

excised leaves was reasonably synchronous since most units of the population attained optimum of each phase within 4 hr period of its beginning (table I). Also, penetration and colonization processes on excised leaves were shortened appreciably compared with that of cleared leaves (figure 1). This was apparently because (i) the clearing process results in loss of nutrients¹³, (ii) *D. sorokiniana* requires exogenous nutrients for penetration and colonization¹⁰ of host leaves and (iii) nutrients leach out of excised barley and wheat leaves into the infection drops¹⁴ and are reabsorbed by *D. sorokiniana* spores¹⁵. This viewpoint gets further support by the shortening of penetration and colonization phases on cleared leaves when the infection drops were enriched (table I). Because of its fairly well-defined kinetics on excised host leaves, this host-parasite combination lends itself to qualitative and quantitative biochemical studies of early host-parasite interactions. It is possible to predict what portion of the parasite population is in a particular stage of development at a particular time.

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IN VITRO HYBRIDIZATION IN AN INCOMPATIBLE CROSS — BLACKGRAM × GREENGRAM

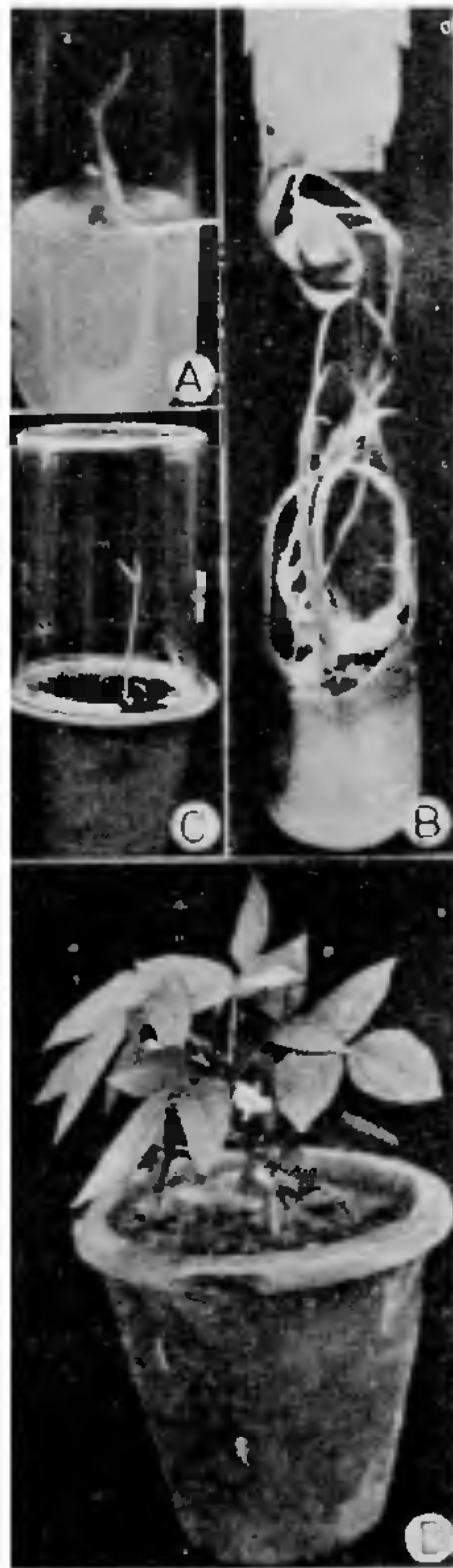
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DURING the last decade although considerable progress has been made in the improvement of grain-legumes (pulses), yet their yield has remained almost static. This is attributed to the lack of sufficient genetic diversity in the base populations of these crops. Thus need is felt to increase the genetic variability by resorting to means other than conventional¹. The present communication is, therefore, a part of the project undertaken to achieve wide hybridization in pulses using *in vitro* techniques. By combining the application of growth regulators and embryo culture, interspecific hybrids have been obtained in an otherwise incompatible cross^{2,3}, involving two important Indian pulses, blackgram (mash) × greengram (mung). The ultimate objective is to combine the desirable traits of these two parents. Whereas blackgram is resistant to the yellow-mosaic virus and seed shattering, greengram possesses larger number of seeds per pod and contains more easily digestible and relatively high protein⁴.

The emasculated flowers of blackgram (*Vigna mungo* cv. Mash 1-1) pollinated with pollen of greengram (*V. radiata* cv. Russian Mung) were treated with a distilled-water solution containing naphthalene acetic acid (25 mg/l + gibberellic acid (100 mg/l) and kinetin (5 mg/l) twice a day, for a week. The hybrid embryos were excised from the developing pods (14 days after pollination), and cultured on Murashige and Skoog's medium⁵ (MS) supplemented with indoleacetic acid (1 mg/l) + kinetin (0.2 mg/l) + coconut water (70 ml/l). All manipulations were conducted aseptically in a Laminar Flow Cabinet (Klenzaid, Bombay), and the cultures were maintained at 25 ± 2° C.

The daily application of growth regulators to the hybrid pods delayed their abscission⁶, prevented the early abortion of the embryos, and thus encouraged their development to a stage when they could be excised and cultured. The retention of pods in the control and hormone-treated ones was 8% and 20% respectively.

The excised embryos of both the parents and those of the hybrid in cultures resumed growth within 4



Figures A-D. *In Vitro* culture of the hybrid embryos of a cross *Vigna mungo* × *V. radiata*, and the subsequent transfer of the hybrid plant from test tube to a pot. **A.** Plantlet from a hybrid embryo 3 weeks after culture on MS + IAA (1 mg/l) + kinetin (0.2 mg/l) + coconut water (7%). **B.** A 5-week-old culture showing the formation of multiple shoots from the hybrid embryo. **C.** Five-week-old embryo-derived plant, 4 days after transferring to the pot containing autoclaved soil. **D.** A hybrid plant (10-week-old) grown in broad day light; note the flowering.

TABLE I

In vitro growth response of parental as well as hybrid embryos cultured on MS + IAA (1mg/l) + kinetin (0.2 mg/l) + CW (70 ml/l).

Embryo (parentage)	No. of embryos cultured	No. of embryos forming plants	Percentage of embryos forming plants
<i>Vigna mungo</i>	30	24	80.00
<i>V. radiata</i>	30	21	70.00
<i>V. mungo</i> × <i>V. radiata</i>	30	19	63.33

days, and complete plants were obtained in 4–5 weeks (figure A–C). The frequency of embryos developing into plants was more in the parents as compared to the hybrids (Table 1). The hybrid plants have been raised to maturity (figure D) and were somewhat intermediate between their parents. Whereas the flower colour in blackgram and green-gram is bright yellow and greenish-yellow respectively, the hybrid flowers were dull yellow. The segregants F₂ were partially fertile due to the meiotic irregularities. The seeds showed green, black and other intermediate colours, suggesting gene complementation in the hybrids. These seeds will be sown in the field to raise further progenies, and the plants will be incorporated into the pulse improvement programmes.

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