OCCURRENCE OF PARTHENOGENETIC POLYHAPLOID IN A CROSS, C-4X C. OLITORIUS X C. PASCUORUM

RASHIDA MUGHAL AND A. S. ISLAM
Department of Botany, University of Sind.

Although extensive hybridization work has been done in recent past to elucidate the relationships between the different diploid species of *Corchorus* (Islam. 1965), few attempts have so far been made to determine the relationships between diploid and tetraploid *Corchorus* species. The only published account of such crosses is between *C. siliquosus* L. and *C. sidoides* F. von Muell. reported by Datta and Sen (1961).

The present work was undertaken with a view to determining the extent of crossability between *C. olitorius* var. C.G. and a natural tetraploid *C. pascuorum* from Australia.

Review of Literature

Roy (1962) reported for the first time the chromosome number of *C. pascuorum* to be 28. This brought the total number of natural tetraploid species of *Corchorus* to be 3, the first 2 being *C. siliquosus* and *C. hirtus* reported by Rao and Datta (1953), Islam and Qaiyum (1961) respectively.

Crosses between *C. olitorius* var. C.G. and *C. siliquosus* ex-Jamaica have been attempted by Islam and Abbasi and the results reported elsewhere (1966).

Material and Methods

(*a*) *Species used*

(*i*) Colchicine induced tetraploids of *C. olitorius* L.var. C.G.; crosses of species with the same chromosome number succeed more easily; hence colchicine induced tetraploids were used.

(*ii*) *C. pascuorum* Domin., an Australian natural tetraploid; the seeds were obtained through the courtesy of S.L. Everist, Botanic Museum and Herbarium Brisbane, Queensland, Australia.

(*b*) *Crosses*

The flowers of *C. olitorius* and those of *C. pascuorum* open at 8-0 a.m. and 2-30 p.m. respectively. Because of the difference in anthesis, the freshly opened

flowers of *C. olitorius* were kept inside a refrigerator until used after having been placed in a desiccator containing silica gel. The flowers were emasculated in the evening; plastic bags were used to cover the emasculated flowers. These were pollinated the following morning. IAA, when used was applied in 300 p.p.m. strength in lanolin paste.

(c) Cytology

P.M.Cs. were fixed in acetic alcohol in the proportion of 1 : 3, stored in 70 per cent alcohol and studied in 2 per cent aceto-carmine.

Experimental results

(a) Crosses

The results of reciprocal crosses between c-tetraploid *C. olitorius* and *C. pascuorum* are shown in table I. Using c-tetraploid *C. olitorius* as the seed parent, the number of crosses made in 1964 was 200, in which IAA was applied in 150 crosses and in the remaining 50, no hormone was used. Only hormone applied crosses yielded 30 fruits. The number of seeds per fruit ranged from 4-18. A total number of 100 seeds was obtained; eighty reciprocal crosses yielded no fruit even though IAA was used. When the same number of crosses in the same direction was repeated in 1965 using Indole-Butyric acid, 8 fruits were obtained.

(b) Germination

Of 100 seeds obtained, only 45 appeared full and of these, 25 were sown in sterilized soil, and from the remaining 20 hybrid seeds, embryos were dissected and grown in test tubes containing White's medium supplemented by 4 per cent sugar and solidified in 0.75 agar.

Only 2 from a total of 25 seeds sown in the sterilized soil germinated as against 95 per cent germination in the control. The number of days taken by the hybrid seeds to germinate from the date of sowing was 12 as against 6 in the control. The 2 seedlings grew to adult stage and flowered and fruited. Out of 20 embryos implanted in the White's medium, 16 grew and 4 did not show any sign of growth. Of the 16 seedlings, none were found to be albino as reported by Islam (1964) in *C. capsularis* (female) x *C. olitorius* (male) hybrids or in c-tetraploid *C. olitorius* x *C. siliquosus* hybrids reported by Islam and Abbasi (1966). Six of the 16 seedlings were soon lost due to contamination and the remaining 10 grew for 22 days by which time the third leaf appeared in the majority of hybrids. All the 10 hybrids were unfortunately lost during transfer from the tube to the soil. The death of the seedlings appeared to result from the shock following transplantation

TABLE I

TABLE 2

Comparison of characters of the two parthenogenetic diploids with diploids and c-tetraploid control, C. olitorius var. C. G.

Characters	*C. olitorius* (tetraploid)	*C. olitorius* (diploid)	Plant (a)	Plant (b)
Height	250-300 cm	350-400 cm	152.4 cm	190.6 cm
Branching	Rare	non-branching	non-branching	Sparingly branched
Flowers	Yellow	Yellow	Pale yellow, smaller	Yellow, as large as diploid
Percentage of pollen stainability	90	98	6.4	34
Fruit length	6.1-7.2 cm	6.35-7.5 cm	2.5-4.1 cm	5.8-6 cm
No. of fruits per node	1-2, rarely 3	1-2, rarely 3	Mostly 2-3	1-2, rarely 3
No. of seeds per fruit	120-170	200-300	8-25	11-55

CamScanner

rather than on genetic grounds. Improved techniques of transplantation will be used this year to transfer the hybrid seedlings from the test tube to the soil.

(c) *Morphology of the plants from the cross, C. olitorius x C. pascuorum*

The two plants R/500-64-1 (*a*) (Fig. 2) and R/500-64-2 (*b*) resembled the female *C. olitorius* closely (Fig. 3). They differed from the control c-tetraploid *C. olitorius* (Fig. 1), by being relatively weak and of smaller height. The size of petals in (*a*) was smaller than that of the c-4x *C. olitorius* control. The percentage of stainable pollen grains was 6.4 (Figs. 5 & 6) and the fruits it bore were much smaller, 2.5-4.1 cm compared to the control 5.8-6.0 cm (Figs. 4 a, b, c). The number of seeds in this plant ranged from 8-25 as compared to 120-170 in the control.

The other plant (*b*) was sparingly branched but the size of petals was not different from that of c-4x control *C. olitorius*. The percentage of stainable pollen grains in this plant was 34 much higher than that of plant (*a*) in which the stainability was 6.4. The fruit size was larger in plant (*b*) than in (*a*), the size being 5.8-6 cm compared to 2.5-4.1 cm in (*a*). The same was true of the number of seeds. As against 8-25 seeds per fruit in plant (*a*), the number of seeds in plant (*b*) ranged from 11-55.

In table 2, a comparison of the two plants has been made with diploid and c-tetraploid *C. olitorius*.

On chromosome examination both the plants were found to have the diploid chromosome number *i.e.* 14. The diploid chromosome number indicated beyond any reasonable doubt that none of the plants were true hybrids and possibly they arose from a diploid egg of the c-tetraploid *C. olitorius* through parthenogenesis stimulated by the pollen of *C. pascuorum*.

(*d*) *Cytology*

Study of the diakinesis revealed 7 bivalents in the p.m.cs. of both the plants. In anaphase I studied in the p.m.cs. of plant (*a*), 2 lagging chromosomes were seen. Sterility of pollen grains as high as 94 per cent cannot be explained on the basis of such chromosomal irregularities alone.

Discussion

Two plants were obtained from the cross, c- tetraploid *C. olitorius* x *C. pascuorum* using the latter as pollen parent. These two plants on chromosome examination turned out to be diploid. The crosses were made between 2 tetraploid species and therefore the chromosome number in these plants, if they were hybrids, must have been at tetraploid level. With 14 as their somatic chromosome number these 2 plants cannot therefore be regarded as hybrids. Both the plants resembled

CamScanner



Comparative height and habit of the parthenogenetic diploid with normal diploid control of *C. olitorius* & c-4x *C. olitorius*. Fig. 1. c-4x *C. olitorius*. Fig. 2. Parthenogenetic diploid. Fig. 3. Diploid control *C. olitorius*. Fig. 4. Comparative size of fruits of c-4x *C. olitorius* (*a*), parthenogenetic diploid (*b*), diploid control *C. olitorius* (*c*).

CamScanner

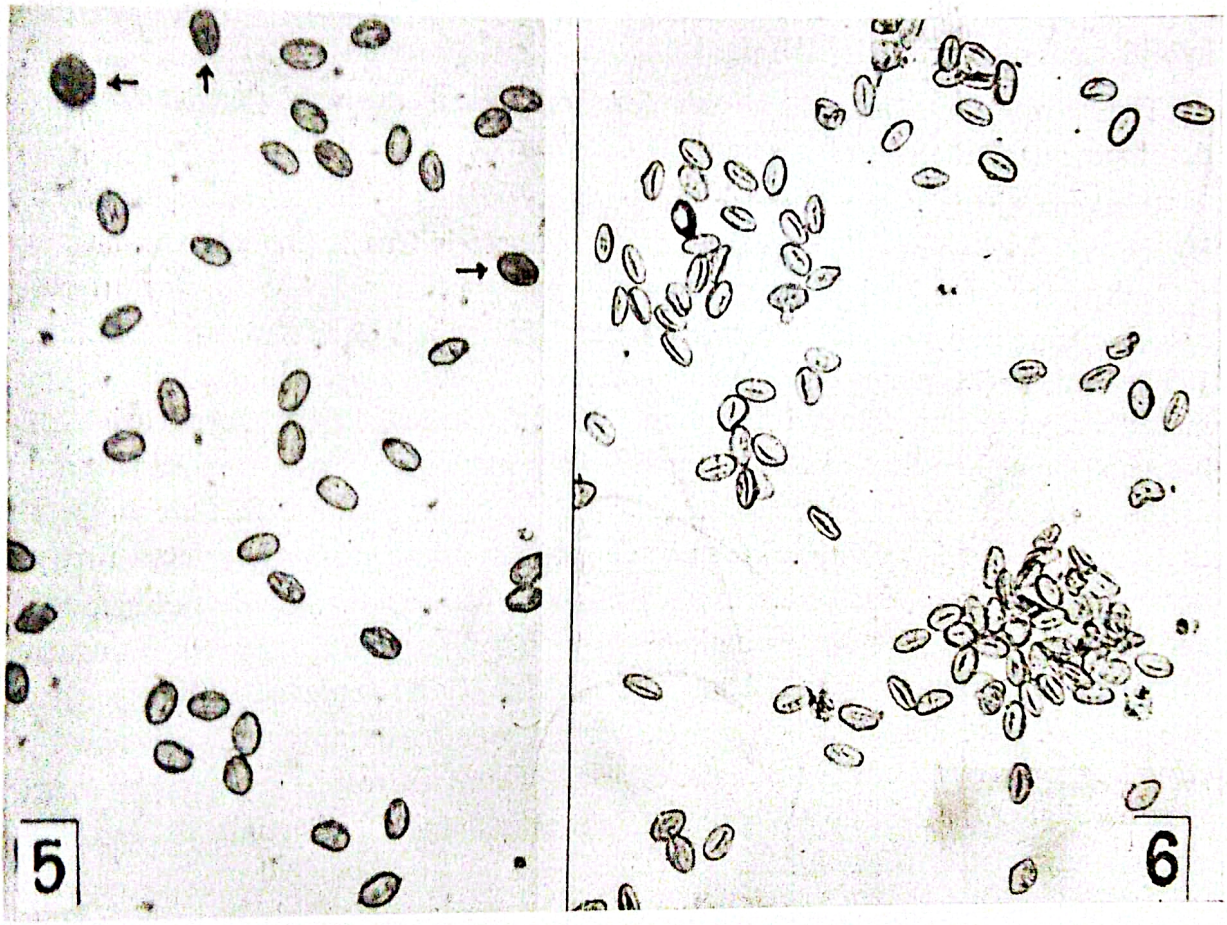

Fig. 5. Stainability of pollen grains of c-4x *C. olitorius*. Note the pollen grains of large size (arrow marked).

Fig. 6. Stainability of pollen-grains of parthenogenetic diploid. Note that almost all the pollen grains are sterile.

closely the diploid *C. olitorius*. Could they be therefore contaminants? The very low percentage of stainability of pollen grains in both the plants (6 and 3 per cent in plant (*a*) and (*b*) respectively), smaller fruits and relatively smaller flowers a fewer number of seeds per fruit indicated beyond doubt that they cannot be regarded as diploid contaminants. Use of sterilized soil for germinating the hybrid seeds also precludes the possibility of any contamination.

These two plants may have therefore originated through parthenogenetic development of the egg of c-tetraploid *C. olitorius*.

Parthenogenesis may occur spontaneously as well as by the stimulus provided by the pollen of some other species; which of these is true in the present case is difficult to say since haploids are known to arise spontaneoulsy in the progeny of colchicine induced polyploids e.g. in c-tetraploid maize (Randolph and Fischer 1939), Triticale (Muntzing *et al.*, 1963) as well as they may be stimulated to development through interspecific hybridization (see Maheshwari, 1950). Application of hormone on the pedicel of the crossed flowers may however be an additional factor in inducing parthenogenesis. The results reported here further suggest that the two, namely, *C. olitorius* and *C. pascuorum* are distantly related firstly because no fruit is obtained when crosses are made between them without hormone and secondly when plants are obtained through hybridization they are found to be of parthenogenetic origin. Islam and Abbasi (1966) reported recently that they had obtained 2 hybrids between *C. siliquosus* and c-tetraploid *C. olitorius* by using IAA on pedicels of the pollinated flower. From the results reported by them it is clear that of the 2 tetraploid species *C. pascuorum* and *C. siliquosus*, the latter is more closely related to *C. olitorius*.

The high sterility of pollen grains in one of the plants (plant a) is hard to explain on the basis of chromosomal irregularity alone. It is possible that during megasporogenesis in the tetraploid *C. olitorius*, the megaspore which developed into the polyhaploid plant might have received some semilethal combinations of genic material and as such it showed high sterility of pollen grains. The sterility factors did not however operate to the same extent on the formation of female gametes in the polyhaploid since the fruits borne by both the polyhaploids contained a good proportion of viable seeds.

Summary

Two parthenogenetic polyhaploids were obtained unidirectionally from the cross, c-4x *C. olitorius* (female) x *C. pascuorum*—a wild natural tetraploid from Australia. Fruit set was obtained only with the use of IAA in lanolin paste on the pedicel of pollinated flowers.

CamScanner

Of the 2 diploids one showed as much as 93.6 per cent pollen sterility and the other 66 per cent. The fruits in both the polyhaploids were smaller than the normal diploid *C. olitorius* with a fewer number of seeds.

References

Datta, R. M. & Sen, S. K. 1961. Interspecific hybridization between *C. sidoides* F. v. Muell & *C. siliquosus* L.—a natural polyploid, *Euphytica* **10:** 113-119.

Islam, A. S. 1964. A rare hybrid combination through application of hormone and embryo culture, Nature **201:** 320.

————, & Qaiyum, F. 1961. Some more chromosome numbers in the species of *Corchorus*. Curr. Sci. **30:** 433.

————, 1965. Interspecific hybridization in the genus *Corchorus*. S. U. Sci. Res. J. **1:** 71-112.

————, & Abbasi, Z. 1966. Interspecific hybridization in the genus *Corchorus*. *C. olitorius* L. var. C. G. *C. siliquosus* L., S. U. Sci. Res. J. **2:** 21-28.

Katayama, Y. 1934. Haploid formation by X-rays in *Triticum monococcum*. Cytologia **5:** 235-237.

Maheshwari, P. 1950. An introduction of Embryology, McGraw Hill Book Co.

Muntzing, A., Hrishi, N. J. & Tarkowski, C. 1963. Reversion to haploidy in strain of Hexaploid and octoploid Triticale. Hereditas **49:** 78-90.

Randolph, L. F. & Fischer, H. E. 1939. The occurrence of parthenogenetic diploid in tetraploid Maize Proc. Nat. Acad. Sci. Wash. **25:** 161-162.

Rao, N. S. & Datta R. M. 1953. Chromosomes of the genus *Corchorus*. Nature **171:** 754.

Roy, K. 1962. Chromosome number of *Corchorus pascuorum* Domin., a wild jute of Australia. Curr. Sci. **31:** 205.

CamScanner