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Prospects of Using *Cicer canariense* for Chickpea Improvement

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There are eight annual and 34 perennial wild species in the genus *Cicer* (van der Maesen 1987). Many of these wild species are known to possess resistance genes to important biotic and abiotic constraints. Wild species placed in primary and secondary gene pools are crossable by conventional techniques (Ladizinsky and Alder 1976, Pundir and Mengesha 1995). There are wild species which are placed in tertiary gene pool based on non-crossability with cultivated chickpea (*Cicer arietinum*). One of the bottlenecks for the failure to produce hybrids was the absence of a suitable technique to save aborting embryos from interspecific crosses. Recent efforts at ICRISAT, Patancheru, India have led to the development of techniques to save aborting embryos from failing crosses (Mallikarjuna 1999). As a result hybrids have been produced between *C. arietinum* and *C. pinnatifidum*.

There is no report of successfull crossing of perennial wild species with cultivated chickpea and hybrid production. Mercy and Kakkar (1975) crossed perennial wild species *C. songaricum* with *C. arietinum*, and in spite of carrying out 4200 pollinations hybrid seeds were not obtained. Pundir et al. (1993) reported that none of the perennial wild species can be successfully grown to set seeds under the environmental conditions of ICRISAT research center, except *C. canariense*, a perennial wild species from Canary Islands (Fig. 1a).

Experiments were initiated at ICRISAT to cross kabuli chickpea cv ICCV 6 and desi cv GL 769 using *C. canariense* as the male parent. Emasculations and pollinations were carried out in the morning between 8.00 am and 10.00 am. Cross pollinated flowers were tagged and an aqueous mixture of growth regulators consisting of gibberellic acid, naphthalene acetic acid, and kinetin (7:1:1) was applied to the base of the pollinated pistils five hours after pollination.

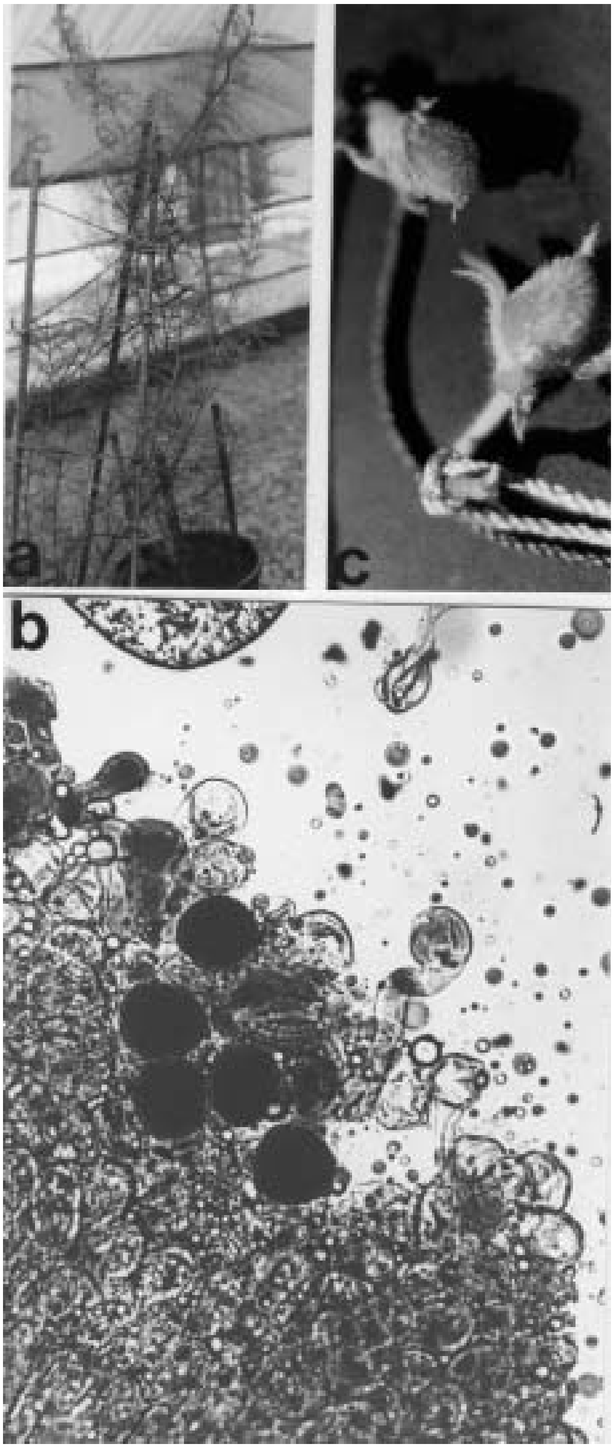

Figure 1. Interspecific hybridization between *Cicer arietinum* and *C. canariense*. a. The male parent *C. canariense* plant raised in the greenhouse. b. *C. canariense* pollen grains germinating on *C. arietinum* stigma. c. Growth regulators induced hybrid pod development in the cross *C. arietinum* × *C. canariense*.

Table 1. Success of pollinations in crosses between cultivated chickpea *Cicer arietinum* and the wild species *C. canariense*.

Chickpea cultivars	Pollinations	Pod set	Pod size (3–4 mm width)	Ovule size (1–2 mm width)
ICCV 6	25	11	6	5
GL 769	19	8	3	2

Light and fluorescent microscopic studies showed that the pollen grains germinated normally on the stigma (Fig. 1b). Swelling of the pollen tubes was rarely observed. Pod initials were observed at 6 days after pollination (DAP). By 14 DAP, yellowing of the pods was observed (Fig. 1c); hence pods were harvested at 14–18 DAP. The maximum pod size obtained was 4 mm × 4.5 mm and ovule width was 2 mm (Table 1). Pods were surface sterilized and green ovules of 2 mm width were aseptically cultured on the ovule culture medium, which consisted of ML-6 basal medium with 3% sucrose, zeatin (1.0 mg L^{-1}) and 0.25 mg L^{-1} indole acetic acid (IAA) (Mallikarjuna 1999).

Ovules did not show growth even after 45 days of culture. Ovules which were green at the time of culture had bleached. The ovules were dissected and the embryos were isolated. Globular embryos were observed. This indicated that the barrier to hybridization between *C. canariense* and *C. arietinum* was mainly post-zygotic. This is supported by the fact that pollen grains germinated normally on the stigma and development of the pod was dependent on growth regulator. In the pollinated pistils where growth regulators were not applied, development of pod initials was not observed.

About 45–50% of the pollinations do not form pods in the compatible cross *C. arietinum* × *C. echinospermum*. Hence, a large number of pollinations is a requisite for the success of a cross involving wild species of chickpea. In the crossing experiment involving the annual incompatible wild species *C. pinnatifidum*, large number of cross pollinations resulted in many hybrid pods but only few aborting ovules were large enough for culture and pod formation was dependent on growth regulators (Mallikarjuna 1999). Paucity of *C. canariense* pollen prevented large number of pollinations with cultivated chickpea. This could be one of the limiting factors for the success of this cross. Although hybrid plants were not obtained, information is now available on the nature of barriers operating in the cross involving cultivated chickpea and perennial wild species *C. canariense*.

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Anther Culture of Chickpea

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Since the discovery by Guha and Maheshwari (1964, 1967) the immature pollen could be induced to bypass normal development within the anther and the production of haploid plants was first realized in *Datura innoxi*. Since then, haploid plant production has been reported in more than 200 species (Dunwell 1986). Today, androgenetic haploids have been developed in economically important plants such as vegetable crops and cereals