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Short Communication

Interspecific hybridization between *Cicer arietinum* and *C. pinnatifidum*

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With 2 figures

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Abstract

An interspecific hybrid between *Cicer arietinum* cv. GL 769 and a wild species *C. pinnatifidum* was obtained after emasculation, pollination and application of growth regulators. Ovules were cultured and embryos were later dissected to obtain hybrid plants. These plants were albinos and morphologically resembled *C. pinnatifidum*. Shrivelled seeds were also obtained in 2% of the crosses, which on germination gave rise to albino plants. These plants did not survive beyond 20 days. The hybrid nature of these plants was confirmed by esterase isozyme studies. Hybrid shoots obtained from germinating embryos were cultured on modified ML-6 medium with BAP 2 mg/l, IAA 0.5 mg/l, where they turned green after 3–4 weeks. Transmission electron microscopy (TEM) studies on leaf sections from green hybrid shoots showed an improvement in the chloroplast structure, with better organized grana.

Key words: *Cicer arietinum* — *C. pinnatifidum* — chloroplast — embryo rescue — grafting — interspecific hybridization — ovule culture

The genus *Cicer* comprises of 33 perennial and eight annual wild species, in addition to the cultivated *C. arietinum*. The primary gene pool comprises of *C. arietinum* and *C. reticulatum*, which cross freely to produce fertile hybrids with normal chromosome segregation (Ladizinsky and Alder 1976). The secondary gene pool comprises only *C. echinospermum*. Among the species of primary and secondary gene pools, interspecific hybrids can be easily obtained with conventional crossing techniques and gene transfer is possible. Pundir and Mengesha (1995) obtained hybrids between cultivated chickpea and *C. echinospermum*, but with reduced fertility.

None of the wild species from the tertiary gene pool has been successfully crossed with cultivated chickpea (Pundir and van der Maesen 1983). Naik (1993) reported crossing *C. arietinum* with *C. pinnatifidum* (a member of tertiary gene pool), and obtained shrivelled seeds which, upon germination, gave rise to albino seedlings, that did not survive beyond 20 days of growth. *C. pinnatifidum* was chosen as the wild species in the present study because it has many desirable characters (ICARDA 1989). The present investigation reports the results of the study and discusses the prospects of obtaining hybrids between *C. arietinum* and *C. pinnatifidum*.

Seeds of *C. arietinum* cv. GL 769 and *C. pinnatifidum* (ICCW (38) were sown in the field. Crosses were carried out in October 1992 to February 1993 (post rainy season), June to September 1993 (Rainy) and October 1993 to February 1994 (post rainy season). Flower buds were emasculated and tagged between 15.00 and 16.00 hours. They were pollinated with fresh pollen of *C. pinnatifidum* the following morning between 08.00 and 09.00 hours. A mixture of growth hormones (GA 75 mg/l + napthalene-1-acetic acid (NAA) 10 mg/l + KN 10 mg/l) was applied to the base of the peduncle of the pollinated buds after pollination to prevent premature pod abscission. This was repeated for three consecutive days after pollination. Selfed pods on the same branch were removed. Pods which began to turn yellow were harvested and dissected so that the ovules could be cultured on ovule culture medium consisting of B_5 basal salts (Gamborg et al. 1968) with 0.25 mg/l indole-3-acetic acid (IAA) and 1 mg/l zeatin (ZN). After 10 days of culture, the embryos were dissected and cultured on embryo growth medium consisting of ML-6 medium (Kumar et al. 1988) with BAP 2 mg/l and IAA 0.5 mg/l.

Hybrid shoots which were more than 3 cm in length were transferred to ML-6 basal rooting medium (Sheila and Moss 1992) to induce roots. The following were the rooting media used in the present study. (a) rooting medium (RM) ML-6 basal salts + IBA 0.2 mg/l; (b) RM basal salts + IBA 0.2 mg/l + 0.2% charcoal (c) 1/2 MS (Murashige and Skoog 1962) basal salts + IBA 0.5 mg/l.

Hybrid shoots 2–3 cm long were grafted on to chickpea cv. GL 769 stocks. Grafts were maintained under 90–95% humidity until established.

Polyacrylamide gel electrophoresis (PAGE) was carried out on 10% gels to isolate esterase isozymes. The gels were stained according to the method of Scandalios (1969).

Transmission electron microscopy (TEM) studies of normal green leaves of chickpea cv. GL 769, *C. pinnatifidum* and albino leaves of the hybrid were carried out according to the method followed by Reddy et al. (1995) and Hayat (1992), and the sections were examined under Phillips CM 20 transmission electron microscope.

Pod set in the cross GL 769 and *C. pinnatifidum* varied with the season. It was 22% in the post rainy season (October 1992–February 1993), 27% in the rainy season (June 1993–September 1993) and 23% in the post rainy seasons (October 1993–February 1994). Application of hormones to the interspecific pollination was mandatory in order to obtain pods. In 2% of the pods, seeds that were dark brown and shrivelled were collected 30 days after pollination. These seeds, upon germination, gave rise to albino seedlings (Fig. 1a, c) which did not survive beyond 20 days of growth. Pods obtained after cross pollination began to abscise 8 to 20 days after pollination. Externally, the pods appeared normal, and most of them contained an ovule between 1 and 3 mm 7–8 days after pollination (DAP). None of these ovules responded to the in-ovule embryo culture techniques developed for chickpea. Pods 18 DAP which turned yellow on the plant contained ovules which were 7–8 mm, which were cultured on the ovule culture medium. After 10 days of ovule culture, 6 mm embryos were dissected out and cultured

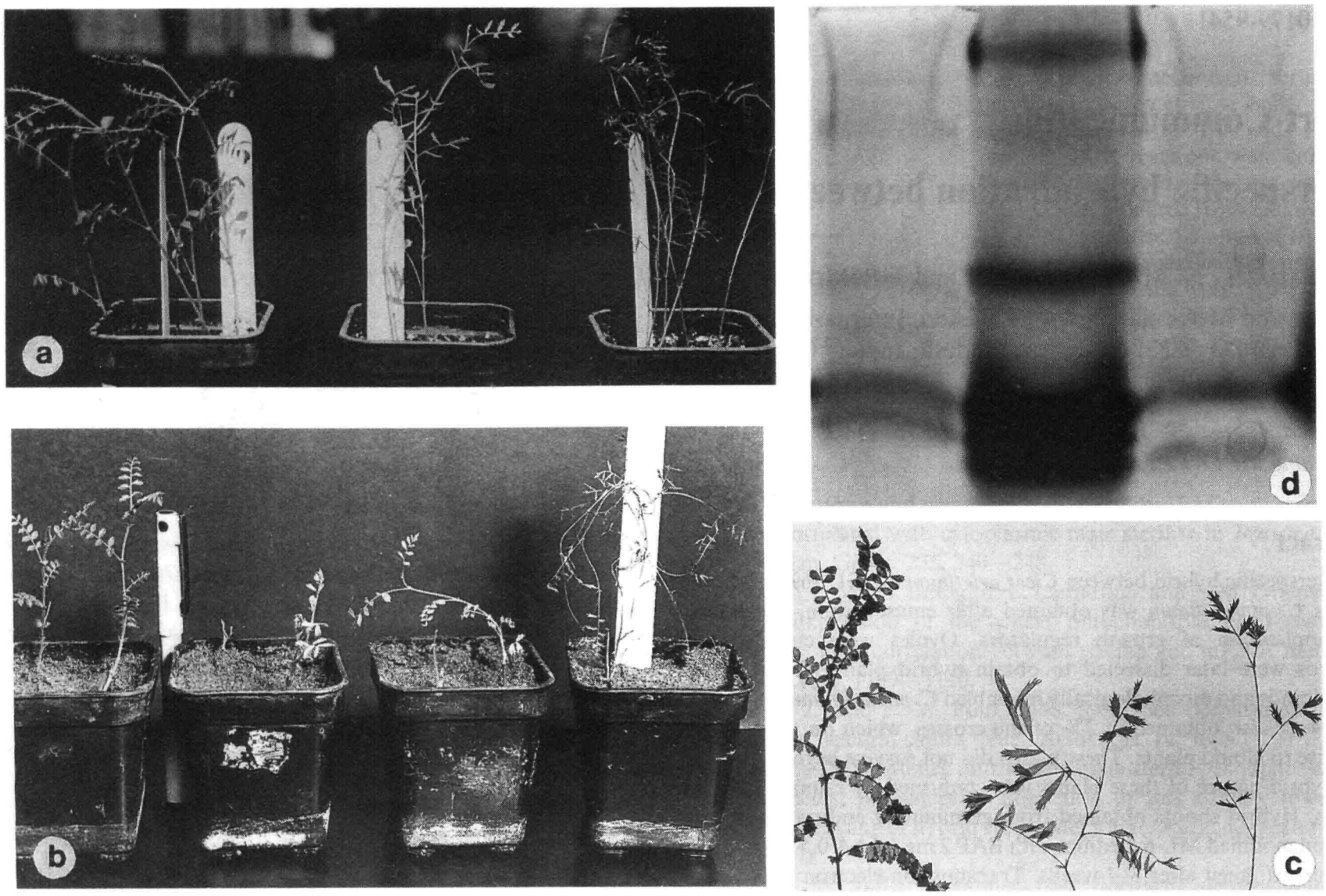

Fig. 1 a. Parents *Cicer arietinum* (left), *C. pinnatifidum* (right) and hybrid plant (*C. arietinum* × *C. pinnatifidum*) (centre); b. hybrid shoots grafted on *C. arietinum* stocks (two pots at the centre), with the parents on either side; c. leaf morphology of the parents and the hybrid (centre); d. esterase isozyme pattern of hybrid (centre) with *C. pinnatifidum* (left) and *C. arietinum* (right)

on the embryo growth medium. Only two embryos germinated, giving rise to albino seedlings which grew on the growth medium, giving rise to multiple albino shoots. These shoots were again subcultured on embryo growth medium and, after 3–4 weeks of culture, green pigmentation was observed. The new green shoots with the shoot buds were subcultured and a large number of green and pale green shoots were obtained. In order to obtain hybrid plants, green shoots were transferred to a root induction medium. All three rooting media induced roots on cv. GL 769, whereas the hybrid shoots did not develop healthy roots. Maximum rooting response was observed when the shoots were rooted on $\frac{1}{2}$MS basal salts supplemented with IBA 0.2 mg/l, and 0.2% activated charcoal, where 25% of the cultured shoots formed roots. The roots were thin and without the secondary roots and these plants, upon transfer to soil, gradually wilted and did not survive beyond 12 days.

Green hybrid shoots were grafted on to cv. GL 769 shoots of 12-day-old seedlings. Thirty-one percent of the grafts (20 out of 64 hybrid shoots grafted) established themselves on the stocks and six grafts survived 45 days of grafting. Two grafts grew to a height of 12 cm (Fig. 1b).

Esterase isozyme analysis of the parents and the hybrid shoots revealed that three bands of the hybrid were common to *C. pinnatifidum*. One band of the hybrid was common to cv. GL 769 and one band to both of the parents. Two bands were unique to the hybrid (Fig. 1d). This confirmed the hybridity of the material.

TEM of the leaf sections from hybrid shoots which were albino showed abnormal chloroplast structure with poorly developed thylakoids containing few and disorganized grana (Fig. 2a). TEM photographs of sections of leaves from green hybrid shoots showed improvement in chloroplast structure, with the grana being more regularly stacked with the thylakoids (Fig. 2b) similar to that of *C. pinnatifidum* (Fig. 2c).

There are no prezygotic barriers in the cross *C. arietinum* and *C. pinnatifidum* (Ahmed et al. 1988), hence hybridization between the two species is possible but the present study shows that there are very strong postzygotic barriers. This is exemplified by the fact that pods are obtained only after hormone application to the pollinated pistils, and the hybrid plants are obtained by in-ovule embryo rescue techniques.

One possible reason for the yellow hybrid shoots turning green upon culture could be the conversion of plastids to chloroplasts. According to Chory et al. (1991), light incubation conditions coupled with the cytokinin in the culture medium has a very important role to play in the conversion of etioplasts to chloroplasts in *Arabidopsis thaliana* mutants. Initially, the plastids were abnormal, giving a yellow colour to the plant. This was supported by TEM studies where abnormal thylakoids were observed. Upon continuous culture, plastids reverted to normal green chloroplasts, giving a green colour to the hybrid plants. Reddy et al. (1988) observed that plastids in yellow tissues of a *Pennisetum americanum* mutant showed no internal thylakoid membrane differentiation.

The technique to root *C. pinnatifidum* and cv. GL 769 shoots has been standardized, with a rooting efficiency of 30% and 25%, respectively (Badami 1995) but this technique did not make it possible to root the hybrid shoots obtained in the present study. Similar results have also been observed by Singh and Singh (1989) where they failed to produce roots on shoots

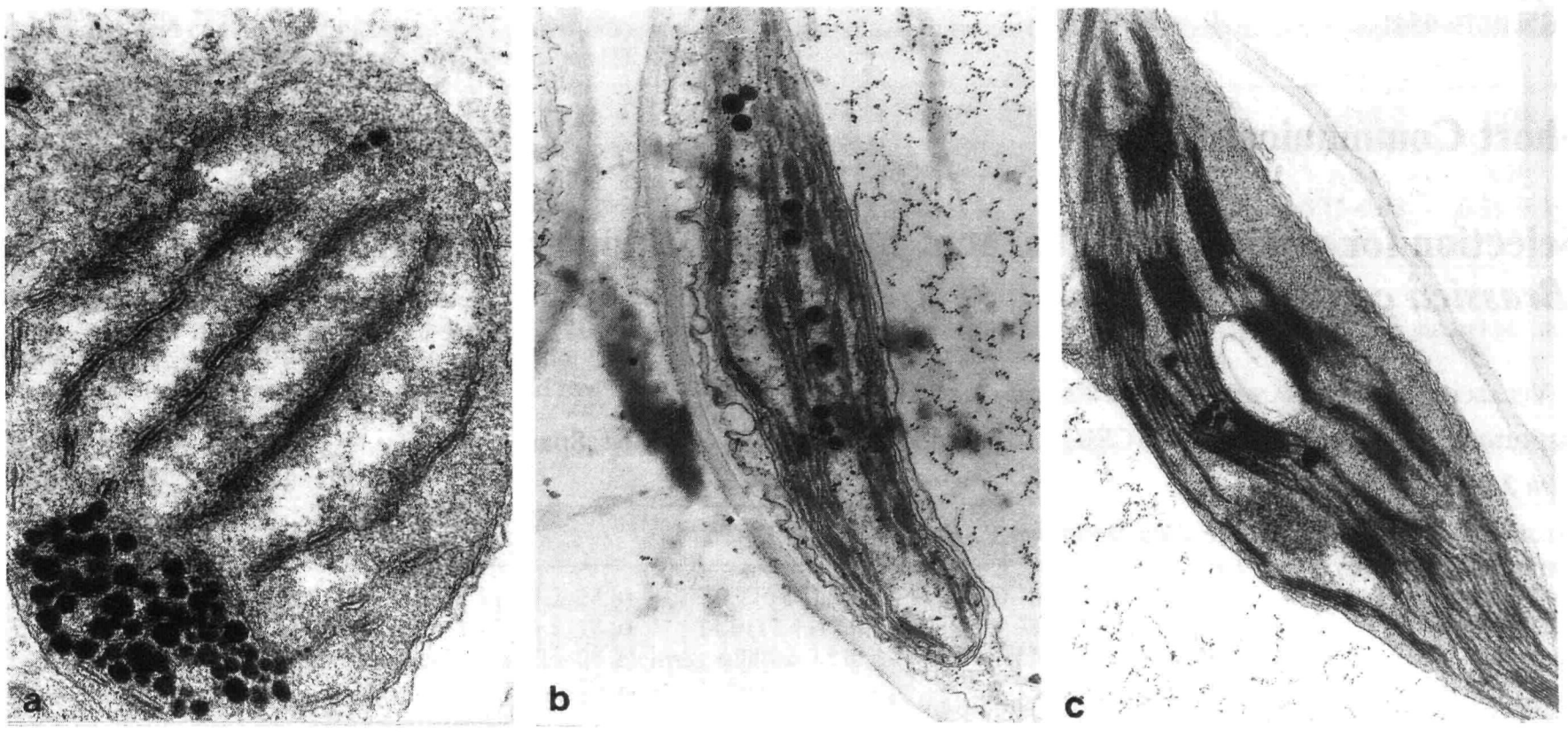

Fig. 2 a. Chloroplast of hybrid showing few and disorganized thylakoids (× 60 800); b. improved thylakoid organization in chloroplast of *in vitro* cultured shoots of hybrid (× 33 000); c. normal chloroplast of *C. pinnatifidum* (× 40 000)

obtained by crossing *C. arietinum* with *C. pinnatifidum* and *C. cuneatum*.

The technique whereby hybrid shoots which do not root well in culture are grafted to the female parental stocks works very well for groundnut (N. Mallikarjuna, unpublished data). The technique to graft hybrid shoots (cv. GL 769 × *C. pinnatifidum*) to cv. GL 769 stocks was initially successful and 9% of the grafted plants grew for more than 1 month, but after reaching a height of 12 cm began to wither. There is clearly some post-zygotic incompatibility between the genomes of *C. arietinum* and *C. pinnatifidum*, resulting in low seed set, high frequency of hybrid embryo necrosis and albinism in surviving hybrid shoots and their poor adaptability to form roots. However, the ability of the albino shoots to form normal chloroplasts *in vitro* raises hopes that hybrids can be produced from this cross.

The work described above opens up avenues to be used with other wild species from the tertiary gene pool, and possibly the perennial wild species, for broadening the genetic base of chickpea.

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