chloride (0.2%) for 5 min. After a thorough wash with autoclaved distilled water the sheaths were removed and inflorescence segments measuring about 1 cm were inoculated on callusing medium containing Murashige and Skoog's (MS) nutrients supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D, 1 mg/l), 3-indoleacetic acid (IAA, 5 mg/l) and kinetin (0.5 mg/l). The cultures were incubated under a 16-h photoperiod at 25±2°C.

Callus initiation started within 4-6 days and good proliferating calli were derived by the end of 21 days. Nearly all parts of the inflorescence segments, except the glumes, showed proliferation. The calli produced were white, soft, compact and slow-growing. Most of the cultures had generated white, hard structures on the surface at the end of 10 weeks (figure 1A).

Subcultures were usually carried out every 20-24 days on the same medium. After 12-14 weeks of growth the calli showed numerous dome-shaped, greenish, localized areas from which green, leafy shoot buds developed in due course of time. Often the green areas were found to be flanked by green, leafy structures, which were actually the enlarged scutella of the precociously germinating embryos. The frequency of shoot bud formation ranged between 5 and 9 per culture. Further development of shoot buds was achieved after transferring them to a medium devoid of growth regulators (figure 1B, C). On this medium, the cultures with shoot buds showed profuse rooting. The majority of the cultures developed several roots (figure 1C, D). Of these, some roots indicated direct link with the shoots. These in vitro produced plantlets were successfully transferred to soil, and up to 60% of the plantlets survived to maturity.

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RECOVERY OF RARE INTERSPECIFIC HYBRIDS OF GRAM CICER ARIETINUM × C. CUNEATUM L. THROUGH TISSUE CULTURE

R. P. SINGH and B. D. SINGH
Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi 221 005, India

The genus Cicer has a total of 43 species of which only one, Cicer arietinum L., is cultivated while the rest are wild. C. arietinum (chickpea) crosses readily with only C. reticulatum. Attempts to hybridize it with other wild Cicer species have so far been unsuccessful. Bridge hybridization among wild species was also not successful. This has seriously hampered utilization of germplasm from wild relatives of chickpea in breeding programmes. Embryo rescue is a powerful technique to obtain hybrid plants from sexually incompatible crosses. Several rare hybrids have been obtained from the crosses of sexually incompatible species using the embryo culture technique. An attempt was therefore made to cross chickpea with C. cuneatum.

More than 2000 crosses were made but no seed-bearing pods were obtained. Manipulations like removal of stigma and part of style, pollination at bud stage, and delayed pollination were not beneficial. However, in one cross ten flowers were retained on the plant up to the 5th day. Generally, pollinated flowers dropped 4-5 days after pollination. Bassari et al. suggested that interspecific hybrids could be obtained through embryo culture from incompatible crosses of Cicer species. Hence, the ten pollinated flowers retained on the plant till the 5th day were surface-sterilized with 8% calcium hypochlorite for 15 min and washed with sterile distilled water 5-6 times. The ovaries of these flowers were dissected under a dissecting microscope and transferred to culture tubes containing embryo regenerating medium, conditioned with chickpea embryo culture (figure 1, B5, basal medium containing 0.5 mg/l BA, 0.1 mg/l naphthaleneacetic acid (NAA) and 100 ml/l coconut milk). The cultures were maintained at 26±2°C under a cool fluorescent light (4000 lux) in a 12-h photoperiod.

Eight of the cultures showed callus formation while the other two showed shoot-bud formation accompanied by small amount of callus. These shoot buds produced 10-15 shoots in each culture tube after 8-10 days (figure 2). Each of these shoots with small amount of callus, on further transfer to the
Figures 1–3. 1, Multiple shoot formation from young embryo (8-day-old) of chickpea. 2, Regenerated shoots from shoot bud developed from fertilized ovule of the cross *C. arietinum* × *C. cuneatum*. 3, Complete plantlet regeneration.

same medium in culture tubes, produced many shoots/plantlets within 20 days. In most of the cases shoots were accompanied by roots. Profuse root regeneration was achieved on transferring the shoots to B5 medium containing 1 mg/l NAA alone. The plantlets were transferred to small plastic cups, with 40% success. The surviving plants were transferred to pots. The hybrid plants were morphologically more similar to *C. cuneatum*, showing small linear leaflets (figure 3), although chickpea (*C. arietinum*) was the female parent. This confirms that the regenerated plants are hybrid and not selfed plants; selfed plants would be similar to chickpea morphologically. The hybrid plants did not flower.

15 October 1988; Revised 24 January 1989


**IN VITRO MASS-SCALE PROPAGATION OF ROSA HYBRIDA CV. LANDORA**

G. R. ROUT, B. K. DEBATA and P. DAS
Regional Plant Resource Centre, Bhubaneswar 751 012, India

Conventionally, most of the garden roses are propagated by budding or grafting of the scions on to suitable rootstocks. Of late, tissue culture techniques have been applied for rapid propagation of many hybrid rose cultivars and it has been suggested that roses can be propagated through tissue culture on a commercial scale and can be grown successfully on their own roots. It has also been stressed that tissue culture practices offer the possibility of eliminating pathogens from infected stocks, and also reduce the time and financial inputs for propagation. In this investigation tissue culture protocols for mass-scale propagation of Rosa hybrida cv. Landora have been standardized. This cultivar shows considerably poor multiplication rate in conventional methods.

Young branches with dormant axillary buds were cut from field-grown plants of Rosa hybrida cv. Landora and brought to the laboratory with their cut ends dipped in distilled water. The terminal portion with 5–6 nodes of each branch was discarded and the subsequent 10–12 nodes were taken for culture. The axillary buds with a small part of the stem (~2 mm) on both sides were cut off and surface-sterilized in 0.1% mercuric chloride solution for 25 min. The sterilized materials were rinsed four times in sterile distilled water and aseptically inoculated on to solidified MS medium. The basal medium was supplemented with different concentrations of 6-benzylaminopurine (BAP), gibberellic acid (GA$_3$), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) singly or in combinations, and 30 g/l of sucrose, and gelled with 8 g/l of agar (Glaxo, India). The pH of the medium was adjusted to 5.8 and the medium autoclaved for 20 min at 121°C and 1.06 kg/cm$^2$. All cultures were raised in 250 ml Erlenmeyer flasks (Borosil, India) and incubated at 25 ± 2°C under 14-h photoperiod provided by cool white fluorescent light (ca. 3000 lux). For induction of rooting microshoots were cultured on a filter paper bridge placed in liquid medium.

On a hormone-free MS medium the explants showed bud-break after about 22–30 days and the buds elongated a little but the subsequent growth of the explants was totally suppressed. In BAP-supplemented, or (BAP and GA$_3$)-supplemented media, early bud-break in about 6–10 days was noticed and multiple shoots were formed subsequently (table 1). Addition of BAP (0.25–1.0 mg/l) alone to the medium resulted in feeble callusing at the cut ends of the explants and the shoot elongation was considerably slow, though the average number of shoots produced in a 60-day culture period was around 4 per explant. Explant response remained between 63 and 80%. Incorporation of GA$_3$ at low concentrations (0.25–0.5 mg/l) in the BAP-supplemented medium gave encouraging results.

<table>
<thead>
<tr>
<th>Treatment (mg/l)</th>
<th>No. of explants cultured</th>
<th>Cultures with multiple shoots (%)</th>
<th>No. of shoots per explant (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>126</td>
<td>71.4</td>
<td>4.08 ± 0.75</td>
</tr>
<tr>
<td>BAP (0.25)</td>
<td>252</td>
<td>80.0</td>
<td>4.00 ± 0.08</td>
</tr>
<tr>
<td>BAP (0.5)</td>
<td>240</td>
<td>63.0</td>
<td>4.33 ± 0.17</td>
</tr>
<tr>
<td>BAP (1.0)</td>
<td>276</td>
<td>95.0</td>
<td>3.25 ± 0.59</td>
</tr>
<tr>
<td>BAP (0.25) + GA$_3$ (0.25)</td>
<td>220</td>
<td>91.6</td>
<td>2.66 ± 0.84</td>
</tr>
<tr>
<td>BAP (0.25) + GA$_3$ (0.5)</td>
<td>240</td>
<td>95.0</td>
<td>5.25 ± 0.92</td>
</tr>
<tr>
<td>BAP (0.5) + GA$_3$ (0.25)</td>
<td>240</td>
<td>90.0</td>
<td>4.75 ± 0.92</td>
</tr>
<tr>
<td>BAP (0.5) + GA$_3$ (0.5)</td>
<td>220</td>
<td></td>
<td></td>
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</tbody>
</table>