DETERMINATION OF GENOMIC RELATIONSHIP IN THE GENUS CORCHORUS, C. OLIOTORIUS X C. DEPRESSUS AND C. CAPSULARIS X C. DEPRESSUS THROUGH IMPROVED TECHNIQUES**

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Abstract

When emasculated under strictly controlled conditions, no hybrid in the cross, C. olitorius x C. depressus, C. capsularis x C. depressus was obtained. Hybrid formation is not possible in the above combinations because in both, the pollen tube of C. depressus fail to reach the ovule of the cultivar. Application of 300 mg/l IAA around the pedicel of the pollinated flower, however, made it possible to obtain hybrids in both combinations. These hybrids were near sterile, more so in the cross, C. olitorius x C. depressus. In the latter hybrid, chromosomes were found to be sticky. Meiotic irregularities characterised the hybrids of both combinations. There was however enough fertility for the F₂ generation to be raised.

Introduction

Baig (1957) reciprocally crossed the ‘Tossa’ jute variety of C. olitorius with wild C. depressus and Memon (1957) crossed the latter with the ‘White’ variety of jute, C. capsularis to transfer the useful genes of the wild species into the cultivars. These genes were for resistance to disease, pest, drought and photoneutrality. They reported success in obtaining hybrids in one direction only, using the cultivar as female. The high fertility of their hybrids and their close resemblance with the respective parent however raised doubts whether theirs were real hybrids or the result of self-pollination. To settle this, crosses in the above combinations were made under rigorously controlled conditions. In this paper the results of such crosses are reported. Another object was to use improved techniques in hybridisation in case ordinary methods failed.

Materials

The following Corchorus species were used: (a) C. olitorius L. var. C.G., (b) C. capsularis L. var. D. 154 and (c) C. depressus (= C. anticorhous Rausch) C. Christensen.

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Characters of the parent plants

The details of the morphological characters of the three parents, *C. capsularis*, *C. olitorius* and *C. depressus* are given in Bentham and Hooker (1874). Only the salient features used to compare the hybrids with the parents are described here.

Cultivar: *C. olitorius* var. C.G. (2n = 14)

_Morphological_: Unbranched; height when grown in 1' dia pot is 10-12' and shows occasionally 1-2 branches (Fig. 2). B/L ratio of leaf 0.526 ± 0.916, has two serratures one on each side. Flowers not in cluster as in *capsularis*, larger than other two species; petal size 0.61 ± 0.02 cm. Pod an elongated capsule surfaced with 10 alternate ridges and furrows, seeds 250-300 per pod and smaller than *capsularis* in size.

_Physiological_: Short day plant requiring 10 hr or less photoperiod; strictly annual (six months' life), dies even before all the pods mature.

Cultivar. *C. capsularis* var. D. 154 (2n = 14)

_Morphological_: Grows slightly smaller (in field 10-12') than *olitorius*. Flowers in cluster; smaller than *olitorius* in size; petal size 0.36 ± 0.07 x0.10 ± 0.02 cm, larger than those of *depressus* (Fig. 3). Pod is a spherical capsule with highly ruffled surface, seeds larger than *olitorius* in size; contains 40-50 seeds.

_Physiological_: Short day, less sensitive to photoperiod, strictly annual (six months' life), dies before the maturation of the last harvest of fruits.

Wild: *C. depressus* (2n = 14)

_Morphological_: Perennial herb, prostrate hugging the ground, much branched and confined to one plane because of their contact with the ground (Fig. 1). Flowers smallest of the 3 species; petals 5. Pod elongated capsule but much smaller than *olitorius* pod (Fig. 4 right), with ridges and furrows, clearly visible only under high magnification.

_Physiological_: Experiments carried out by I.I. Chaudhri (personal communication 1959) at California Institute of Technology, Pasadena, U.S.A., showed that *depressus* is photoperiodically neutral.
Methods

(a) For tracing of pollen tube inside the style, the cross-pollinated flowers were fixed in acetic-alcohol (1:3) in two lots at 6 and 24 hrs interval.

(b) Since in both *olitorius* and *capsularis* anthesis takes place in the morning flowers were emasculated in the previous afternoon. From a mature bud, anthers were pulled out one by one and each time the anther was examined under a compound microscope to see whether any pollen was sticking out of the anther. If any pollen was seen outside the anther, the flower was not used in the cross. This method first described by Islam (1954) though tedious and time consuming proved a fool proof method against any chance self-pollination in *Fragaria* interspecific crosses. Therefore, this method was adopted in the present investigation.

(c) *Use of indoleacetic acid (IAA) in lanolin paste*: In the beginning, the untreated flower dropped 2-3 days after pollination. In the subsequent crosses, therefore, a thin layer of lanolin paste containing 300 mg/l IAA was smeared around the pedicel of the pollinated flower to prevent flower drop.

(d) The entry of pollen tube was studied according to the method described by Islam and Ali (1966).

(e) Pollen stainability was studied in 2%, acetocarmine.

(f) For meiotic study PMCs were squashed in 2% acetocarmine.

Experimental Results

Experiment I

(i) *Tracing the course of pollen tube in the crosses*, C. *olitorius* × C. *depressus*, C. *capsularis* × C. *depressus*

After fixation and staining, the material was dissected out to see the growth rate of the pollen tube (PT) in the alien style. The results presented in Table 1, prepared from 155 observations, indicate that *depressus* PT stops growth after 18 hrs. This may be more of a mechanical nature than genetical; the *depressus* style is much shorter in length compared with that of *olitorius* and *capsularis* and *depressus* PT does not have inherent capacity for enough growth to reach; the ovule of *olitorius*.
TABLE 1: Growth rate of pollen tube (PT) in interspecific crosses

<table>
<thead>
<tr>
<th>Parents</th>
<th>Length of PT in</th>
<th>Length of PT in</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>microns in hrs</td>
<td>microns after hormone treatment in hrs</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Male</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>olitorius depressus</td>
<td>31.4</td>
<td>131.2</td>
<td>131.2</td>
</tr>
<tr>
<td>capsularis depressus</td>
<td>28.9</td>
<td>---</td>
<td>120.8</td>
</tr>
</tbody>
</table>

(ii) Effect of IAA on pollen tube growth in the alien styles

In this experiment the flower pedicels were smeared with 300 mg/l IAA after pollination. The fixation was made at 6 and 24 hrs intervals.

The results of the experiment are shown in Table 1. The depressus PT elongated under the influence of IAA, reached the ovule of olitorius (Fig. 14) and capsularis and subsequently effected fertilization.

The application of IAA prevented flower drop in both combinations of crosses. Also a good number of mature fruits was obtained from each (cf. Tables 2 & 5).

Experiment II

(A) *C. olitorius × C. depressus*

In Table 2 the results of crosses have been summarised. In all 81 crosses made by using hormone, there was nearly 25% fruit set but no fruit set was obtained in 30 crosses made without hormone. The fruit set was obtained only in one direction, namely, when olitorius was used as female.

TABLE 2: Results of crosses, *C. olitorius × C. depressus*

<table>
<thead>
<tr>
<th>Parents</th>
<th>No. of crosses made</th>
<th>Whether IAA applied</th>
<th>No. of fruit set</th>
<th>No. of fruits harvested</th>
<th>No. of full seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>olitorius depressus</td>
<td>81</td>
<td>1-</td>
<td>40</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>capsularis</td>
<td>30</td>
<td>---</td>
<td>Nilt</td>
<td>Nil</td>
<td>Nilt</td>
</tr>
</tbody>
</table>
(b) Germination: The hybrid seed germinated poorly and over a long period (Table 3). Among 26 hybrid seedlings seven were albino, six xantha, five virescent and others green (Table 3). The xantha and virescent seedlings turned green later but the albinos did not. Eventually only one hybrid plant grew to adult stage (Fig. 6).

Table 3: Germination of hybrid seeds, C. olitorius × C. depressus

<table>
<thead>
<tr>
<th>No. of seeds</th>
<th>Period over which germination took place</th>
<th>No. of seeds germinated</th>
<th>No. of seedlings</th>
<th>No. of adult plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>sown</td>
<td></td>
<td></td>
<td>Albino</td>
<td>xantha</td>
</tr>
<tr>
<td>40</td>
<td>Six weeks</td>
<td>26</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

(c) Extent of fertility of the hybrid

Unlike the hybrid reported by Memon (1957), this was near sterile. The pollen stainability was only 3.4% (cf. Fig. 10). However three to four small fruits were obtained towards the end of growing season. Viable seeds from these produced an F₂ population of 13 plants.

(d) Comparison of some important characters of the hybrid with its parents

The hybrid did not show the decumbent habit as reported earlier by Memon (Fig. 6). Although weak, it lived two months longer than the female parent. The genes for perennial habit contributed by the male parent must be responsible for this greater longevity. It was 1/3rd (105 cm) as tall as the female parent (300 cm), and was sparingly branched and in these two characters the hybrid may be considered as intermediate. The branches showed a tendency to grow at the same angles and plane as those of depressus (Fig. 6). These are dominant characters transmitted into the hybrid from the male. The majority of leaves were malformed (Fig. 8) having dentate margin. Some of them had crinkle surfaced leaf. The stem had warty projections throughout their length reminding one of tumourous joints (Fig. 7) characteristic of some interspecific hybrids e.g. Nicotiana (Stebbins 1958) and thurberi - anomalous hybrid in Gossypium obtained in this laboratory. The hybrid flowered for nine months showing dominance of the male in respect of flowering. The flowers in the hybrid were
remarkably variable, no two being alike. Besides the variation in the number of petals which ranged from 5-7 (Fig.9), the other peculiarities of the flowers were: (1) two flowers in one pedicel (Fig. 9, upper centre), (2) fasciated carpel in twin flowers, (3) adhesion of sepal with petal (Fig. 9, lower centre) and (4) presence of staminodes. In size the hybrid flower showed the dominance of the female; the development of large sized petals might have been more due to sterility rather than mere dominance. The anthesis time, however, coincided with that of the female parent, the buds opening in the morning. Since the fruit in both the parents are elongated no decision could be taken as to which kind of elongation got transmitted into the F₁ hybrid.

(c) Cytology

Meiosis of the hybrid was studied by Islam and N. Bhutto (unpublished). They observed stickiness in the chromosomes of the hybrid. Both at diakinesis and M I the chromosomes were faintly stained.

In a few PMCs studied for M1 (Bhutto, N. unpublished), only 2-4 bivalents were seen, the rest being univalents. It is only in the study of A1 that the number of chromosomes could be clearly determined and they added to 14 in every cell. The number of laggards as expected was due to the presence of a large number of univalents. Anaphase II was also erratic. In two PMCs three and in one two laggards were seen being left outside the 4 newly constituted nuclei. These cytological data (reported for the first time) establish beyond doubt that chromosome homology between olitorius and depressus is small: highly irregular sporads (cf. Table 4: Fig. 13) and as low as 3.4% pollen stainability was obviously the result of highly irregular meiosis.

| Table 4: Frequency of different-celled sporads in the F₁, C. olitorius : C. depressus |
|---------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| No. of sporads examined                    | Frequency of cells in | Percentage of normal |               |               |               |               |               |               |
| Triad                                         | Tetrad                | Pentad              | Hexad          | Heptad         | Octad          | Ennad          | Decad          | tetrads         |
| 84                                            | 6                      | 32                   | 17             | 8              | 11             | 5              | 4              | 1              |

Fig. 1. and a f length hybrid showing malformed
and malformed
Fig. 1. Corchorus depressus showing the prostrate habit. Fig. 2. A mature plant of C. olitorius showing erect habit and a few branches at the top. Fig. 3. C. capsularis showing erect habit and lack of any branch. Fig. 4. Comparative length of fruits of (left) C. olitorius, (centre) hybrid, C. olitorius x C. depressus, (right) C. depressus. Fig. 5. F₁ hybrid seedlings, C. olitorius x C. depressus. Fig. 6. Adult F₁ hybrid, C. olitorius x C. depressus. Fig. 7. Same hybrid showing warty projections on the stem. Fig. 8. Variability in leaf shape in the same hybrid: some of leaves are malformed. Fig. 9. Flower shape and size in the same hybrid. Note two flowers in the same pedicel in the centre of upper row and the reduced small flowers in the centre of bottom row. Fig. 10. Highly sterile pollen grains in the
Fig. 13. Many called sporads in the hybrid, C. olitorius × C. depressus. Fig. 14. Entry of C. depressus pollen tube into ovule of C. olitorius under the influence of 300 mg/l IAA.

(B). C. capsularis × C. depressus

As in olitorius and depressus cross, here also only the application of IAA in lanolin paste yielded fruits and the fruit set was unidirectional. namely, with capsularis as female (Table 5).

**Table 5 : Results of crosses, C. capsularis × C. depressus**

<table>
<thead>
<tr>
<th>Parents</th>
<th>No. of crosses made</th>
<th>Whether IAA applied</th>
<th>No. of fruit set</th>
<th>No. of fruits harvested</th>
<th>No. of full seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>capsularis</td>
<td>depressus</td>
<td>16</td>
<td></td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>29</td>
<td>1</td>
<td>19</td>
<td>9</td>
</tr>
</tbody>
</table>
(b) Germination: In this combination also seed germinated over a longer period and its rate of germination was slightly lower (60%). None of the seedlings were albino or xantha as observed in the F₁ seedling population of C. olitorius × C. depressus crosses. The results are shown in Table 6.

<table>
<thead>
<tr>
<th>No. of seeds sown</th>
<th>Period over which seeds germinated</th>
<th>No. of seeds germinated</th>
<th>No. of seedlings</th>
<th>No. of adult plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>6 weeks</td>
<td>18</td>
<td>15</td>
<td>2</td>
</tr>
</tbody>
</table>

Although initially eighteen seedlings started growing ultimately all of them except two died at various stages of development.

(c) Fertility of the F₁ hybrids

The two F₁ hybrids were different in the extent of their pollen stainability. In plant No. X the percentage of the stainability was 20 and in the other i.e. B it was 19. In both, the fruits contained fully developed seeds and their number ranged from 2 to 35.

<table>
<thead>
<tr>
<th>Plant no.</th>
<th>Triad</th>
<th>Tetrad</th>
<th>Pentad</th>
<th>Hexad</th>
<th>Heptad</th>
<th>% of tetrads</th>
<th>Total no. of sporads</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>2</td>
<td>48</td>
<td>29</td>
<td>4</td>
<td>3</td>
<td>55.8</td>
<td>86</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>60</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>77.9</td>
<td>77</td>
</tr>
</tbody>
</table>

Seed germination was good and unlike the F₁ seedlings, some F₂ seedlings were albino and some xantha.

Because of the good pollen fertility it was possible to backcross capsularis with the pollen of both the F₁s. The results of backcrosses are given below (Table 8):
TABLE 8: Results of backcross, C. capsularis (female) × F₁,  
(C. capsularis × C. depressus)

<table>
<thead>
<tr>
<th>No. of backcrosses</th>
<th>Parents</th>
<th>% Pollen stainability</th>
<th>No. of fruit set</th>
<th>% fruit set</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td>XF₁ capsularis</td>
<td>20</td>
<td>7</td>
<td>9.09</td>
</tr>
<tr>
<td>51</td>
<td>BF₁ capsularis</td>
<td>49</td>
<td>4</td>
<td>7.84</td>
</tr>
</tbody>
</table>

(d) Morphology of the two hybrids and their comparison with the parents

The two F₁ hybrids, although of the same parentage, were strikingly different from each other in their morphology, pollen stainability and seed fertility. The male parent was not made homozygous and its heterozygosity evidently accounts for their diversity in morphological and other characteristics of the two F₁s. This F₁ hybrid was also very much unlike the one reported by Baig (1957). In his F₁, Baig observed dominance of globular fruit of the female over the elongated fruit of the male whereas the two hybrids reported here showed the dominance of the elongated fruit of the male. The dominance of elongated over globular shape of the pod was also observed in the F₁ of the cross, C. olitorius × C. capsularis (Islam and Rashid 1960, Swaminathan and Iyer 1961). Haque and Islam (1966) also noted the same character relationship in olitorius-capsularis cross. Unlike olitorius-depressus hybrid these two F₁s were normal in growth and vigour and lacked warty projections. Both of them were profusely branched showing the dominance of the male parent. Also the branches were multidirectional unlike the male parent and the olitorius-depressus hybrid. Another male character found dominant in the hybrids was their flowering over a longer period. This prolonged period may be related to their acquiring semi-perennial habit from the male. That both the F₁s lived for two more months than capsularis, must be because of transmission of genes for perennial habit from depressus—which sired the cross. In the background of capsularis genotype, however, these genes did not have their full penetrance and as such the perennial habit did not find full expression.

(e) Cytology: In plant no. X, meiosis was slightly irregular (Bhutto, N. unpublished). Of the 13 cells analysed for A₁ in 10 there were no laggards, in two there were two and one in the remaining cell (Table 9).
Table 9: Frequency of laggards in PMC's of hybrids

<table>
<thead>
<tr>
<th>Plant no.</th>
<th>No. of PMCs examined</th>
<th>No. of cells with no laggard</th>
<th>No. of cells with one laggard</th>
<th>No. of cells with two laggards</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>13</td>
<td>10</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No clear M I at side view was available for study. Whatever cells were examined, chromosome units in excess of 7 were seen indicating the presence of univalents. Judging from the $7 + 7$ distribution at A I in 10 out of 13 cells, the tetrads were found more irregular than expected (Table 7); so also the pollen stainability which was only 20%. The high sterility of pollen may be on account of the disharmonious assortment of chromosomes resulting from lack of bivalent formation at M I.

In plant no. B, 10 M I were examined: in all of them there were 7 II. In 8 cells examined for A I, no laggards were seen (Table 9). The sporad analysis showed that they were much more regular than the plant X. In spite of regular meiosis only 49% pollen was stainable indicating cryptic structural differences between the two species.

Discussion

That examination of each and every emasculated anther under high magnification prevents self-pollination has been shown by Islam (1960). Using this technique he showed that the reported instances of maternal inheritance in the interspecific hybrids in Fragaria (East 1927) were the results of accidental self-pollination and not the examples of unique mode of inheritance. The present hybrids obtained through the same technique as employed by Islam were very different from those of Memon (1957) and Baig (1957) who reported high pollen and seed fertility in their hybrids, olitorius × depressus and capsularis × depressus respectively.

The olitorius-depressus hybrid was near sterile with only 3.4% pollen stainability. It showed the characteristic branching pattern of male and also absence of one or two serratures from some leaves—a again a character of the male parent (Fig. 8). The stipule was conspicuously larger than either parent and might have resulted through the interaction of genes causing transgression. The abnormality and variation in petals both in their number.
adhesion, the presence of twin flowers and jointed carpels, warty projections on the stem represented the same phenomenon that the genomes of \textit{oltiorius} and \textit{depressus} are unable to function together harmoniously. The genetic physiologic system got so much disturbed on account of disharmonious combination that abnormalities of all kinds appeared in the hybrid. Variation in petal abnormalities was obviously due to different degrees of penetrance. That the genomes were highly incompatible was also unmistakably clear from the presence of the significant proportion of albino, \textit{xantha} seedlings among hybrid progeny. Further proof of it also came from the observation made in the study of meiosis. The chromosomes were sticky, the maximum number of bivalents ranged from 2-4 and both anaphase I and II contained a large number of laggards resulting in the formation of sporads with as high as 10 cells. The above characteristic in the plant established beyond doubt that it was true hybrid. Another strong point against accepting Memon's plant as hybrid was that in the cross without hormonal treatment no pollen tube of \textit{depressus} was found to grow beyond halfway through the style of the female parent. There are however some points which are irreconcilable even if it is accepted that Memon's was a self-pollinated plant. Firstly why the plant stem divided a little distance above the ground and why were there drooping branches as if simulating the decemvent habit of \textit{depressus}, the male parent. Secondly, there was some sterility in her hybrid. One might be tempted to regard the drooping branches to have been transmitted through chance hybridization between cultivar and much branched wild \textit{oltiorius} which occurs as a common place wild plant in the open and cultivated field wherever there is some moisture. But this consideration does not seem to hold much water in the light of the results of Haque (1970) whose F₁ and F₂ population in the cross, wild \textit{x} cultivar \textit{oltiorius} did not contain any individual like Memon's drooping F₁. What actually happened in her cross is anybody's guess; the phenomenon of merogyn may explain the presence of a male character but confirmation through embryological studies will be necessary before any conclusion of this kind is drawn. Suspecting merogyn, however, will presuppose the entry of pollen tube into the embryo sac of female parent. This was not observed in the present investigation. Indirect evidence that merogyn may result comes from the crossing data, c-4x \textit{oltiorius} \textit{x} \textit{C. hirtus} (tetraploid), in which a polyhaploid originated inheriting only branching character from the male parent (Mughal 1969).

Regarding Baig's \textit{capsularis-depressus} F₁ it would have been straightway dismissed as self-pollinated \textit{capsularis}, had it not been for the fact that the branching of the main stem was seen to have started below the ground. Other than this and the branching in the F₁ hardly there was any character by which its hybrid nature could be ascertained. It had spheroidal fruits exactly like those of female whereas both the F₁s in the present study bore elongated fruits like those
of male. Elongated shape was found dominant over spherical in *olitorius-capsularis* (Islam and Rashid 1960), *tridocularis-capsularis* (Sharif 1961) cross and as expected the same dominant-recessive relationship was obtained in the present F₁s. The presence of spherical fruits, in Baig’s F₁, therefore strongly militates against accepting it as a hybrid. Its regular meiosis, cent per cent normal tetrad and high pollen (92.8%) and seed fertility as against irregular, meiosis, a sizable proportion of abnormal tetrads, high pollen sterility in the two present F₁s further strengthen the point made above. It is possible that what Baig thought to be branching below the ground might have been the result of two plants growing very close together giving one the impression of branching. This point should have been settled by Baig by digging the F₁ at some stage of the development before claiming this plant to be hybrid.

The two present F₁s bore *olitorius* like fruits raising doubt in one’s mind that perhaps these were hybrids of *olitorius × depressus* rather than *capsularis × depressus*. Comparative study of the cross section of the fruit of the hybrids and the two parents removed this doubt. In cross section the contour of the fruit was more like *capsularis* with shallower ridges and furrows than *olitorius*.

The two F₁s as pointed out under experimental results were morphologically somewhat different. Their differences were, however, more pronounced in respect of meiosis, extent of pollen and seed fertility. In one, laggards were seen, in the other absent in the small number of PMCs examined (Table 9). In the one with laggards, tetrads were abnormal. In the former the pollen stainability was 20% and in the latter without laggard 49%. As has been explained earlier the *depressus* parent used in the cross was not made homozygous before crossing. This might have been responsible for the differential cytological behaviour of the two plants. It is well known that genes such as those found in 3 B of hexaploid wheat control pairing (Riley and Kempna 1965) and the one (plant X) with more univalents might have lacked genes responsible for pairing while the plant ‘B’ had those.

Genomic relationship

In the light of the results reported here certain changes need to be made in the crossing polygon suggested by Islam (1965). Firstly, no hybrid is possible to obtain between *olitorius* and *depressus* or between *capsularis* and *depressus* as has been shown in the polygon. Since hormonal treatment yields unidirectional hybrid in both as reported earlier with cultivar as female, crossing polygon needs to be changed only to the extent to show that the application of hormone is necessary for the formation of such hybrids. The type of line connecting the two pairs of species *olitorius*, *depressus* and *capsularis*, *depressus* is also to be changed since much greater sterility was observed in these two hybrid
combinations than that reported by Baig and Memon. From the present results it can be concluded that each of the above three species is a cenospecies by itself and limited unidirectional gene exchange is possible only through the intervention of some suitable techniques.

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