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Characterization of a fertile backcross progeny derived from inter-specific hybrid of *Momordica dioica* and *M. subangulata* subsp. *renigera* and its implications on improvement of dioecious *Momordica* spp

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ABSTRACT

A fertile female backcross progeny was produced from an inter-specific hybrid [teasel gourd (*Momordica subangulata* Blume subsp. *renigera* (G. Don) WJJ de Wilde) × spine gourd (*Momordica dioica* Roxb)] by backcrossing the F_1 with the female parent. Agro morphological and biochemical traits of the first generation backcross (F_1 ♀ BC) confirmed interchanges of genetic material. The F_1 ♀ BC bear more resemblance to maternal parent than the paternal parent and exhibited substantial heterosis for number of flowers and yield per plant. In F_1 ♀ BC, fruit set was significantly higher when pollinated with teasel gourd (84%) while the fruit set was very low upon sibbing (36%) and pollination with spine gourd (64%). The fruits of F_1 ♀ BC had little variation for shape from teasel gourd when pollinated with teasel gourd pollen, while fruits obtained from sibbing and dusting of spine gourd pollen were deformed and smaller in size. The proximate and phytochemical analysis revealed that the F_1 ♀ BC was intermediate between the original parents in its biochemical traits and antioxidant activity while mineral content was found to be higher or similar to its parents. The present study shows the potential for improving *Momordica* species through inter-specific hybridization followed by restoration of fertility by backcrossing.

1. Introduction

Spine gourd (*Momordica dioica* Roxb. ex Willd.) is a highly nutritious (Gopalan et al., 1982) high value wild edible vegetable with domestication potential. Commercial cultivation of spine gourd is not picking up mainly due to lack of standard propagation technique for mass multiplication. Seed dormancy and pre-flowering sex determination are major limiting factor constraining commercial cultivation of spine gourd (Bharathi et al., 2007). Though spine gourd can be propagated through rooted stem cuttings (Maharana and Tripathy, 1996; Ram et al., 2001, 2002) and tuberous roots (Ram et al., 2002; Bharathi et al., 2007), the tuberous roots produced from rooted vine cuttings do not have ratoon potential and mass multiplication through tuberous root cuttings is difficult (Joseph et al., 2009). Teasel gourd (*M. subangulata* Blume subsp. *renigera* (G. Don) WJJ de Wilde), on the other hand, is a commercially cultivated vegetable in many South East Asian countries, having adventitious root tubers through which a large number of plants can be produced. In addition to its high propagation efficiency, teasel gourd also possess favourable traits like bigger fruits (40 to 100 g), higher fruit yield (8–10 kg/plant) and extended cropping period (Joseph, 2008).

Movement of genes responsible for adventitious root character from *M. subangulata* subsp. *renigera* to *M. dioica* through hybridization for improved propagation efficiency has been made possible and the tuberous adventitious root character was expressed in F_1 hybrids (Bharathi, 2010). However, interspecific hybridization has not been successfully utilized for the improvement of dioecious *Momordica* species as the hybrids produced were highly sterile (Mohanty et al., 1994; Maharana and Tripathy, 1996; Mondal et al., 2006; Bharathi et al., 2010, 2012). Chromosome doubling of F_1 or backcrossing of F_1 to its parents may help to restore the fertility of the sterile hybrids developed between teasel gourd and spine gourd

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(Bharathi et al., 2012). Therefore, the present study was taken up to restore the fertility of the sterile F_1 hybrid through backcross.

2. Materials and methods

2.1. Production of F_1 ♀ BC

The parental species and their inter-specific (F_1) hybrid [teasel gourd (P_1) × spine gourd (P_2)] developed in 2008 (Bharathi et al., 2010) were used for developing the backcross progenies. A total of 1000 back crosses were attempted between F_1 hybrid and parents. Pollination of female flower of the F_1 hybrid was done in the morning hours (6–7 AM) using freshly collected pollen from *M. subangulata* subsp. *renigera* and stored pollen (7 °C) of *M. dioica* collected in previous day evening at anthesis (6–7 PM). The seeds extracted from successful crosses were cleaned and dried. Before sowing, the seeds were stored for approximately three months to overcome any dormancy. The seed coat was removed and the seeds of both male and female parent backcross were sown in polypropylene trays with appropriate potting media (80% decomposed coir dust coco-peat:vermiculite:river sand, 2:1:1). Thirty-day-old seedlings were transplanted in the main field. Subsequently, the plants were multiplied through vine cuttings and used for further evaluation.

2.2. Agro-morphological traits

The seeds of F_1 ♀ BC only germinated while none of the seeds extracted from male parent backcross germinated. Fifty plants each of parental species and F_1 ♀ BC were grown in 5 replicates of 10 plants each in Randomized Block Design (RBD) over a period of 2 years. A random sample of 5 plants per replication was taken for recording the different agro morphological traits. The pollen of the parents and hybrid progeny were collected on glass slides by gentle tapping of the anther and was stained in 2% acetocarmine solution on first day of anthesis to determine the fertility. Pollen fertility was calculated based on the number of densely stained pollen and total number of pollen. Twenty-five flowers of F_1 ♀ BC were sib-mated. Further, 25 flowers of F_1 ♀ BC were pollinated with each of the parents to determine the fruit set. Data on fruit set was expressed as fruits developed in proportion to flowers pollinated. The fruit biometric characters were recorded 15 days after pollination and yield of the parents and hybrid progeny were recorded.

2.3. Nutritional traits

Five plants were selected at random each from parents and F_1 ♀ BC for fruit sample collection for biochemical analysis. A part of this sample was kept aside under refrigeration until use for antioxidant assay. The other part of the samples was thoroughly washed with water, dried at 65 °C, ground and used for mineral analysis.

Dried samples were digested using nitric:perchloric acid (1:3) until clear solution was obtained, made to 100 ml and used for mineral analysis. Phosphorus and calcium levels were evaluated by the Association of Official Analytical Chemists' methods (AOAC, 2005). Flame photometry was used for Na and K and atomic absorption spectrometry (AAS) for the remaining minerals studied and the results were expressed on dry weight basis.

The moisture, crude protein and fat contents were determined by Association of Official Analytical Chemists' methods (AOAC, 2005). Moisture was determined using the oven dry method, by drying 5 g sample in an oven at 105 °C for 3 h. Protein was estimated by micro-Kjeldahl method using 6.25 factor to calculate protein content from nitrogen content. Fat was determined by petroleum ether extraction in a Soxhlet apparatus. Total and reducing sugars (Ranganna, 2001), total phenolic compounds and total flavonoids (Shivashankar et al., 2012) were estimated as described. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was assayed according to Brand-Williams et al. (1995), with some modifications. Ferric reducing antioxidant power (FRAP) was estimated following the method of Benzie and Strain (1996), based on the increase in absorbance at 593 nm.

2.4. Statistical analysis

Data on agro-morphological and biochemical traits of parents (P_1, P_2) and F_1 ♀ BC were subjected to one-way ANOVA and Fishers LSD multiple comparison test was applied to compare the factor level differences. The analyses were performed using SPSS (SPSS software for Windows release 17.0, SPSS Inc., USA).

3. Results

3.1. Production of F_1 ♀ BC

The inter-specific F_1 hybrid was sib-mated and backcrossed to the parents. There was no fruit set with viable seeds in F_1 hybrids upon sib mating. Though most of the ovaries were swollen a little to give small fruits but, none of them was with any seeds. These swollen ovaries turned yellow and subsequently dried. Only three

Table 1a
Agro-morphological characters of *M. subangulata* subsp. *renigera* (P_1), *M. dioica* (P_2) and 1st generation backcrosses to *M. subangulata* subsp. *renigera*. Data is presented as mean ± SE.

	P_1	F_1 ♀ BC	P_2
Month of sprouting	Feb.–Mar.	Feb.–Mar.	Apr.–May
Dormancy period	Nov.–Jan.	Nov.–Jan.	Oct.–Mar.
Anthesis time	5.00–6.00 h	2.30–3.30 h	17.00–19.00 h
Leaf lobing	Entire	Angled to lobed	Deeply lobed
Petal spot	Present	Present	Absent
Corolla venation	Embossed	Embossed	Less prominent
Calyx colouration	Purple	Purple	Light cream yellow
Thecae colour	Black	Black	White
Shape of receptacle tube	Saucer	Saucer	Cup
Tuberous adventitious roots	Present	Present	Absent
Fruit set (%)	15.33[a] 98.00[b]	02.00[a] 36.00[b] 84.00[c] 64.00[d]	79.92[a] 94.50[b]
Pollen fertility (%)	80.66 ± 1.16	13.60 ± 0.76	89.70 ± 0.42
Fruit weight (g)	48.12 ± 1.15	12.51[b] ± 0.35 38.30[c] ± 0.79 20.20[d] ± 0.70	11.01 ± 0.49

[a] Natural pollination.
[b] Manual pollination (sib).
[c] Manual pollination with teasel gourd.
[d] Manual pollination with spine gourd.