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Phylogenetic Relationship of Different Kakrol Collections of Bangladesh

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Abstract: Collections of two different types of wild kakrol and cultivated kakrol were grown in the experimental field, described and identified. The two wild kakrol types were detected as *Momordica dioica* (small fruit type) and *M. cochinchinensis* (big fruit type), while the cultivated type was found to posses similarities and dissimilarities with both the types. Genetic analysis indicated that the cultivated kakrol as completely isolated from both the wild kakrol species. The big fruited *M. cochinchinensis* is phylogenetically more different than *M. dioica* from cultivated kakrol. Both pre-and post-zygotic isolation has been indicated. However, cultivated kakrol and *M. dioica* proved to be isolated more by post-zygotic mechanisms. The hybrids could be recovered at a low rate which were sterile and failed to reproduce successfully. The cultivated kakrol this proposed a separate status. The name *Momordica hybrida* has been proposed as the cultivated kakrol has traits common to both the wild types, seemed to be an amphidiploid arising from *M. dioica* and *M. cochinchinensis*. Morphological characters of the cultivated type are common to both of the species: *Cochinchinensis* and *Dioica*. Large leaf, white flower, eye spot at petal, base, gland absent in leaves, position of bract, cylindrical fruits, grey-black sculptured seed length of petiole, 2-fid filament, fruit size. The sterility of the progeny from crosses supported this view in that two wild kakrol types are diploid and the cultivated kakrol derived tetraploid. The scope of tissue culture in rescuing the hybrid embryos gave somewhat negative result. The wild kakrol types were observed to set fruits and seeds normally in nature. The cultivated kakrol flowers lack pollinations and need artificial pollen transfer. The presence of pollinator guides as bright spots near the petal base suggest possibility of origin at a stable insect pollinator.

Key words: *Momordica*, hybridization, kakrol, Bangladesh

INTRODUCTION

Teasle gourd (*Momordica dioica* Roxb.) locally known as kakrol is a popular vegetable in Bangladesh. Recently teasle gourd has become a major vegetable in Bangladesh because of the high export potential and demand in the internal market. It has good nutritional value as well as having 33 mg Ca, 42 mg P, 4.6 mg Fe and 1620 μg carotene per 100 g of edible portion and a high amount of vitamin C (Bhuiya *et al*., 1977). As a crop, kakrol has a number of problems, including poor natural pollination of female flowers and low yield. Fruits become inedible at maturity owing to the presence of large number of hard seeds. Production of tuberous root pieces per plant is low; 10-20 tuberous pieces are produced per year. Germination of seeds is very difficult or impossible because of the hard seed coat (Rashid, 1976b). Moreover, it is impossible to predict the sex of seed-produced plants before flowering. The plants are dioecious requiring artificial transfer of pollen from male to female flower. There being no natural pollinator, hand pollination is necessary. The wild genotypes of kakrol are observed to set plenty of fruits in nature, thus may have pollinators in the nature. Under cultivation, both the male and female plants are often grown together at a ratio of 1 male: 15 female (Rashid, 1976b; Vijay, 1978) to ensure enough pollen. Flowers started to appear 1-2 months after planting and continue until the senescence of the vines (Hossain and Rashid, 1974). There are some close relatives of kakrol, like *M. charantia* Linn., *M. balsamina* L., *M. cochinchinensis* Spring and *M. tuberose* Roxb. Study of pollination, fruit-setting and interspecific as well as intraspecific crossability is important for the improvement of kakrol. There may be scope of transfer useful characters from its close relatives, for example in between *M. diocia* (cultivated) and small wild kakrol (collected from Barind area in Bangladesh). The study of reproductive biology of the cultivated and wild kakrol was therefore undertaken.

This study was undertaken with a view to understand reproductive biology and interrelationship between various collections of teasle gourd (*Momordica dioica* Roxb.). The information will be utilized to improve quality and production of rates of kakrol. Eaelier studies indicated that production of hybrids will be difficult due to germination failure of the hybrid seeds.

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MATERIALS AND METHODS

The materials consisted of seven female and five male plants of small wild kakrol (root tuberous), were collected from wild populations at North Western district of Chapai Nawabganj in Bangladesh during 2001 and 2002. Local farmers and village people reported that the plant was locally known as Bon-kakrol (= wild kakrol). The fruits used as a vegetable of poor womenfolk. Six female and one male plants of cultivated kakrol (root chain tuberous) were collected from Brahmanbaria were used. Seeds of large wild kakrol were collected from the district of Bagerhat in Bangladesh during the winter season of 2002. During the summer of 2003, the parents as well as seeds obtained from crosses between these were grown in the Botanical Garden of Rajshahi University. The tuberous of small wild and cultivated kakrol genotypes were planted in pots, containing a soil medium of sand and decomposed cow dung of equal proportion during 2002. The tuberous roots began to sprout within 4-12 days after planting. Seeds of big wild kakrol were also planted in earthen pots. The seedlings were transplanted in the field after hardening. Kakrol is a dioecious plant, so there was no need for emasculation. All the selected male and female flowers to be used for pollination were bagged. Pollen transfer was made by taking the male flower to the female plants and by rubbing the dehisced other to the stigma of the female flower. After pollen transfer, the female flower was labeled and bagged. The various morphological, floral, fruit and seed characters were recorded from the flowering plants and mature fruits/seeds. The characters scored were: vine characters, leaf characters, fruit and seed measurements, length, weight of fruits and seed germination. The technique of somatic regeneration from callus through tissue culture was attempted to rescue embryo from hybrids and to establish a protocol for rapid generation and standard media were adopted. The collected data were analyzed following standard bio-statistical techniques.

RESULTS

Taxonomic description

The small wild kakrol: The wild kakrol studied in the study-sites is an annual plant, appearing before the onset of the monsoon during the month of March. The plants flower and fruit till the end of the monsoon and die after November/December leaving the under ground tuberous roots for perennation. The plant is a medium climber 5 to 7 m in length growing on the shrubs and tall grasses. The lower stem is brown/grey in the colour and corky, the young stem in green. The leaves are cordate, more or less 3 to 5 lobed, glabrous, delicate, with petiole. The plants are dioecious, flowers unisexual, solitary on long peduncles. Bract is large and copious in male flowers and is near the base of the peduncle. Flowers small, the petals are pale yellow, without any spots at the base. Male flowers with three stamens, the two anthered filament is bi-fid half any down. Female flowers have globes ovary, long style and three lobed stigma. Ovules are many on horizontal placentas, fruits are globes with soft hairs, green when unripe but bright yellow when ripe. The ripe fruits open in three irregular pieces, seeds 10-20, compressed, smooth surfaced with bright red aril. The description of the present materials agrees well with the account of *Momordica dioica* of Prain (1903) and Hooker (1961). But the wild kakrol was found to differ from the cultivated kakrol also classified as m. dioica (Hossain and Rashid, 1974; Rashid, 1976a; Hossain *et al*., 1987a, b; Ali *et al*., 1991; Fakir *et al*., 1990, 1992).

Comparison with the cultivated kakrol: Though a plant of the same season, the cultivated kakrol, also identified previously as *Momordica dioica*, is different from the wild kakrol in several important morphological, floral and fruit characters (Table 1 -3). The cultivated kakrol is more vigorous, the vines had almost unlimited growth. The leaves are more thick, large and the lamina is entire. Remarkable differences are observed in flowers colour and size, the cultivated kakrol bears large, white, showy flowers. The three upper-whorl petals bear very distinct black, shiny spot at the base. The peduncle of the female cultivated kakrol are much longer than the wild female flowers. The fruits of the cultivated forms are much larger with twice as many larger seeds than small wild kakrol form. The fruits of the cultivated kakrol are oblong with larger and harder spines than those of the small wild fruit. The tuberous of the cultivated kakrol are smaller than the wild kakrol.

Comparison with large wild kakrol (*M. cochinchinensis*): There was another wild kakrol with a very large climber, large fruits and pointed spines on the fruit. The description of *M. cochinchinensis* as given by Prain (1903) agrees quite well with the collected big wild plant (Table 4).

The studied materials and previous taxonomic description: C.B. Clarke's description of kakrol given under two identified species *M. dioica* and *M. cochinchinensis* (Prain, 1903) are compared with the description of three collected materials (wild small, cultivated and wild large kakrol) in Table 4. From the

Table 1: Measurements (mean with SD) of stem and leaf characters of wild (s), cultivated and wild (b) kakrol

Types of plants	Inter node length (cm)	Stem diameter (cm)	Leaf shape		Leaf blade size		Leaf petiole	
			Young	Older	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)
Wild female	6.94±1.69	0.36±0.05	4-sected	2-sected	5.45±0.61	6.21±0.63	Entire 3.72±0.32	0.16±0.009
Wild male	6.93±1.16	0.41±0.05	6-sected 2-sected	4-sected Entire	7.08±0.38	7.77±0.43	3.57±0.24	0.22±0.03
Cultivated female	5.19±0.54	0.80±0.07	4-sected Entire	2-sected Entire	10.49±1.21	10.82±1.19	7.65±2.27	0.29±0.03
Cultivated male	5.55±0.79	0.47±0.10	Entire	Entire	10.81±0.99	11.06±0.98	7.48±0.93	0.33±0.02
Big wild female	11.12±1.16	0.83±0.13	2-sected 4-sected	4-sected	17.58±1.00	17.92±1.04	8.52±0.34	0.66±0.07
Big wild male	11.51±0.96	0.87±0.15	2-sected 6-sected	4-sected	17.85±1.06	18.21±1.11	8.78±0.41	0.66±0.05

Table 2: Measurements (Mean with SD) of wild cultivated and flowers (N = 10)

Types of plants	Peduncle length (cm)	Bract position (cm)	Bract	
			Length (cm)	Breadth (cm)
Wild female	1.91±0.38	0.74±0.13	0.17±0.05	0.23±0.03
Wild male	6.54±1.02	6.12±1.03	1.04±0.02	1.47±0.18
Cultivated female	16.72±3.15	2.15±1.74	0.35±0.06	0.21±0.04
Cultivated male	15.49±2.24	14.49±2.24	2.09±0.24	3.71±0.36
Big wild female	4.21±0.28	2.09±0.21	0.21±0.05	0.28±0.05
Big wild male	11.21±1.58	10.50±1.49	3.21±0.33	3.97±0.30

Types of plant	Calyx		Corolla		Flower
	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Diameter (cm)
Wild female	0.57±0.04	0.08±0.01	1.93±0.13	0.82±0.07	2.94±0.15
Wild male	0.57±0.04	0.16±0.02	2.02±0.27	0.97±0.01	3.24±0.25
Cultivated female	0.85±0.13	0.18±0.04	5.65±0.54	3.72±0.04	8.21±0.52
Cultivated male	1.40±0.08	0.68±0.04	5.54±0.21	3.71±0.30	8.08±0.58
Big wild female	1.34±0.07	0.75±0.05	6.45±0.35	4.10±0.16	8.60±0.33
Big wild male	2.02±0.15	1.04±0.07	7.20±0.22	4.36±0.14	8.90±0.42

Table 3: Measurements (mean with SD) of female and male sex organ of wild and cultivated kakrol

Types of plants	Stigma		Style		Ovary		Anther		Filament	
	Length (cm)	Diameter (cm)	Length (cm)	Diameter (cm)	Length (cm)	Diameter (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)
Wild female	0.34±0.0	0.35±0.02	0.48±0.03	0.16±0.01	0.82±0.03	0.49±0.03-	-	-	-	
Wild male	-	-	-	-	-	-	0.36±0.02	0.35±0.017	0.26±0.03	0.21±0.03
Cultivated female	0.46±0.0	0.54±0.03	0.61±0.03	0.03±0.02	1.81±0.12	0.83±0.06	-	-	-	-
Cultivated male	-	-	-	-	-	-	0.37±0.01	0.69±0.029	0.47±0.04	0.48±0.03
Big wild female	0.52±0.0	0.56±0.02	0.71±0.02	0.34±0.01	2.38±0.09	1.04±0.03	-	-	-	-
Big wild male	-	-	-	-	-	-	0.63±0.01	0.71±0.01	0.55±0.01	0.82±0.02

comparison it appeared that description of wild large kakrol and *M. cochinchinensis* agrees quite well whereas those of wild small kakrol and *M. dioica* fits well. The characters proved important in distinguishing these are:

Thus the comparison of the morphological attributes through indicated the big wild kakrol as *M. cochinchinensis*; the small wild kakrol as *M. dioica*; puts the cultivated kakrol in an ambiguous position. The large leaf, bract size, white flowers with eye-spots on petals, cylindrical fruits and seed shape indicates its affinity towards *M. cochinchinensis* but peduncle length, absence of glands in leaves, position of bract on male flowers, anthers with filament 2-fid half way down, fruits size are more common with *M. dioica*. The characteristics of cultivated kakrol which are common to the two different species are: *Cochinchinensis* and *dioica*. Large leaf, white flower, eye spot at petal base, cylindrical fruits, grey-black sculptured seed gland absent in leaves, position of bract, length of petiole, 2-fid filament, fruit size.

Thus, the phylogenetic relationship between these kakrol types should be determined to understand the genetic similarity and prospect of improvement of this vegetable crop by breeding and selection.

Crosses between the different types: A large number of crosses were made between three different kakrol types i.e., the small wild, the cultivated and the big wild kakrol to determine phylogenetic relationship between these.

Table 4: Comparison between description of different types of kakrol with Clarke's description

Items	Clarke's description		Description of materials studied		
	M. dioica	*M. cochinchinensis*	Wild (small)	Cultivated	Wild (big)
Habit	Climber	Large climber	climber	Medium climber	Large climber
Leaves	2-6 inch long and broad, cordate, ovate, entire, acute, denticulate, more or less 3-5 lobed	4-5 inch diameter, cordate, ovate, usully 3-lobed glabrous	2-3 inch long and broad, entire, 2-6 sected	4 inch long and broad, entire, cordate, ovate	7 inch long and broad,3 or 5 lobed, margin little dentate
Petiole	1-2 inch. gland absent	2-3 inch glandular	1-5 inch long, gland absent	3 inch long, gland absent	3 inch long, glandular
Peduncle	Male peduncle 2-6 inch, female peduncle as long as the male	Male peduncle 2-6 inch, female peduncle 1-2 inch	Male peduncle 2-5 inch, female peduncle 1 inch	Male peduncle 6 inch, female peduncle 6 inch	Male peduncle 3-5 inch, female 1.5 inch
Flower colour	Yellow	White	Yellow	White	White
Bract (male)	Large, couple like, embracing the expanded flower	Small, embracing the expanded flower	Small 0.41 inch long in male flower.	Large, 0.81 in.long in male flower.	Large, 1.1-1.5 inch long in male flower.
Petals	1 inch yellow	1-2 inch, white tinger with yellow, 3 with black spots at the base, 2 with yellow glands	0.76-0.80 inch, yellow	2.18 inch, white, 3 with black spots at the base and 2 with tiny black spots with yellow hair.	2.54 inch, white, 3 with black spots at the base and with yellow hair
Anthesis	-	-	After sunset	Morning	morning
Anther	The two anthered filaments two fid half way down	The two anthered filaments not 2 fid	The two anthered filaments 2 fid half way down	The two anthered filaments 2 fid half way down.	The two anthered filaments not 2 fid
Fruit	1-3 inch. long, densely covered with soft spines ellipsoid	4-5 inch, muricated, ovate, pointed, bright red, very fleshy, covered with conical points.	1 inch long, densely covered with soft spines ellipsoid	4-5 inch long, densely covered with soft spines cylindrical	5-6 inch long, covered with hard spines, ovate bright red
Seed	Nearly 0.5 inch small, many compressed, broad oblong, with a few well-marked corrugations on the margins, nearly smooth on the surface	0.13 by 0.63 and 0.20 in thick, large many irregular, ovate, compressed, black, corrugated on the margins, sculptured on the faces	About 0.26 inch long, smooth on the surface, round, not compressed, very ill-developed smooth on the surface, round, not compressed very ill-developed corrugations on the margins, blackish grey, no 15-20	0.36-0.38 inch long, grey in well marked corrugations irregular sculptured on the faces, grey in colour, thick 0.20 inch no 30-35	0.79-0.89 inch long, many and well-marked corrugations on the faces. No. 30-35

Characters	Big wild/*cochinchinensis*	Small wild/*dioica*	Cultivated
Habit	Large climber	Climber	Large climber
Leaf	Large	Small	Medium
Petiole	Glandular	Gland absent	Gland absent
Peduncle	Male 3-5 inch Female 1-2 inch	2-3 inch-2.6 inch	6 inch 6 inch
Flower colour	White	Yellow	White
Bract/male	Large	Small	Small
Spot on petal	Bright black spot	No spot	Bright black
Anther	Filaments not 2 fid	Filaments 2 fid	Filaments 2 fid
Fruit	Covered with hard spine	Densely covered with soft spines	Densely covered with soft spines
Shape/size	Ovate-big	Globose-small	Cylindrical-medium
Seed shape	Large-sculptured	Small-smooth	Large-sculptured
Seed number/fruit	15-20	30-35	30-35

The results of these pollination are described. Here all the parents are separate plants of unrelated parentage.

Pollination within the types: To determine the rate of success of pollinations and fertility of various kakrol types, pollinations were made among plants belonging to different types i.e., (small wild × small wild), (big wild × big wild) and cultivated × cultivated). After three weeks, all the pollinations proved success and 100% seed-set was recorded (Table 5).

Pollination between big wild×ultivated kakrol: In all 57 crosses were made (Table 5) between big wild × cultivated and its reciprocal. None of the pollinated flowers set any fruits and seed, proving these two parents not crossable.

Pollination between small wild×big wild: The cross (small wild×big wild) gave 11 fruits out of 46 pollinations. But the reciprocal cross i.e., (big wild × small wild) failed to produce any fruit (Table 5).

Table 5: The success of pollinations among the types (2004)

Types of cross	Total No. of cross	Fruits dropped		Fruits developed	Percentage of fruit developed
		Within 7 days	Within 21 days		
♀Big wild ×♂ big wild	4	-	-	4	100
♀Small wild × ♂ small wild	9	-	-	9	100
♀Cultivated × ♂ cultivated	11	-	-	11	100
♀Big wild × ♂ small wild	21	21	-	-	-
♀Small wild × ♂ big wild	46	28	7	11	23.9
♀Big wild × ♂ cultivated	11	9	2	-	-
♀Cultivated × ♂ big wild	46	34	12	-	-
♀Cultivated × ♂ small wild	64	10	6	48	75
♀Small wild × ♂ cultivated	33	12	4	17	51.5

Cross between small wild × cultivated: A total 33 pollinations between small wild × cultivated and 64 between cultivated × small wild were made. Both the cross and its reciprocal were partially successful. Small wild×cultivated cross has a success rate of 51% while the reciprocal cultivated × small wild has a 75% success (Table 5). Thus, from the above results, we can summarize the following points:

- There is complete reproductive isolation between cultivated and big wild kakrol
- There is only partial compatibility between big wild and small wild kakrol,

only one way cross between these two, (small wild × big wild) gave 24% fruit setting. The reciprocal i.e., (big wild × small wild) were completely unsuccessful.

- The other cross i.e., (small wild × cultivated) pollinations and its reciprocal were proved partially successful.

These results indicated that the three types are genetically different and are isolated by barriers of varying degrees. In the following section, details of progeny from the crosses will be given.

Results of pollinations between small wild and cultivated kakrol

Success of cross: The results from fruit set data in the previous section indicated partial isolation between the small wild and the cultivated kakrol. More extensive genetic analysis in this cross combination has been done. The number of plants were small in 2002, so that only a limited number of pollinations were possible. Progeny from these parents were raised in 2003 and a fairly large number of pollinations (in two sets) were made in 2004 (Table 5).

The results of these pollinations again confirmed previous observation:

- Within type i.e., (wild × wild) and (cultivated × cultivated) were almost fully successful;
- Between types crosses i.e., (wild × cultivated) and (cultivated × wild) were partial successful (48-64% 0;
- (cultivated × wild) combination was fertile than (wild × cultivated) cross.

Flower, fruit and seed characters: The fruit characters were scored when the fruits were mature and ripe. The fruits formed after pollinations with pollen from different types, were always smaller than these from crosses within type (Table 6 and 7). This indicate lower degree of compatibility between types than within types. The seed characters scored from mature seeds also indicated strong differences is seed number per fruit, seed size, weight, colour and sculpture pattern (Table 7). More important is the differences in seed per fruit, small wild had 14 to 16 seeds per fruit when pollinated by small wild pollen but only 5 to 8 seeds when pollinated with cultivated kakrol pollen. Similarly, cultivated kakrol 29 to 30 seeds per fruit when pollinated with cultivated kakrol pollen but only 10 to 13 seeds when pollinated with small wild kakrol pollen (Table 7). This observation too suggested fairly strong isolation between the two types. In both cases, the number of undeveloped seeds were high in intertype pollinations (Table 7) than within type pollination.

Germination of seeds and plant establishment: When seeds were germinated next year, the within type pollinated wild kakrol and cultivated kakrol had 75 and 67% seed germination. Nearly 100% of these plants attained maturity (Table 8). But the cross pollinated (between types) seeds had only 60% germination, though all 6 seedlings flowered in (wild ×cultivated) cross, only 2 out of 6 seedlings were able to flower in the reciprocal cross (cultivated×wild). The seed size data is also indicative that within type pollination produces healthier seeds than between types pollinations (Table 8).

Sex of the progeny plants: The total number of progeny succeeded to reach flowering were 24, of which only 7 were female and 17 were male. This ratio between male and

Table 6: Fruits developed after crosses between small wild and cultivated kakrol in two sets

Types of cross	Total No. of cross	Fruits dropped		Fruits develop	Percentage of fruit developed
		Within 7 days	Within 21 days		
Wild × wild	14	1	-	13	92.86
Cultivated × cultivated	2	-	-	2	100.0
Wild × cultivated	7	2	3	2	28.57
Cultivated × wild	8	4	1	3	37.50
Wild × wild	97	1	1	95	97.94
Cultivated × wild	63	3	1	59	93.65
Wild × cultivated	80	28	14	38	47.50
Cultivated × wild	96	12	23	61	63.54

Table 7: Fruits characters (mean with SD) of crosses made small wild and cultivated kakrol in two sets

Types of cross	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (cm)	Fruit stalk length (cm)	Fruit stalk breadth (cm)	Repining time (days)
Wild × wild	2.86±0.23	2.61±0.22	7.43±0.84	2.40±0.72	0.14±0.01	25.69±2.56
Cultivated × cultivated	7.25±0.61	3.42±0.31	34.32±7.56	15.00±1.41	0.24±0.014	26.50±7.07
Wild × cultivated	2.31±0.67	2.21±0.47	6.14±0.73	2.04±0.21	0.14±0.007	25.00±2.82
Cultivated × wild	6.88±1.47	3.41±0.63	26.05±7.94	19.73±2.00	0.25±0.005	26.33±3.06
Wild × wild	3.34±0.37	2.66±0.23	10.47±1.98	2.17±0.37	0.17±0.024	27.19±1.01
Cultivated × wild	8.20±1.27	4.16±0.68	59.14±2.86	17.89±4.31	0.29±0.027	28.61±1.78
Wild × cultivated	2.32±0.42	1.91±0.36	4.22±1.90	1.91±0.41	0.15±0.02	27.69±1.49
Cultivated × wild	5.60±1.25	3.07±0.65	24.77±9.34	14.18±3.91	0.25±0.04	28.11±1.67

Table 8: Seed characters (mean with SD) of wild and cultivated kakorl in two sets (N = 20)

Types of cross	Seed length (cm)	Seed breadth (cm)	Seed weight (cm)	Seed per fruit	
				Mature	Immature
Wild × wild	0.66±0.56	0.60±0.04	0.07±0.01	15.92±1.98	3.00±1.08
Cultivated × cultivated	0.97±0.05	0.73±0.02	0.16±0.02	29.50±2.12	3.50±0.71
Wild × cultivated	0.64±0.06	0.56±0.03	0.07±0.01	5.50±1.95	5.50±2.12
Cultivated × wild	0.97±0.04	0.69±0.33	0.14±0.01	13.00±5.81	4.67±0.58
Wild × wild	0.65±0.04	0.55±0.03	0.07±0.01	13.82±2.53	2.81±1.21
Cultivated × wild	0.99±0.03	0.69±0.03	0.17±0.01	29.20±8.59	3.82±2.48
Wild × cultivated	0.66±0.03	0.58±0.02	0.09±0.007	4.45±1.24	8.13±2.14
Cultivated × wild	0.92±0.05	0.66±0.06	0.13±0.02	10.11±4.27	6.15±3.42

female (17:7) is different from 1:1 (χ^2 = 4.16, df = 1, significant at 5% level). There was a relative deficiency of female progeny in the experiment. The time required for flowering by the male and female plants are also shown in Table 9. The male plants, in general, flowered earlier than the female plants. The exception being the cultivated kakrol where female plants flowered on average 5 days earlier than male plants.

The fertility of the hybrids: There were two female hybrids (one from wild × cultivated and the other from cultivated × wild) which managed to flower. However, there were six male hybrid plants which flowered. In all 47 pollinations could be made, none of the pollinated female flowers produced mature fruits and seeds. Most of their flowers dropped off before 21 days after pollination. Thus, the results of pollination within hybrids revealed that the hybrids are fully sterile, no fertile seed was set when F1 progeny were crossed among themselves.

Description of the hybrids between wild and cultivated kakrol: The male and female progeny derived from the crosses between wild and cultivated kakrol produced smaller and week plants. The floral characters of these hybrids are summarized in Table 10. For comparison the parental measurements were also included. When compared with the parents, most of the characters of the hybrids were found to be intermediated between the two parents and showed variation in leaf morphology. However flower size (both petal length and flower diameter) of the hybrids exceeded those of the larger parent i.e., the cultivated kakrol. Ovary and anther sizes of the hybrids also increased over the better parent (Table 10.). Thus, for most of the characters, polygenic control was indicated. The hybrid plants however were less vigorous and produced fewer flowers.

Back cross between parents and hybrids: When the hybrid progeny from the (wild × cultivated) and reciprocal cross were crossed with the parents (both cultivated×wild) the sterility of the hybrids were again became apparent (Table 11). None of the two hybrids set any fruits when used as female parent indicating female sterility. The ovaries were morphologically abnormal in the hybrid plants. When cultivated kakrol was used as

Table 9: Rate of Seed germination and their sex (mean with SD) -1993

Types of cross	No. of seed sown	Germination time (days)	No. of seed germinated	Sex of plants		Flowering time after germination (days)	
				Female	Male	Female	Male
Wild × wild	8.0	11.80±2.59	6.0 (75%)	2.0	4.0	113.00±2.83	98.75±2.50
Cultivated × cultivated	15.0	14.60±2.30	10.0 (66.67%)	3.0	7.0	104.33±2.08	109.57±1.82
Wild × cultivated	10.0	23.67±2.34	6.0 (60%)	1.0	5.0	103.00	98.8±2.28
Cultivated × wild	10.0	21.67±3.67	6.0 (60%)	1.0	1.0	110.00	104.00

Table 10: Floral characters of the male and female hybrids of crosses (wild × cultivated and reciprocal (mean with SD)

Breadth	Length	Petiole length(cm)	Position of bract (cm)	Size of bract (cm)		Corolla size (cm)		Floral diameter (cm)	Anther length (cm)	Ovary length (cm)
				Length	Breadth	Length	Breadth			
Wild×	Male	7.90±0.60	7.2±0.52	1.70±0.13	2.8±0.20	4.60±0.23	3.90±0.02	6.60±0.30	0.32±0.02	-
cultivated	Female	5.4±0.52	0.80±0.20	0.24±0.01	0.16±0.02	3.70±0.30	2.20±0.12	5.60±0.20	-	1.33±0.13
Cultivated×	Male	10.30±0.70	9.00±0.60	1.83±0.30	3.17±0.51	6.80±0.50	5.30±0.30	11.50±0.80	0.45±0.02	-
wild	Female	13.10±1.40	0.80±0.14	0.12±0.02	0.22±0.01	4.90±0.90	3.20±0.40	7.00±1.20	-	2.00±0.21
Wild parent	Male	6.54±1.02	6.12±1.03	1.04±0.02	1.47±0.18	2.02±0.27	0.97±0.01	3.24±0.25	0.36±0.03	-
	Female	1.91±0.38	0.74±0.13	0.17±0.05	0.23±0.03	1.93±0.13	0.82±0.07	2.94±0.15	-	0.82±0.03
Cultivated	Male	15.50±2.24	14.50±2.25	2.09±0.24	3.71±0.40	4.54±0.21	3.71±0.30	8.08±0.58	0.37±0.02	-
parent	Female	16.72±3.15	2.15±1.04	0.35±0.02	0.21±0.01	5.65±0.50	3.72±0.04	8.21±0.52	-	1.81±0.12

Table 11: Reproductive performances of progeny derived from crossed between wild and cultivated kakrol (1993), pollinations were made within female and male members of progeny

Types of cross	Progeny crosses	Total No. of cross	Fruit dropped		Fruit developed
			Within 7 days	Within 21 days	
	FP'4/MP'1-93	3	3	-	-
	FP'4/MP'2-93	4	3	1	-
Wild×cultivated	FP'4/MP'3-93	3	3	-	-
	FP'4/MP'5-93	2	2	-	-
	FP'4/MP'6-93	3	3	-	-
Cultivated×wild	FP"6/MP"4-93	10	7	2	1
	FP"6/MP'1-93	5	4	1	-
	FP"6/MP'2-93	3	3	-	-
Reciprocal crosses	FP"6/MP'3-93	3	3	-	-
	FP"6/MP'5-93	5	5	-	-
	FP"6/MP'6-93	3	3	-	-
	FP'4/MP"4-93	3	3		

Table 12: Crosses between hybrids and plants

Crosses (back cross)	No. of pollinated 1	Fruit developed	% of fruit developed	Fruit weight (g)	Seed per fruit 2
♀(wild × cultivated) with♂ cultivated	5	-	-	-	-
♀(wild × cultivated) with ♂ wild	7	-	-	-	-
♀cultivated with ♂(wild × cultivated)	61	-	-	-	-
♀wild with ♂(wild × cultivated)	61	-	-	-	-
♀ (cultivated × wild) with ♂ cultivated	7	-	-	-	-
♀(cultivated × wild) with ♂ wild	6	-	-	-	-
♀cultivated ×♂ (cultivated × wild)	21	-	-	-	-
♀wild ×♂ (cultivated × wild)	14	1	7.1	8.5	9

1.Limited number of hybrid flowers was available. 2. None of seeds germination

parent, there were no fruit setting then. The cultivated kakrol plant was same plant used in previous crossing programme and proved to be fully sterile (Table 12). Thus, partial fertility between the male hybrids and their parent was indicated.

Pollen of hybrid progeny: When anthers from the hybrid male parents were examined in squash preparations very low pollen fertility (7-8%) was noted. There was no difference in pollen size but only 12-14% pollen grains were found to germinate on hybrid stigmas whereas 71-71% pollen germination was noted on parent stigmas.

Rescue of hybrid embryos through tissue culture: The tissue culture technique was employed to rescue hybrid embryos from immature seeds developed on F1 plants. Murashige and Skoog (1962) medium was used with 50% dilution and in combinations with various hormones. The developing embryos were cultured in different medium. But only positive response occurred 33 and 10% in small wild and wild × cultivated cross flower.

DISCUSSION

Taxonomic dilemma: The two types of wild kakrol reported in the present study are two distinct: the wild small fruited and wild large fruited. Taxonomic descriptions of these two agrees well to *M. dioica* (small fruited wild) and *M. cochinchinensis* (large fruited wild) (Prain, 1903). But problem arises when the cultivated

kakrol has also been identified as *M. dioica* (Rashid, 1976b; in all other reports). But the cultivated kakrol has been demonstrated to be very different from M. *dioica* in several important characters i.e., white, large showy flowers opening during morning, distinct, eye-spots at the base of three outer petals and large cylindrical fruits with larger corrugated seeds. To determine the taxonomic status of cultivated kakrol detailed hybridization programme were undertaken and presented here.

Isolation between three kakrol types: Hybridization within the three types (small wild, large wild and cultivated) were successful indicating normal fertility of these types. The big wild (*M. cochinchinensis*) and cultivated kakrol proved non-crossable. Though big wild (*M. cochinchinensis*) × small wild (*M. dioica*) proved unsuccessful, the reciprocal crosses (*dioica* × *cochinchinensis*) gave partial success. The other combination i.e., small wild (*M. dioica*) × cultivated and its reciprocal both proved partially successful. Thus there was complete reproductive isolation between cultivated kakrol and *M. cochinchinensis*. The two wild kakrol (*dioica* × *cochinchinensis*) cross gave some fruit setting (24%) while the reciprocal (*cochinchinensis* × *dioica*) fully sterile. However, (*dioica* × cultivated) and reciprocal were partially fertile (50 to 70%). These indicated partial genetic isolation between dioica and cultivated kakrol.

Further analysis of *dioica*×cultivated kakrol crosses: The loss of vigor of the hybrid fruits, reduced size and reduced number of seed setting also indicated distance between *dioica* and cultivated kakrol. The healthy seeds from hybrid fruits too had low germination (60%) and lower rate of success, growth and development. The small number of hybrids which did manage to reach reproductive maturity and flowered set no fruits at all. Thus full hybrid sterility was indicated.

Phylogeny of cultivated kakrol: The development of reproductive isolation between populations is required for species formation. The history of evolution of kakrol and the cause of isolation of the cultivated and the wild kakrol types are not known. In fact, very little is known about the genetic system (number of genes and chromosomes, details of meiosis and pairing, breeding system, sex determination, regulation of gene activities etc.) of this species. Genetic systems vary considerably from species to species (Grant, 1958) and were subject to natural selection to become adapted to its environmental conditions (Grant, 1963). The reproductive isolation noted in the present study includes pre-zygotic mechanisms preventing the two species to mate and produce viable seeds. Here the pollen stigma interaction i.e. physiological isolation may be involved.

The post-zygotic mechanisms include the failure of the zygotic to develop, the lower viability of the hybrid and full sterility of the hybrid. The complete sterility of the hybrid may indicate chromosome differences. These are some of the aspects to be looked into the future research. Here the hybrid embryos could not be rescued through tissue culture indicating complete sterility of the hybrid progeny. Thus, from the existing evidences, it is obvious that the cultivated kakrol should not be included in *M. dioica*. A distinct species status should be assigned to this taxon i.e. the cultivated kakrol. Character combinations suggest that the cultivated kakrol may be an amphidiploid between *M. dioica* and *M. cochinchinensis*, arising through hybridization and chromosome doubling. The name *Momordica hybrida* is suggested.

CONCLUSIONS

The result of the present study indicated that hybridization between types has very low potential in improving the crop. Collection of cultivated kakrol made by the author indicated appreciable variability and this variability can be exploited. The difficult of the pollination is another problem which also proved difficult to solve. The small wild kakrol flowers at night and is observed to be pollinated by nocturnal moths. Thus the prospect of cultivated kakrol being pollinated by an insect pollinator during day time needs extensive searching and coadeptation of the plant and pollinating animal species. As the flowers are equipped with pollinator guides in the form of spots at the base of petals, this seems realistic. The prospect of micri-propagation through tissue culture has long been indicated a bright one in vegetables. The economics of the prospect, however, requires demonstration. Patronization from commercial cultivators willing to utilize this technology is required.

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