</i> var. <i>muricata</i> (Self)				
12	351	330.67 (94.20)	280.00 (84.80)	173.00 (52.41)
24	323.33	303.00 (93.72)	254.33 (83.89)	162.67 (53.64)
48	307.33	288.67 (93.95)	246.67 (85.46)	148.33 (51.38)
Total	981.67	922.33	781	484
Mean		(93.96)	(84.72)	(57.48)
<i>Hybrid-2</i> (<i>M. charantia</i> var. <i>muricata</i> × <i>M. charantia</i> var. <i>charantia</i>)				
12	330	288.67 (87.44)	72.66 (73.78)	137.67 (47.85)
24	313	261.33 (83.49)	70.94 (73.75)	126.00 (48.23)
48	285.33	238.33 (83.56)	74.27 (76.38)	115.33 (48.36)
Total	928.13	788.33	587.67	379
Mean		(84.43)	(74.64)	(48.15)

* Figures in parentheses denote percentage value and bold typeface indicate percentage of average value calculated on total pollen grains germinated.

Selfed pistil of both the groups showed >90% of pollen germination on stigma as compared to their cross combinations. However, in both direct and reciprocal crosses, it showed >80% pollen germination and no incongruity of pollen behaviour was noticed, thus these results explained that there is high per cent of pollen-pistil compatibility between these two intra-species. The per cent of pollen tube reached to ovule was also sufficient and it is presumed that the pollen tube was observed till micropyle were fertilized, as suggested by Liedl *et al.* (1996). The pollen tubes reached ovule within 6–8 h after pollination and completed fertilization within 18–20 h of pollination. The results of present experiment confirmed previous reports of Wang *et al.* (1997).

The morphological traits of *M. charantia* var. *charantia* and *M. charantia* var. *muricata* and their hybrids were analysed and compared for both qualitative and quantitative

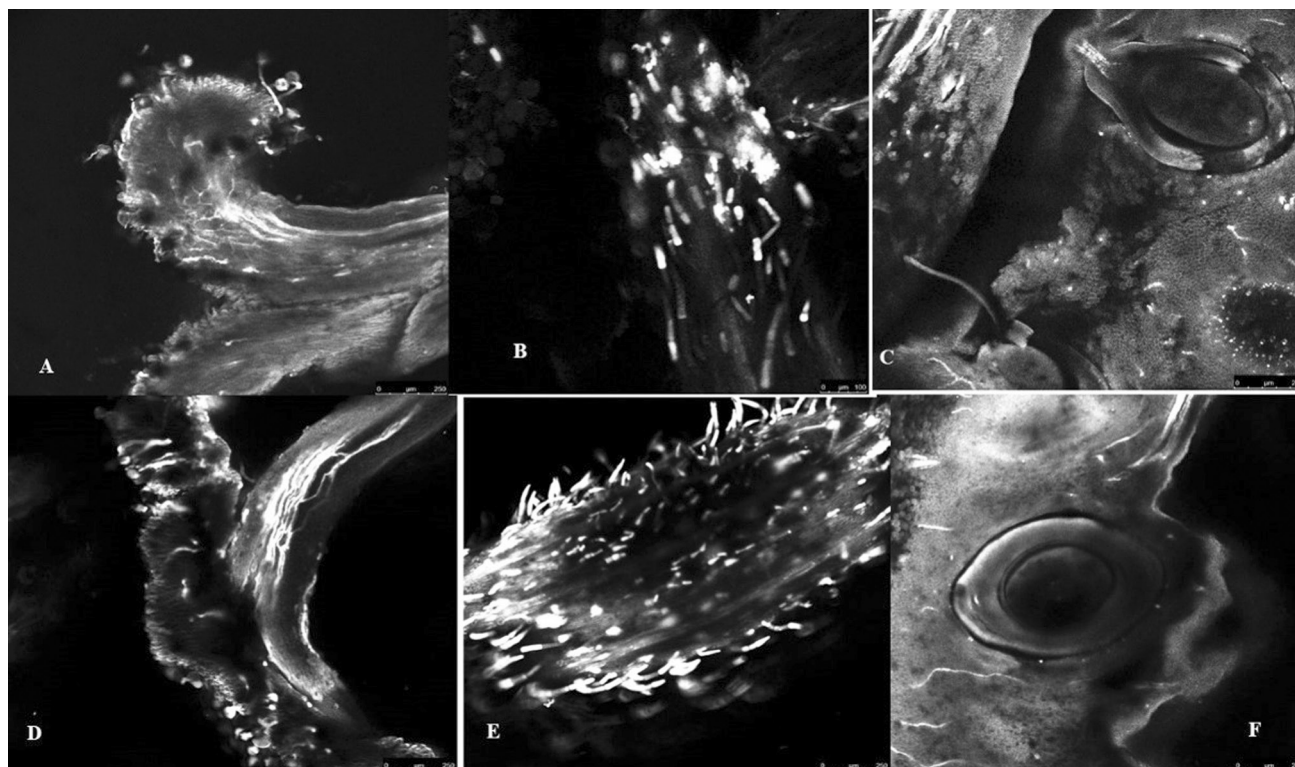


Fig 1 Pollen - pistil interaction in *M. c. var charantia* × *M. c. var muricata* (Hybrid-1) (A, B, C) and *M. c. var muricata* × *M. c. var charantia* (Hybrid-2) (D, E, F) (A & D-Pollen grains adhesion in direct and reciprocal cross, B&E-Pollen tube growth in the style region in direct and reciprocal cross, C& F- Pollen tubes reached to ovule for fertilization in direct and reciprocal cross.

Table 3 Qualitative characters of the *M. charantia* var. *charantia*, var. *muricata* and their F1 Hybrids

Character	<i>M. charantia</i> var <i>charantia</i>	<i>M. charantia</i> var. <i>muricata</i>	Hybrid-1	Hybrid-2
Vine length	Long	long	long	Long
Internodal length (cm)	15.30	10.28	11.70	13.17
Leaf colour	Light green	Green	Green	Green
Leaf Length (cm)	12-15cm	6-8cm	10-12m	10-12cm
Male flower bract position	Bellow middle	Bellow middle	Bellow middle	Bellow middle
Male flower colour	Deep yellow	Deep yellow	Deep yellow	Deep yellow
Stigma Colour	Green	Green	Green	Green
Ovary length	Long (60-90mm)	Short (10-12mm)	Medium (18-21 mm)	Medium (18-21mm)
Ovary surface	smooth	Spine	Spine	Spine
Stigma arrangement	Tight	Tight	Tight	Tight
Ovary colour	Green	Green	green	Green
Fruit shape	spindle	Disc	spindle	Spindle
Fruit pedicel length (cm)	12-15	12-15	10-13	10-14
Fruit colour at ripening	Yellow	yellow	yellow	Yellow
Fruit Length (cm)	20-28cm	5-7cm	10-13cm	10-13cm
Fruit tubercles	Few	Medium	Medium	Medium
Tubercles prominence	Non-conspicuous	Conspicuous	Conspicuous	Conspicuous
Ridges	Continuous	Discontinuous	Discontinuous	Discontinuous
Seed shape	Subtridentate	Subtridentate	Subtridentate	Subtridentate
Seed colour	Brown	Yellowish brown	Brown	Yellowish brown

Table 4 ANOVA of quantitative morphological characters of the *M. charantia* var. *charantia*, *M. charantia* var. *muricata* and their F₁ hybrids

Treatment	Vine length (cm)	Internodal length (cm)	Number of Primary Branches	Leaf length (cm)	Leaf width (cm)
<i>M. charantia</i> var. <i>charantia</i>	6.50 ± 0.31 ^a	15.30 ± 0.42 ^a	14.67 ± 0.28 ^c	10.98 ± 0.24 ^a	14.39 ± 0.52 ^b
<i>M. charantia</i> var. <i>muricata</i>	4.60 ± 0.16 ^b	10.28 ± 0.08 ^d	23.67 ± 0.33 ^a	4.89 ± 0.40 ^b	6.55 ± 0.19 ^c
<i>M. charantia</i> var. <i>charantia</i> × <i>M. charantia</i> var. <i>muricata</i>	5.20 ± 0.13 ^b	11.70 ± 0.2 ^c	23.00 ± 0.58 ^a	10.48 ± 0.13 ^a	16.78 ± 0.04 ^a
<i>M. charantia</i> var. <i>muricata</i> × <i>M. charantia</i> var. <i>charantia</i>	5.50 ± 0.52 ^b	13.17 ± 0.17 ^b	19.67 ± 0.62 ^b	11.07 ± 0.11 ^a	16.91 ± 0.10 ^a
<i>F</i> value	8.59 ^{**}	72.45 ^{***}	101.51 ^{***}	144.49 ^{***}	299.11 ^{***}
	<i>DFF</i>	<i>NFF</i>	<i>Ovary length (mm)</i>	<i>Ovary dia (mm)</i>	<i>No. of petals (♂ & ♀)</i>
<i>M. charantia</i> var. <i>charantia</i>	73 ± 1.16 ^a	20.00 ± 0.680 ^a	87.86 ± 1.16 ^a	2.83 ± 0.08 ^b	5.00
<i>M. charantia</i> var. <i>muricata</i>	47 ± 0.58 ^c	9 ± 0.58 ^d	9.86 ± 0.16 ^c	3.86 ± 0.12 ^a	5.00
<i>M. charantia</i> var. <i>charantia</i> × <i>M. charantia</i> var. <i>muricata</i>	63 ± 0.42 ^b	17 ± 0.31 ^b	19.46 ± 0.47 ^b	2.89 ± 0.13 ^b	5.00
<i>M. charantia</i> var. <i>muricata</i> × <i>M. charantia</i> var. <i>charantia</i>	49.33 ± 0.88 ^c	11.67 ± 0.88 ^c	20.62 ± 0.36 ^b	2.54 ± 0.11 ^b	5.00
<i>F</i> value	214.12 ^{***}	56.06 ^{***}	3951.09 ^{***}	111.27 ^{***}	-
	<i>Fruit length (cm)</i>	<i>Fruit diameter (cm)</i>	<i>Fruit weight (g)</i>	<i>No. of fruits/ vine</i>	<i>Yield/vine (g)</i>
<i>M. charantia</i> var. <i>charantia</i>	26.74 ± 0.88 ^a	2.69 ± 0.09 ^c	121.57 ± 5.74 ^a	16.67 ± 0.33 ^d	2037.4 ± 97.18 ^b
<i>M. charantia</i> var. <i>muricata</i>	5.23 ± 0.34 ^d	3.76 ± 0.24 ^a	9.28 ± 0.22 ^c	52.67 ± 0.88 ^a	719.15 ± 20.10 ^c
<i>M. charantia</i> var. <i>charantia</i> × <i>M. charantia</i> var. <i>muricata</i>	10.40 ± 0.43 ^c	3.23 ± 0.06 ^b	66.26 ± 2.03 ^b	40.33 ± 0.67 ^c	2430.65 ± 59.44 ^a
<i>M. charantia</i> var. <i>muricata</i> × <i>M. charantia</i> var. <i>charantia</i>	14.63 ± 0.56 ^b	3.38 ± 0.09 ^{ab}	68.76 ± 3.31 ^b	43 ± 0.58 ^b	2459.76 ± 57.71 ^a
<i>F</i> value	242.687 ^{***}	10.215 ^{**}	163.11 ^{***}	615.12 ^{***}	160.19 ^{***}

NS, Non-significant; DFF, Days to first female flower appearance; NFF, Node at first female flower appearance. *, ** and *** significant at 5 %, 1% and .01% probability levels, respectively. In each column, any two means having a common letter are not significantly different at P=0.05 based on Duncan's multiple range test.

characters (Table 3 and 4). Both botanical varieties exhibited significant variance between themselves for most of the traits studied. The direct cross and reciprocal cross showed intermediate trait expression of their parents but significant differences were recorded for quantitative traits. The earliest flowering (days to first female flower) was noticed in *M. charantia* var. *muricata* and Hybrid-2 followed by Hybrid-1, whereas DBGS-2 showed late flowering, similar fashion was noticed for node to first female flower appearance. Significant difference was recorded for ovary length between *M. charantia* var. *muricata* (9.86 mm) and *M. charantia* var. *charantia* (87.76 mm) but both hybrids showed intermediate ovary length. In contrast, ovary diameter was bigger in *M. charantia* var. *muricata* (3.86 mm) compared to *M. charantia* var. *charantia*, Hybrid-1 and Hybrid-2. Fruit length was longer in *M. charantia* var. *charantia* followed by Hybrid-2 and Hybrid-1, however smallest fruits were borne in *M. charantia* var. *muricata*. The similar observation was noticed for fruit weight of these cross combinations, however yield per plant was higher in Hybrid-1 and hybrid-2 followed by cultivated variety and

lowest were recorded in *M. charantia* var. *muricata* (719.15 g per plant). The intermediate nature of F₁ hybrids has been reported in *Momordica* spp (Roy *et al.* 1996).

Meiotic studies were carried out to observe the chromosomal behaviour of the *M. charantia* var. *charantia* and *M. charantia* var. *muricata*. Meiosis was regular in Pollen Mother Cells (PMC) of *M. charantia* var. *charantia* and *M. charantia* var. *muricata* with the chromosome number (2n=22). In the intervarietal hybrids, the meiosis was regular and did not show any chromosomal abnormalities. Pollen fertility was recorded based on staining frequency of pollen grain. The pollen grains of Hybrid 1 and Hybrid 2 showed 95% and 96.25% pollen fertility with per cent pollen germination of 69.50 and 73.51, respectively (Table 1). Cytological studies on intra-specific hybrid between *M. charantia* var. *charantia* and *M. charantia* var. *muricata* revealed that there is regular meiosis in both parents and hybrids. The occurrence of 11 bivalent chromosome per PMCs in both parents and hybrids suggested that the complete homology between the cultivated and wild varieties of *Momordica*. The results are in accordance with reports

of Bai and Beevy (2012).

Hybrid identification was performed using SSR molecular assay. A total 534 SSR markers were surveyed for parental polymorphism and of which 10 markers were polymorphic (Table 4) between the parents. The cytological and molecular studies strongly suggest these two varietal groups are relatively close to each other.

In this experiment, intra-specific hybrids were produced among *M. charantia* var. *charantia* and *M. charantia* var. *muricata*. The attempts were made to identify the cause for low per cent fruit set and closeness between these two varietal groups. There was no significant difference observed for pollen viability and per pollen germination in both the species which reconfirmed the close relationship between both the species. The results showed that *M. charantia* var. *muricata* is the wild progenitor of cultivated bitter gourd. The hybrids derived from both the species showed intermediate pattern of character expression. Therefore, the variability presents for quantitative and qualitative traits *M. charantia* var. *muricata* along with high crossability behaviors holds an immense potential for broadening the gene pool of cultivated bitter gourd. The polymorphic SSR markers identified between the parental lines of *M. charantia* var. *charantia* and *M. charantia* var. *muricata* could be utilised in marker assisted selection for mapping of economic traits.

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