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Full Length Research Paper

Cytomorphological and molecular characterization of interspecific F_1 hybrid of *Momordica dioica* Roxb. × *Momordica subangulata* subsp. *renigera* (G. Don) de Wilde

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Interspecific hybrid (F_1) between spine gourd (*Momordica dioica*) and teasle gourd (*Momordica subangulata* Blume subsp. *Renigera* (G. Don) WJJ de Wilde) produced through bagging and hand pollination showed vigorous growth habit with less fertility. Morphological parameters along with meiotic, mitotic chromosome behaviors and Randomly Amplified Polymorphic DNA (RAPD) analysis, confirmed interchanges of genetic characters in the new hybrids. The interspecific hybrid showed a vigorous foliage with intermediate flower characters than that of parental species. Self pollination and backcrossing of F_1 plants to either parent confirmed presence of both male and female sterility, that might be due to improper meiotic chromosome pairing. The characters like petal spot and time of anthesis (early morning) were similar to that of pollen parent while the diameter and size of leaves and flowers, pedicel length and plant growth were intermediate as compared to parents. Somatic chromosome analysis revealed 2n = 28 in *M. dioica* – the female parent, 2n = 56 in *M. subangulata* ssp. *renigera* - the male parent and 2n = 42 in F_1 hybrid of *M. dioica* × *M. subangulata* ssp. *renigera.* RAPD analysis of both the parents and F_1 hybrid confirmed more genetic affinity (60.95%) with female parent than the male parent (32.35%). Primer specific DNA markers confirmed the introgression of male genetic elements into the newly developed amphidiploids. Incorporation of male specific DNA markers into the F_1 hybrid is discussed. Preliminary evaluation indicates that the F_1 plants sprouted early, which is lacking in the mother parent and this character could be exploited by restoring of fertility by chromosome doubling.

Key words: *Momordica*, cytomorphology, F_1 hybrid, karyotype, RAPD markers.

INTRODUCTION

Spine gourd (*Momordica dioica* Roxb..) is an important vegetable with high food value, containing good amount of carotene (162 mg/100 g) and protein (3.1 g/100 g) amongst all Cucurbitaceous vegetables (Gopalan et al., 1982). It is of high market demand with a special delicacy for the people of Eastern India, it fetches premium price in the market. Organized cultivation of spine gourd is lacked, despite its demand, mainly due to its short harvesting period, low yield, tuber dormancy and lack of standard propagation technique. It sprouts during March to April and produces fruits from September to October and becomes dormant during winter (Manik et al., 2001; Joseph et al., 2009). Roots are tuberous, leaves are 4 to 10 cm long, cordate, acute, more or less 3 to 5 lobed and

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flowers with 5 sepals, gamosephalus, 5 petals, gamopetalus, 3 stamens and one inferior ovary. Fruits are 2.90 to 5.67cm long, shortly beaked, densely covered with soft spines. Individual fruit weights varied from 10 to 20 g.

Teasle gourd (*Momordica subangulata* Blume subsp. *Renigera* (G. Don) WJJ de Wilde) popularly known as kakrol, lacks extended harvest period but having, uniform and early sprouting and bigger fruit size (60 to 80 g). Leaves are 8 to 10 cm long, usually 3 lobed; flowers dioecious, large, whitish; fruit 7 to 10 cm long, ovate or oblong, covered with conical points, bright red when ripes and weighs 60 to 80 g. *Momordica* fruits with less spine, bigger fruit size with high protein and carotene content could be of high commercial value which can meet the market demand. Keeping this view, in this present study an attempt was made to cross *M. dioica* with *M. subangulata* subsp. *renigera* to seek possibilities of combining desirable attributes of both the species. Moreover, the chromosomal and RAPD analysis of F1 hybrid and the male and female partners were made, to confirm the transfer of parential genetical attributes to the offspring, if any, besides their morphological variations.

MATERIALS AND METHODS

Species of *M. dioica* and *M. subangulata* subsp. *renigera* were collected from the living collection of Central Horticultural Experimental Station (CHES), Bhubaneswar, India. The crossing was made in the center's experimental garden and all the F_1 hybrids were kept at the centre. Young leaves of the plants were collected from the field from both the parents and F_1 hybrids for the extraction of nuclear DNA for the study of random amplified polymorphic DNA (RAPD) analysis. Root tips were collected for chromosome study. Pollen grains were studied from the collected flower buds. The voucher specimens were identified and kept in the herbarium of the Orissa University of Agriculture and Technology, Orissa.

Crossing of species

Both direct and reciprocal crosses were made between *M. dioica* (spine gourd) and *M. subangulata* ssp. *renigera* (teasle gourd). Anthesis of *M. dioica* occurs between 6 to 8 P.M. while that in *M. subangulata* subsp. *renigera* is between 4 to 6 A.M. Therefore, the pollen of teasle gourd was collected at anthesis (morning) and stored at 7°C and used to pollinate female buds of spine gourd in the evening. The pollen of spine gourd was collected at anthesis (evening), stored at 7°C and dusted to the female flowers of teasle gourd in the subsequent morning.

Cytological analysis

Pollen sterility and meiotic analysis

For meiotic studies, staminate buds were fixed between 10.30 A.M. to 11.30 A.M. in 1:3 propionic acid: alcohol for 24 h, hydrolysed in 1N HCl at 60°C for 10 min, stained in Fuelgen solutionfor 3 to 4 h and squashed in 2% aceto-carmine and examined under light microscope and photographed in bright field, under a NIKON E 600 research microscope with an Evolution VF camera. The pollen grains with stained nucleus were counted as viable pollen while without nucleus or unstained pollens were counted as sterile pollen and calculated accordingly.

Somatic chromosome analysis

Fresh healthy ~ 2 mm long root-tips were pre-treated in half-saturated p-dichlorobenzene and asculine mixture for 3 h at 18°C, followed by overnight fixation in propionic acid:ethanol (1:3) for 24 h, hydrolysed in 5 N HCl at 4°C for 6 min, stained in 2% propionic-orcein and squashed in 45% propionic acid. For both the parents and hybrids, 10 well-scattered metaphase plates were selected for chromosome number determination. Well scattered metaphase plates were selected for karyotyping of each specie and hybrid. Total chromosome length and volume of a karyotype was calculated by applying the formula $¶r^2h$, where 'r' and 'h' represent the radius and the length of the chromosome respectively. Form percentage (F%) of individual chromosome was calculated following the method of Das and Mallick (1993). Total form percentage (TF%) of a karyotype was the average of sum total F% of a karyotype. Mean values of total genomic chromosome length and total genomic chromosome volume with standard error were calculated.

RAPD analysis

Nuclear DNA extraction

Genomic DNA was isolated from young leaves of both the male and female parents as well as F_1 hybrids of *Momordica* plants using the standard CTAB protocol as described by Saghai-Moroof et al. (1984). 5 g of leaves were ground to fine powder in liquid nitrogen and suspended in 2 volumes of CTAB extraction buffer [2% CTAB (cetyl trimethyl ammonium bromide), 100 mM Tris HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl and 2% ß-mercaptoethanol]. The suspension was incubated at 60°C for 1 h, extracted with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 10,000 g for 15 min. The aqueous phase was transferred to a new 50 ml tube and DNA was precipitated with two third volume of chilled isopropanol. The DNA was spooled out and dissolved in $T_{10}E_1$ (10 mM Tris-HCl, 1 mM Na_2EDTA, pH 8.0) buffer. The DNA was purified again with RNAse A (Sigma, 10 µg ml^{-1}) at 37°C for 1 h followed by phenol : chloroform (1:1) extraction and precipitation in chilled ethanol (2.5 volume) in the presence of 3 M sodium acetate (pH 5.2). DNA was spooled out, washed in 70% ethanol, air dried and dissolved in $T_{10}E_1$ buffer. DNA concentration was estimated in Fluorimeter (BIO-RAD) with Hoechst 33258 dye and was diluted to a final concentration of 25 ng $µl^{-1}$ using $T_{10}E_1$ buffer to use as template DNA for RAPD analysis.

PCR and RAPD analysis

RAPD profiles were generated by using single decamer primers obtained from Operon Technologies, Alameda, USA, in polymerase chain reaction (PCR) following the standard protocol of Williams et al. (1990). Ten each of four primers of series OPA, OPC, OPD and OPN (10 × 4) were used for RAPD analysis. Each PCR reaction cocktail amplification reaction mixture contained 25 ng of template DNA, 200 µM of each dNTP, 25 ng of primer, 0.5 unit of Taq DNA Polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) and 10 × PCR assay buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, pH-9.0) in a final reaction volume of 25 µl. The reaction was carried out in a GeneAmp PCR System 2400 thermal cycler (Perkin Elmer, USA). The first cycle consisted of denaturation (92°C) for 4 min,

Table 1. Morphological characters of the parents and their F_1.

Sl. No.	Morphological characters	*Momordica dioica* (♀)	F_1	*Momordica subangulata* subsp. *renigera* (♂)
1	Time of sprouting	Mar-Apr	Jan-Feb	Jan-Feb
2	No. of branches / plant	3.00	7.00	4.00
3	Stem circumference (cm)	0.93	1.16	1.53
4	Internodes length (cm)	6.75	8.88	5.17
5	Leaf surface	Smooth	Smooth	Rough
6	Leaf lobe number	Trilobate	Trilobate	Entire
7	Leaf length (cm)	9.66	15.76	10.68
8	Leaf breadth (cm)	6.84	9.14	5.78
9	Pedicel length (cm)	2.22	10.64	6.12
10	Corolla colour	Rich yellow	Rich yellow	Whitish yellow
11	Black spot on the base of corolla	Absent	Present	Present
12	Corolla length (cm)	2.36	4.4	4.9
13	Corolla width (cm)	0.86	2.42	3.28

primer annealing (42°C) for 1 min and DNA polymerisation (72°C) for 2 min. In the next 45 cycles the period of denaturation was maintained at 1 min while the duration of primer annealing and DNA polymerization was same as in the first cycle.

The last cycle consisted of only primer extension (72°C) for 8 min. The amplified products were stored at 4°C and separated by electrophoresis on 1.5% agarose gel in 1 × TAE buffer for 4 h at 55 V. To determine the size of the polymorphic fragments, low range DNA Ruler Plus (Bangalore Genei, India, was used as size standard. DNA fragments were visualized by staining the gel with ethidium bromide and the image was captured in Gel Doc G700 (BioRad, USA) for documentation. Only those amplification products that consistently appeared in three replications were scored for further analysis.

Statistical analysis for RAPD data

In RAPD analysis, the presence or absence of the bands was taken into consideration, but the differences in their intensity were ignored. From RAPD data, a binary matrix was obtained and calculated using the multivariate analysis program NTSYS-pc (Rohlf, 1993). The binary matrix was transformed into a similarity matrix using Jaccard's coefficient. From this matrix a dendrogram was obtained by cluster analysis, following Unweighted Pair Group using Arithmetic Averages (UPGMA) method, using NTSYS version 1.7, Exeter Software, New York, USA.

RESULTS

Cytomorphological analysis

The character of the parents and their progeny was recorded in Table 1. The study indicated the possibility of utilizing the extended harvesting period of teasle gourd to spine gourd. The characters namely diameter and size of leaves and flowers, peduncle length, plant growth habit and fruit character were intermediate (Figures 1, 3a, b and c) while the characters like early sprouting, bulls eye nectar guide corolla and time of anthesis (early morning) found inherited from the male parent (teasel gourd) to the F_1 plants (Figures 2a, b and c) as compared to parents. In F_1 plants, the pollens were mostly sterile (Figures 4a, b and c) with ~16.75% viable pollen (stained with acetocarmine). Meiotic metaphase I of *M. dioica*, showed 14 bivalents (Figure 5a). Pollen mother cells (PMC) of *M. subangulata* subsp. *renigera* at metaphase I, exhibited the presence of both bivalents and quadrivalents with a mean of 25.8 bivalents and 1.1 quadrivalents (Figure 5b). The PMCs of the triploid hybrid between *M. dioica* and *M. subangulata* subsp. *renigera* produced an average of 13.84 univalents, 12.76 bivalents and 0.88 trivalent (Figure 5c). Meiotic abnormalities like unequal distribution of chromosomes and laggards were also observed at anaphase I (Table 2).

Detailed analysis of somatic chromosomes of female (*M. dioica*) and male (*M. subangulata* subsp. *renigera*) showed somatic chromosome number $2n = 2\times = 28$ (Figure 6a) and $2n = 4\times = 56$ (Figure 6b) chromosomes respectively; whereas, the F_1 showed $2n = 42$ (Figure 6c). On the basis of the size of the chromosome and the position of the constrictions, a number of chromosome types were found common with the female, male and hybrid genotypes though they differed from each other in the minute structural details of the karyotype. A general description of the representative types of chromosomes is given as:

Type A: Chromosomes are medium sized with two constrictions in nearly median to median and nearly sub median to sub median in position respectively.
Type B: Medium to small sized chromosomes with nearly median to median primary constrictions.
Type C: Chromosomes are medium to small chromosomes with sub median primary constrictions to nearly terminal.

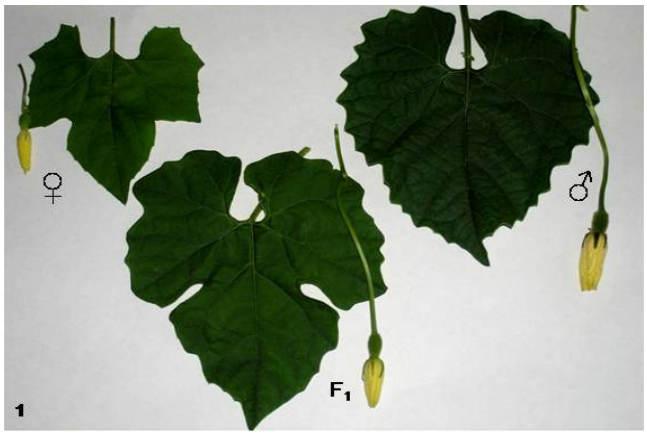


Figure 1. Leaf and peduncle morphology of *Momordica dioica* (♀), *M. subangulata* subsp. *renigera* (♂) & F_1 hybrid.

Figures 2a-c. Flowers characters of (a) *M. dioica,* (b) *M. subangulata* subsp. *renigera* and (c) F_1 hybrid.

Figure 3a-c. Fruit characters of (a) *M. dioica,* (b) *M. subangulata* subsp. *renigera* and (c) F_1 hybrid.

The chromosomes in all the species were found very small in size. Somatic chromosome number was 2n = 56 in *M. subangulata* subsp. *renigera*, while *M. dioica* showed 2n = 28 chromosomes and the F_1 hybrid (*M. dioica* × *M. subangulata* subsp. *renigera*) having 2n = 42 chromosomes (Table 3, Figures 6a, b, c and 7). Detailed karyotype analysis of all *M. dioica*, *M. subangulata* subsp. *renigera*, and interspecific hybrids, showed numerical and structural alterations of chromosomes. Though all the three types of chromosomes were present in all the studied genotypes, numerical differences were more prominent among them.

The karyotype formula of all the genotypes revealed definite differences in the chromosome structure (Table 3). Type A, B and C chromosomes were present in all the genotypes but the dose difference of each type of chromosome was found prominent (Figures 6a, b, c and 7). Two numbers of Type A chromosomes were present in *M. dioica* while in the tetraploid species *M. subangulata* subsp. *Renigera* (2n = 56), two pairs of secondary

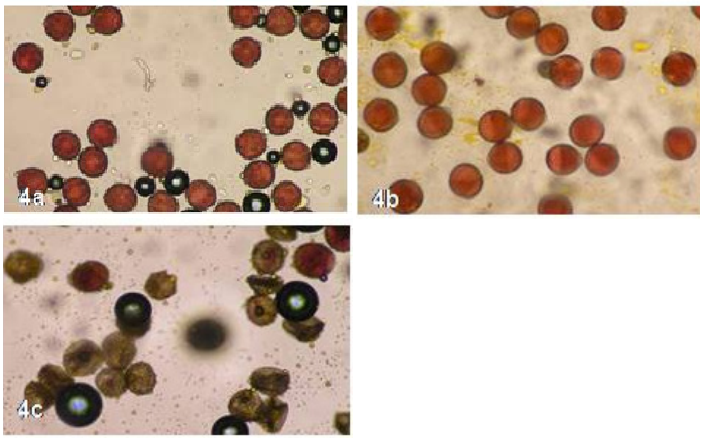
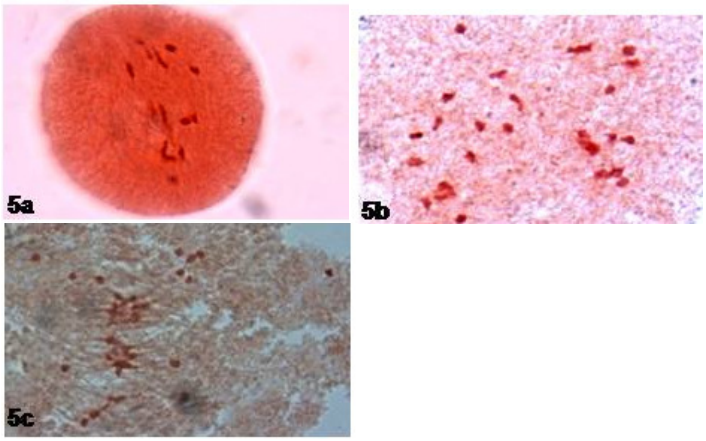
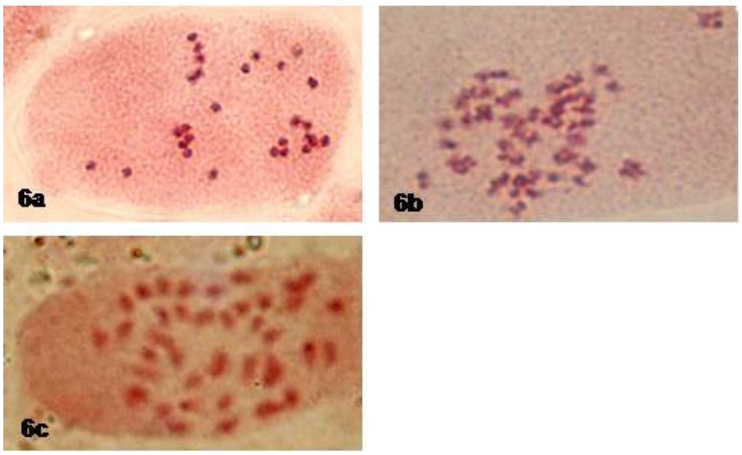


Figure 4a-c. Pollen grains showing meiotic metaphase I of (a) *M. dioica* (n = 14II), (b) *M. subangulata* subsp. *renigera* (n = 28II) and (c) F_1 hybrid (n= 12I + 12II + 2III).



Figures 5a-c. Pollen mother cells showing meiotic metaphase I of (a) *M. dioica* (n = 14II), (b) *M. subangulata* subsp. *renigera* (n = 28II) and (c) F_1 hybrid (n = 12I + 12II + 2III).



Figuress 6a-c. Somatic chromosomes (a) *M. dioica* (2n = 28) (b) *M. subangulata* subsp. *renigera* (2n = 56) and (c) F_1 hybrid (2n = 42).

Table 2. Meiotic chromosome configurations in pollen mother cells of *Momordica* species and their hybrids.

Species/cross	Chromosome association							
	Univalents		Bivalents		Trivalents		Quadrivalents	
	R	M±SE	R	M± SE	R	M	R	M± SE
M. dioica	-	-	14	14	-	-	-	-
M. subangulata subsp. *renigera*	-	-	24-28	25.8±0.35	-	-	0-2	1.1±0.18
F_1 (*M. dioica* × *M. subangulata* subsp. *renigera*)	12-16	13.84±0.22	12-15	12.76±0.25	0-1	0.88±0.13	-	-

R-Range, M-mean, SE-standard error.

constricted chromosomes were present. Variation of Type B and C chromosomes was very prominent too. Total genomic chromosome length and volume varied significantly between two species as well as hybrid (Table 3).

RAPD analysis

Out of the total 40 primers (OPA, OPC, OPD, OPN) used, the 12 most informative primers were selected (Table 4). Figures 8a, b and c depict the RAPD profiles of male and female parents, as well as hybrid of Momordica, amplified by different selected primers showed distinct RAPD profile of both the parents and hybrid. Each RAPD marker locus was expressed as two alleles; presence or absence of the band. Frequency of polymorphic loci was estimated in all genotypes considering three criteria for primer selection: (1) Reproducibility (2) Number of polymorphic loci per assay and (3) Levels of polymorphism detected in parents and hybrids. RAPD analysis showed that amplicons number varied from 6 to10 major fragments with their size range from 200 to 2000 bp. A total of 150 amplification products were obtained out of which 70 were polymorphic. The percentage of polymorphism observed 46.66% in between *M. dioica* and *M. subangulata* subsp. *renigera* × *M. dioica* hybrid, 60.95% in between *M. dioica* and *M. subangulata* subsp. *renigera* and 72.95% in between *M. subangulata* subsp. *renigera* and × *M. dioica* hybrids. As the polymorphisms were detected in RAPD bands the presence or absence of a particular amplicon could be potential dominant markers. In OPA-18 primer a monomorphic band of 1000 bp found common in both the parents and hybrids (Figure 8a) but only a male marker band of ~1150 bp was segregated to F_1 hybrid.

In OPC-05 primer a multiple copy prominent marker band of 900 bp remained same in hybrid, like its female parents (Figure 8a) but distinctly ~1050 bp band found introgression in the new hybrids from male donor which was not found in female parent. Similarly, OPD-20 identified as an introgression of a prominent dominant marker band of *M. subangulata* subsp. *renigera* (male) of 400 bp which was found highly enhanced in F_1 with the presence of almost all the DNA bands of *M. dioica* (female). Incorporation of 850, 1100 bp bands amplified by OPD-02 primer in *M. dioica* in the F_1 hybrid within a female genetic background was also a noticeable feature.

However, in the hybrid missing or decrease of intensity of few bands were noted (like 1250 bp in OPC-05, 1050 and 400 bp in OPD-20, 650 bp in OPD-02) in hybrid as compared to its female parents (Figures 8a, b and c). The dendrogram obtained from the cluster analysis reflected the mixture of genetic characters of both the parents in the new inter-specific hybrid

DISCUSSION

The F_1 hybrid resembles *M. subangulata* subsp. *renigera* for anthesis time (morning), bull's eye nectar guide, petal venation, colour of the calyx and filament, saucer shaped floral receptacle while trilobite leaves and smooth leaf surface characters resembles with *M. dioica*. F_1 hybrid registered higher number of branches / plant, leaf size, internode length and pedicel length than their parents while it was intermediate in corolla length, corolla width and stem circumference. Crosses between tetraploid (female) and diploid (male) *Momordica* species found significantly lower seed setter, as well as germination while it was evident that use of diploid as female parent with tetraploid male in the crossing, resulted in good seed set and high germination. Mohanty et al. (1994) and Mondal et al. (2006) also reported a high success rate (>50% fruit set) between *M. subangulata* subsp. *renigera* and *M. dioica*. The pollen grains of the hybrids were highly sterile. This is to be expected if the chromosomes of the two species were poorly homologous, that might be leading to poor pairing and disjunction of chromosomes during meiosis which we observed. This might be leading to aneuploidy reflecting non fertile pollen formation in F_1 hybrid.

Meiotic analyses of *M. dioica* showed 14 bivalents (Figure 5a) and are in agreement with the earlier report by Richharia and Ghosh (1953). In our study, *M. subangulata* subsp. *renigera* at metaphase I showed bivalents as well as quadrivalents. Bivalents were significantly higher (24 to 28/cell) than quadrivalents (0 to 2/cell) and in a few cells 28 bivalents were also observed.

Table 3. Somatic chromosome parameters and karyotype data of *M. dioica* (♀ parent), *M. subangulata* subsp. *renigera* (♂) parent) and F_1 (*M. dioica* × *M. subangulata* subsp. *renigera*).

Species	2n	No. of SCC	Karyotype formula	Total chromosome length (µm)	Total chromosome volume (µm^3)	Range of chromosome length (µm)	Average chromosome length (µm)	Average chromosome volume (µm^3)	Form percentage (F%)
M. dioica	28	2	2A+16B+10C	38.53±0.95	19.84±0.24	0.85-2.17	1.38	0.71	43.88
M. subangulata subsp. *renigera*	56	4	4A+14B+38C	51.88±1.40	26.71±0.42	0.52-1.26	0.93	0.48	32.26
F_1 (*M. dioica* × *M. subangulata* subsp. *renigera*)	42	2	4A+20B+18C	42.05±1.25	22.15±0.55	0.52-2.10	1.00	0.53	35.42

SSC= number of secondary constricted chromosome.

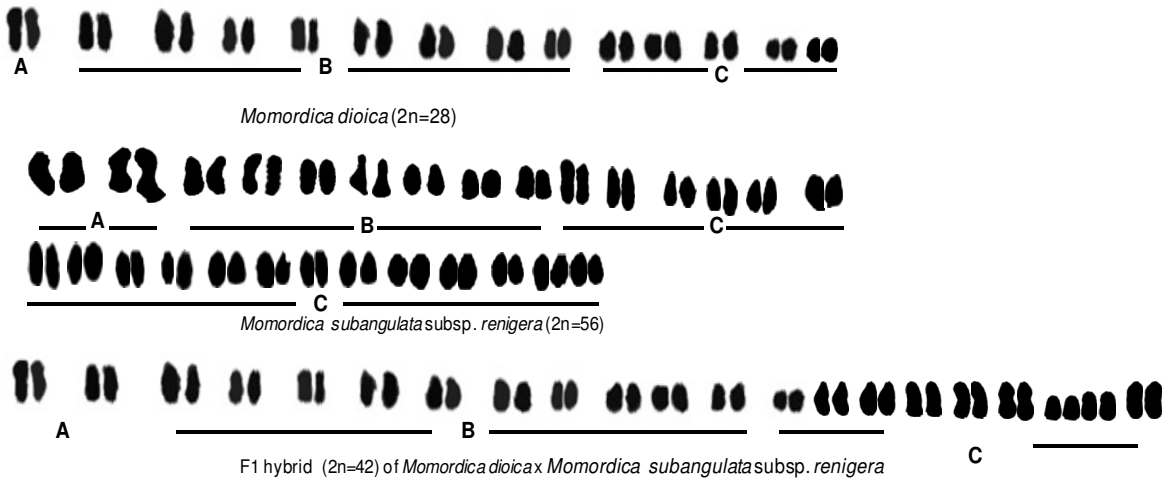


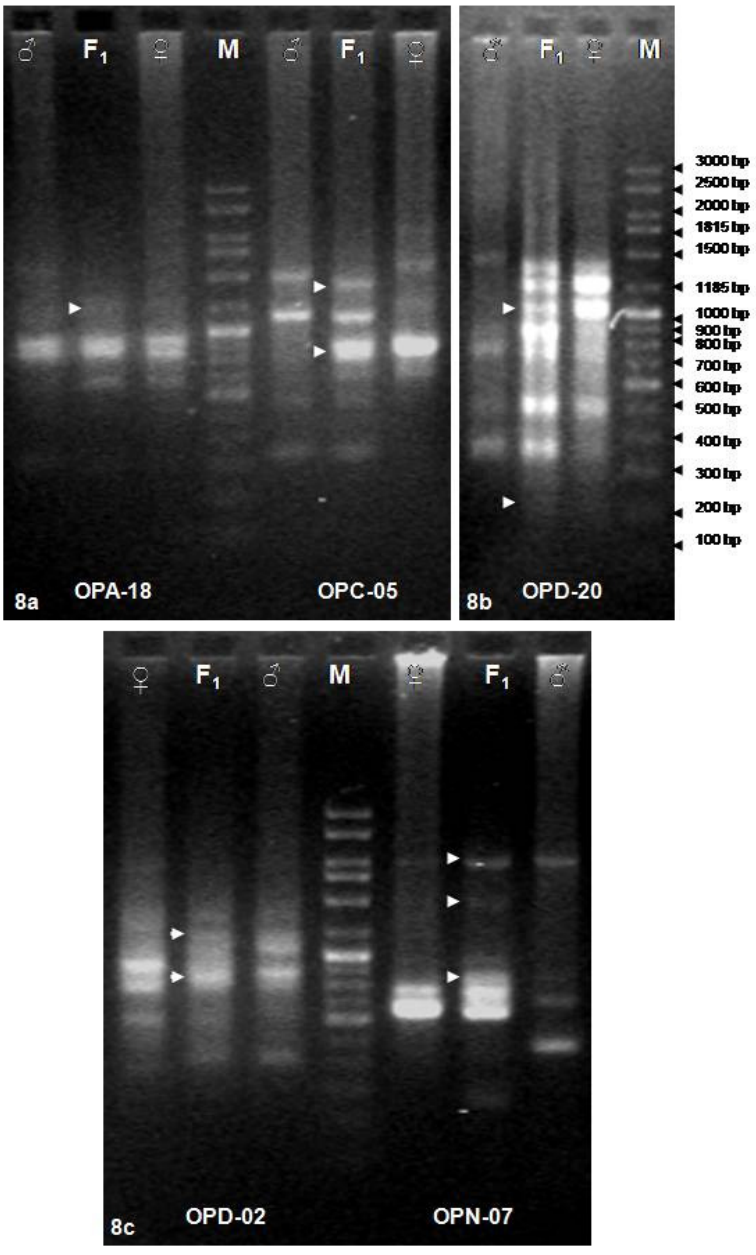
Figure 7. Karyotype of (a) *M. dioica*, (b) *M. subangulata* subsp. *renigera* and (c) F_1 hybrid.

Table 4. RAPD profiles of *M. dioica* (♀ parent), *M. subangulata* subsp. *renigera* (♂) parent) and F1 (*M. dioica* × *M. subangulata* subsp. *renigera*) using different Operan 10mer primers.

Operan primer name	Primer sequence 5'…….3'	No. of total bands	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands
OPA-18	AGGTGACCGT	17	10	6	1
OPA-08	GTCACGTAGG	11	6	5	0
OPA-10	GTGATCGCAG	5	0	5	0
OPC-05	GATGACCGCC	19	6	9	4
OPC-07	GTCCCGACGA	4	0	4	0

Table 4. Contd.

OPC-19	GTTGCCAGCC	8	2	6	0
OPD-02	GGACCCAACC	22	10	11	1
OPD-18	GAGAGCCAAC	9	5	4	0
OPD-20	ACCCGGTCAC	23	13	5	5
OPN-07	CAGCCCAGAG	15	10	2	3
OPN-14	TCGTGCGGGT	10	7	3	0
OPN-18	GGTGAGGTCA	6	1	5	0



Figures. 8a-c. RAPD profiles of *M. dioica* (♀ parent), *M. subangulata* subsp. *renigera* (♂ parent) and F_1 hybrid of *M. dioica* × *M. subangulata* subsp. renigera. M = DNA marker (100bp low range DNA ladder plus, Bangalore Genei, India): (a) amplified with OPA-18 and OPC-05 (b) amplified with OPD-20 and (c) amplified with OPD-02 and OPN-07.

Sinha et al. (1997) reported that, the number of bivalents was significantly higher than those of tri- and quadrivalents with occasional presence of 28 bivalents. In F_1 hybrids few quadrivalents with bivalents and univalents were recorded. Karyotype analysis of F_1 hybrids and both the parents revealed that, F_1 is a triploid (2n = 42) while the female parent was diploid (2n = 28) and male parent was tetraploid (2n = 56). Interspecific hybrid between *Vigna radiata* and *Vigna umbellata* also was reported by Machado et al. (1982) where in Metaphase I 13.40 univalents, 3.95 bivalents and 0.18 trivalents were observed, which are in accordance with our findings. Karyotype formula confirmed that in F_1, chromosome complements were mixed; however, the exact localization of chromosomal segments can be detected with the application of FISH (Flourescent Chromosome *in situ* Hybridization) techniques (Schwarzacher et al., 1992; Heslop-Harrison et al., 2003).

RAPD analysis was used here and did not detect possible changes in allele frequencies, except when the allele detected as the RAPD band was completely lost (Bamberg et al., 2000). Monomorphic bands in F_1 and both parent species at 1000 bp and at 900 bp, amplified with OPA-18 primer and OPC-05 respectively. The male marker band 1150 bp in OPA-18, 1050 bp female marker band in OPC-05 and 400 bp male marker in OPD-20 are the important introgression DNA bands in F_1 hybrid those are responsible for different morphological character combinations. These bands might be interesting for their physical localization on chromosomes both on meiotic and mitotic chromosomes of F_1 as probes. Nevertheless, it was evident that F_1 hybrid was the combination of both characters of female and male progenitor and finally the F_1 fruits size quite large and having more delayed sprouting time which is important from an agronomic point of view. The confirmation of hybridity by chromosome observation and RAPD analysis in interspecific hybrid between leek (*Allium ampeloprasum* L.) and garlic (*Allium sativum* L.) by Yauagino et al. (2004) was in accordance to our findings. Moreover, the hybrid could be propagated vegetatively by planting cuttings; hence, it could be introduced for cultivation as new hybrids, keeping aside the meiotic segregation of the hybrid in F_2 generation.

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