

A 1 per cent solution of cresyl violet is prepared in 50 per cent acetic acid and used as such. After fixation in Farmer's fixative, the root tips are macerated in alcoholic hydrochloric acid for 2-3 min, washed in alcohol and stained in the cresyl violet solution for about 1 min. It is then washed in tap water, squashed under the coverslip and observations made. The structure of the mitotic chromosomes is well demonstrated by cresyl violet, as dense violet coloration against a clear background. In the metaphase plate of the root tip smears of the *Vicia faba* (Fig. 2), chromosomes are seen in their natural morphological condition. The specific heterochromatic segments, due to nucleic acid starvation, are well demonstrated too (arrow). The active nuclei of the meristematic cells are demonstrated in Figs. 3 and 4.

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GENETICS

A Rare Hybrid Combination through Application of Hormone and Embryo Culture

SINCE the first report¹ of unidirectional success of the cross, *Cochorus olitorius* (♀) × *C. capsularis* (♂), Swaminathan and Sulbha² have also obtained hybrids in the same combination. Their reciprocal cross likewise did not yield any hybrid plants confirming the authors' earlier report. The fact that in this combination a small percentage of fruits was set with hormone application in 1958³ encouraged me to repeat the cross at Cornell. The seeds resulting from the hybridization of these two species germinate with difficulty. It was, therefore, decided to develop suitable culture media for growing the hybrid seedlings in case hybrid seeds were obtained. This is to report success in producing a hybrid through the use of embryo culture technique in the hitherto unachieved reciprocal combination, namely, using *C. olitorius* as the pollen parent in the cross, *C. capsularis* × *C. olitorius*.

In the investigation recorded here the hormone, indolyl-3-acetic acid (IAA), was applied in lanolin paste at 300 p.p.m., instead of wrapping the pedicel of the pollinated flowers with cotton soaked in the hormone as described in the previous report¹. This slightly modified technique improved the percentage of fruit set. Of the 87 crosses, *C. capsularis* (♀) × *C. olitorius* (♂), 27 fruits were parthenocarpic with shrivelled seeds and a total of 55 seeds were obtained from the remainder of the fruits.

The seeds were first surface-sterilized in a saturated aqueous solution of calcium hypochlorite for 30 min. These were then washed in sterile water and placed overnight in sterile water inside an incubator set at 25° C. From the sterile water the seeds were transferred to 50 per cent 'S.T. 37' (hexylresorcinol) for 10 min and then kept immersed in 50 per cent ethanol until dissection. Nitsch and White's basic media fortified with 0.1 per cent yeast extract with different supplements were tried to grow the



Fig. 1. A semi-albino hybrid of the cross *C. capsularis* × *C. olitorius* growing in culture media (same size)

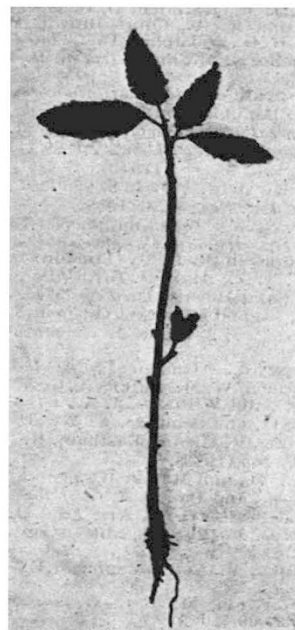


Fig. 2. Eight-month-old F_1 hybrid of the same cross, photographed from the herbarium specimen (× 0.5 natural size)

embryos as shown here. Sucrose at 2 per cent and agar at 0.75 per cent were used in preparing all the media.

From Table 1 it is clear that White's medium supplemented by 0.1 per cent yeast extract and 0.05 p.p.m. each of kinetin and IAA proved to be the best for growing hybrid embryos. Neither of the two media without supplement was suitable for growing the normal embryos.

Of the 15 seedlings that were transplanted to the soil in June 1962, two were semi-albino (Fig. 1) and died soon after their transfer. The remaining 13 seedlings died at different times between 3 and 8 months of age. Only one plant lived for 8 months and during this period it grew to a height of only 12.6 cm (Fig. 2) as against 200-300 cm—the height to which the parents of the hybrid usually grow. The upper four leaves of this plant measured 2.4-3.0 cm in length and 0.8-1.2 cm in width. This hybrid plant showed dominance of the male parent in respect of B/L ratio of the leaves, which was 0.315 ± 0.034 . It was intermediate in respect of characters of serration and serrature. Before it died it produced only a few flower buds, but they dropped before blooming.

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Table 1. PERFORMANCE OF HYBRID EMBRYOS IN DIFFERENT SUPPLEMENTED MEDIA OF NITSCH AND WHITE; CONCENTRATIONS ARE IN P.P.M.

Basic medium	IAA	Kinetin	Gibberelle acid	No. of embryos implanted	No. that grew in the medium
White	0.1	—	1	8	—
Nitsch	0.1	—	1	4	1
White	0.05	—	1	11	—
Nitsch	0.05	0.05	0.05	4	1
White	0.1	0.1	—	9	1
Nitsch	0.1	0.1	—	4	2
White	0.05	0.05	—	11	10
Nitsch	0.05	0.05	—	4	—