

Albinism in F1 Hybrids Hinders Geneflow Between Cultivated Chickpea (*Cicer arietinum* L.) and the Tertiary Gene Pool Species, *Cicer Pinnatifidum*

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Abstract

Wild *Cicer* species, especially those in the tertiary gene pool, carry useful alleles for chickpea improvement. The aim of this study was to evaluate the crossability and geneflow between three chickpea cultivars (as female parents) and four cross-incompatible *Cicer pinnatifidum* accessions (as pollen parents) from the tertiary gene pool. Ten crosses were conducted. One fully developed healthy F₁ seed was harvested *in vivo* from the ICC 4958 × ICC 17269 cross, but the seedling developed an albino phenotype at 4–5 days after germination. Unlike other crosses, those involving the cultivar ICCV 96030 generated a large number of pods with comparatively large ovules. One albino plantlet was obtained from the ICCV 96030 × ICC 17269 cross by embryo rescue. Crosses involving ICCV 10 resulted in flower drop and poor pod set. These variable genotype-specific responses of pod, ovule, and seed development indicate that genetic factors affect the formation of interspecific hybrids. Although pod and seed formation in these interspecific crosses can be improved, geneflow between these materials is hindered by a strong genetic factor conferring albinism in the F₁ hybrids.

Key Message

Pod, ovule, and seed development vary in crosses between chickpea and *C. pinnatifidum*, indicating that genetic factors affect hybrid formation. Albinism of F₁ hybrids restricts geneflow between these materials.

Introduction

Chickpea (*Cicer arietinum* L.), the second most important dietary legume after common bean, is a rich source of proteins, carbohydrates, micronutrients, and vitamins (Jukanti et al. 2012). It is a potential staple food crop in about 55 countries. India is the largest producer of chickpea with an annual production of 9.9 million tonnes (FAOSTAT 2019). Chickpea production worldwide is affected by biotic and abiotic stresses. Because there is limited genetic variation in the cultivated chickpea germplasm, it is necessary to utilize wild *Cicer* species for genetic improvement. Wild *Cicer* species are strongly resistant to major biotic stresses like *Ascochyta* blight (Stamigna et al. 2000; Collard et al. 2001; Croser et al. 2003; Rao et al. 2003; Shah et al. 2005; Pande et al. 2006), *Botrytis* gray mold (Stevenson and Haware 1999; Rao et al. 2003; Pande et al. 2006), and *Fusarium* wilt (Infantino et al. 1996; Croser et al. 2003; Rao et al. 2003), and tolerant to abiotic stresses such as drought (Croser et al. 2003; Kashiwagi et al. 2005; Toker et al. 2007), cold (Croser et al. 2003; Toker 2005; Berger et al. 2012), and combined drought and heat (Canci and Toker 2009). Wild *Cicer* species also have desirable nutrition-related traits such as high seed protein and mineral contents (Rao et al. 2003; Sharma et al. 2021).

Various incompatibility barriers, linkage drag, and poor viability and sterility of F₁ hybrids and progenies mean that potential wild *Cicer* species are underutilized in chickpea breeding programs. Two annual wild *Cicer* species, *Cicer reticulatum* and *Cicer echinospermum*, are crossable with cultivated chickpea. However, the sterility of F₁ hybrids and progenies has limited the utilization of *C. echinospermum* in crossing programs. Little is known about the crossability of the other six annual wild *Cicer* species with cultivated chickpea. To utilize those species in chickpea improvement, specialized techniques such as the application

of growth hormones, ovule culture, and embryo rescue are required (Badami et al. 1997; Mallikarjuna 1999; Mallikarjuna and Jadhav 2008; Lulsdorf et al. 2005).

Few attempts have been made to generate interspecific hybrids between *Cicer arietinum* from the primary gene pool and wild *Cicer pinnatifidum* from the tertiary gene pool (Badami et al. 1997; Mallikarjuna and Jadhav 2008). Systematic crossing efforts involving diverse parental combinations are required to advance the production of viable interspecific hybrids involving tertiary gene pool species. The aim of this study, therefore, was to evaluate the crossability and geneflow between three cultivars of *C. arietinum* and four wild *C. pinnatifidum* accessions originating/collected from Turkey and Syria.

Three chickpea cultivars (ICCV 10, ICC 4958, and ICCV 96030) and four wild accessions (ICC 17126, ICC 17276, ICC 17200, and ICC 17269) belonging to the tertiary gene pool species *C. pinnatifidum* were used (Table 1). The seeds of all wild accessions were scarified by incising the hard seed coat. All seeds were treated with fungicides (2 g thiram + 1 g carbendazim kg⁻¹ seed) before sowing in pots in a 2:1:1 mixture of sterilized black soil, farmyard manure, and sand. Seed sowing was staggered to synchronize the flowering of cultivated genotypes and wild accessions. At 1 month after germination, *C. pinnatifidum* seedlings were exposed to an 18-h light/6-h dark photoperiod with light supplied by 60-W incandescent lights to induce early flowering (Sharma and Upadhyaya 2019). Interspecific crosses were made using the three cultivars as female parents and the four wild accessions as pollen parents. ICCV 10 and ICC 4958 were each crossed with all four *C. pinnatifidum* accessions, and ICCV 96030 was crossed only with two accessions, ICC 17126 and ICC 17269. The flower buds of the female parents were emasculated and tagged between 3:00 p.m. and 4:00 p.m., and then pollinated with fresh pollen from wild accessions the following morning between 8:00 a.m. and 9:00 a.m. Each day for 3 consecutive days, a mixture of growth hormones (50 mg L⁻¹ gibberellic acid + 10 mg L⁻¹ naphthalene acetic acid + 10 mg L⁻¹ kinetin, 1:1:1) was applied to the base of the peduncle of the pollinated buds to prevent flower drop and pod abscission. Selfed pods on the same branch were removed to encourage growth of the crossed pods. We recorded the number of pollinations and number of fully developed pods generated in each cross (Table 2).

Yellowing pods were harvested and the ovules were cultured in liquid Murashige and Skoog (MS) medium containing 3% w/v sucrose, 0.25 mg/L indole acetic acid, and 1 mg/L zeatin. After 3 weeks, the cultured ovules were subcultured into fresh ovule culture medium and cultured until the embryos emerged from the ovules. The embryos were transferred to shoot growth medium (liquid MS containing 3% w/v sucrose, 0.25 mg/L indole acetic acid, and 1 mg/L kinetin). Well-grown shoots were cultured on root-induction medium (half-strength MS basal salts, 1.5% w/v sucrose, and 0.5 mg/L indole butyric acid). We recorded the number of ovules cultured and number of plantlets generated through ovule culture (Table 2).

One fully mature pod with a healthy F₁ seed was harvested from the ICC 4958 × ICC 17269 cross. The F₁ seed resembled that of the cultivated parent (ICC 4958) with respect to size, color, texture, and shape. The mature F₁ seed was sown in a mixture of soil, sand, and vermiculite (3:1:1). The F₁ seedling had a leaf shape similar to that of the wild *C. pinnatifidum* parent ICC 17269, confirming true hybridity (Figure 1). Thus, the generation of a healthy and functional F₁ seed in the ICC 4958 × ICC 17269 cross was not prevented by pre-fertilization barriers such as failure of pollen germination, pollen incompatibility, arrested pollen tube

growth in the stigma or style, failure of the pollen tube to penetrate the ovule, or arrested growth of the pollen tube within the ovule; or by post-fertilization barriers such as embryo abortion, or shriveled or immature F₁ seeds. However, this seedling became albino (lacked chlorophyll) at 4–5 days after germination (Figure 1). This albinism is attributed to defective chloroplasts with poorly developed thylakoids and few and disorganized grana (Badami et al. 1997; Clarke et al. 2011). Our attempts to multiply this albino-type plant by regeneration through callus induction and culture of different explants (leaves, stem cuttings, and nodes) on basal MS medium containing 0.5 mg/L benzylaminopurine and 0.5 mg/L naphthalene acetic acid were unsuccessful. Thus, although gene flow in the ICC 4958 × ICC 17269 cross was not hindered by the pre- or post-fertilization barriers reported elsewhere (Badami et al. 1997; Ahmad and Slinkard 2004; Mallikarjuna and Jadhav 2008; Clarke et al. 2011), it was hindered by the albinism of F₁ hybrid plants. It will be possible to generate more healthy F₁ pods and seeds from this cross by increasing the number of pollinations and using different combinations of plant growth hormones. However, efforts are needed to address the problem of albinism in F₁ seedlings.

Unlike other crosses, interspecific crosses involving ICCV 96030 resulted in fully developed, mature pods (Table 2). However these pods lacked mature seeds. Most of the pods contained minute to small-sized colorless ovules. Thus, the pods developed normally but the ovules inside did not (Figure 2).

Pod development begins after fertilization. In this study, *C. pinnatifidum* pollen successfully fertilized ICCV 96030, leading to the differentiation of the ovary into the pod wall. However, ovules did not successfully differentiate into seeds due to some intrinsic reasons. This kind of hybrid embryo response has not been reported for other chickpea interspecific crosses. The incompatibility between cultivated chickpea ICC 96030 and all the *C. pinnatifidum* accessions used in this study is due to a post-zygotic barrier, specifically defective embryos that could not develop into functional seeds. Post-zygotic barriers hindering interspecific hybridization between *C. arietinum* and *C. pinnatifidum* have also been reported by Badami et al. (1997). Of the three cultivated chickpea cultivars, ICCV 96030 yielded the highest number of mature, fully developed pods, including a few with enlarged embryos, when pollinated with *C. pinnatifidum*. It will be possible to harvest mature pods with seeds from ICCV 96030 × *C. pinnatifidum* crosses by increasing the number of pollinations in each cross, adjusting plant growth hormone treatments to facilitate embryo/seed development, by crossing in different directions, and/or by using other *C. pinnatifidum* accessions, e.g., ICC 17276 and ICC 17200, as the pollen parent. In addition, immature embryos can be rescued by ovule culture.

The aborting ovules were cultured from 7–8 days after pollination. The tiny ovules did not grow upon culturing, but one larger ovule (derived from ICCV 96030 × ICC 17269) grew normally and the embryo regenerated into a seedling (Figure 2). Although the shoot was initially green, the newly formed leaves lacked chlorophyll and the albino seedling died after 2 weeks. Defective chloroplasts are the major barrier in generating interspecific hybrids between *C. arietinum* and *C. pinnatifidum* (Clarke et al. 2011). In the crosses involving ICCV 10, flower drop was the major obstacle. Most of the pollinated flower buds dropped within 1–2 days of pollination, despite the use of plant growth hormones. Although some cross combinations formed a few pods, they turned yellow within 3–4 days of pollination, and ovules from these pods did not develop further *in vitro* because of their small size.

Interspecific hybridization between chickpea cultivars and *C. pinnatifidum* produced one fully mature F₁ seed (from ICC 4958 × ICC 17269). None of the other cross combinations yielded fully mature F₁ seeds. Although ICCV 96030 formed the most pods, followed by ICC 4958, only one ovule from the ICCV 96030 × ICC 17269 cross regenerated into a plantlet in ovule culture. None of the three chickpea cultivars formed pods when pollinated with *C. pinnatifidum* ICC 17276. On the basis of the pod, ovule, and seed formation of the interspecific crosses, we concluded that the chickpea cultivars ICC 4958 and ICCV 96030 and the *C. pinnatifidum* accessions ICC 17269 followed by ICC 17126 and ICC 17200 exhibited good crossability.

To our knowledge, this is the first report of a fully mature F₁ seed derived from an interspecific cross between cultivated chickpea and *C. pinnatifidum* without using embryo rescue. Our results show that the parents' genotypes affect crossability between *C. arietinum* and *C. pinnatifidum*. The successful development of a mature healthy F₁ seed from the interspecific ICC 4958 × *C. pinnatifidum* ICC 17269 cross confirmed the absence of pre- and post-fertilization barriers. Instead, albinism of F₁ hybrids was the major obstacle hindering gene flow between *C. pinnatifidum* and cultivated chickpea. Embryo abortion occurred after interspecific crosses involving the chickpea cultivar ICCV 96030 and all *C. pinnatifidum* accessions. Using an ovule culture technique, one albino plantlet was regenerated from the ICCV 96030 × ICC 17269 cross. The interspecific crosses between chickpea cultivar ICCV 10 and *C. pinnatifidum* accessions were unsuccessful due to excessive flower drop and poor pod formation. These variable genotype-specific responses of pod and seed development suggest that more genotypes should be included when testing for cross-compatibility. The cultivated genotypes used here originate from central and southern agro-geographical areas of India. Including more genotypes from other parts of India may be useful for identifying those that are readily crossable with *C. pinnatifidum*, preferably without producing albino progeny.

Although pod and seed formation in crosses between cultivated chickpea and *C. pinnatifidum* can be improved using various techniques, it will be difficult to improve gene flow between these two species because of the genetic factor that confers albinism in the F₁ hybrids. These results show that different parental genotype combinations have different crossabilities in inter-specific crosses, indicating that some genetic factors are important for the efficient production of interspecific hybrids involving *C. pinnatifidum*.

Declarations

Statements & Declarations

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Data availability statement

All data generated for this study is included in the article.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

SS planned the study; SAL grew the materials and performed the experiments; SS, SAL, and BK analyzed the data and prepared the manuscript. All the authors read and approved the final manuscript.

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Tables

Table 1. Cultivated chickpea and *Cicer pinnatifidum* accessions used in this study.

Accession identity	Species	Biological status	Country of origin
ICC 4958	<i>Cicer arietinum</i>	Advanced/Improved cultivar	India
ICCV 10	<i>C. arietinum</i>	Cultivated variety	India
ICCV 96030	<i>C. arietinum</i>	Cultivated variety	India
ICC 17126	<i>C. pinnatifidum</i>	Wild species	Turkey
ICC 17276	<i>C. pinnatifidum</i>	Wild species	Syria
ICC 17200	<i>C. pinnatifidum</i>	Wild species	Syria
ICC 17269	<i>C. pinnatifidum</i>	Wild species	Turkey

Table 2. Crosses attempted between cultivated chickpea and *Cicer pinnatifidum* at ICRISAT, Patancheru, India

Cross		No. of pollinations	No. of matured F ₁ seeds obtained	No. of F ₁ pods harvested for ovule culture	No. of ovules cultured	No. of plantlets regenerated
Female parent (<i>Cicer arietinum</i>)	Pollen parent (<i>C. pinnatifidum</i>)					
ICCV 10	ICC 17126	95	0	9	9	0
	ICC 17276	55	0	0	0	0
	ICC 17200	58	0	0	0	0
	ICC 17269	75	0	4	4	0
ICC 4958	ICC 17126	95	0	5	5	0
	ICC 17276	55	0	0	0	0
	ICC 17200	58	0	7	7	0
	ICC 17269	85	1	3	3	0
ICCV 96030	ICC 17126	100	0	88	9	0
	ICC 17269	100	0	68	7	1

Figures

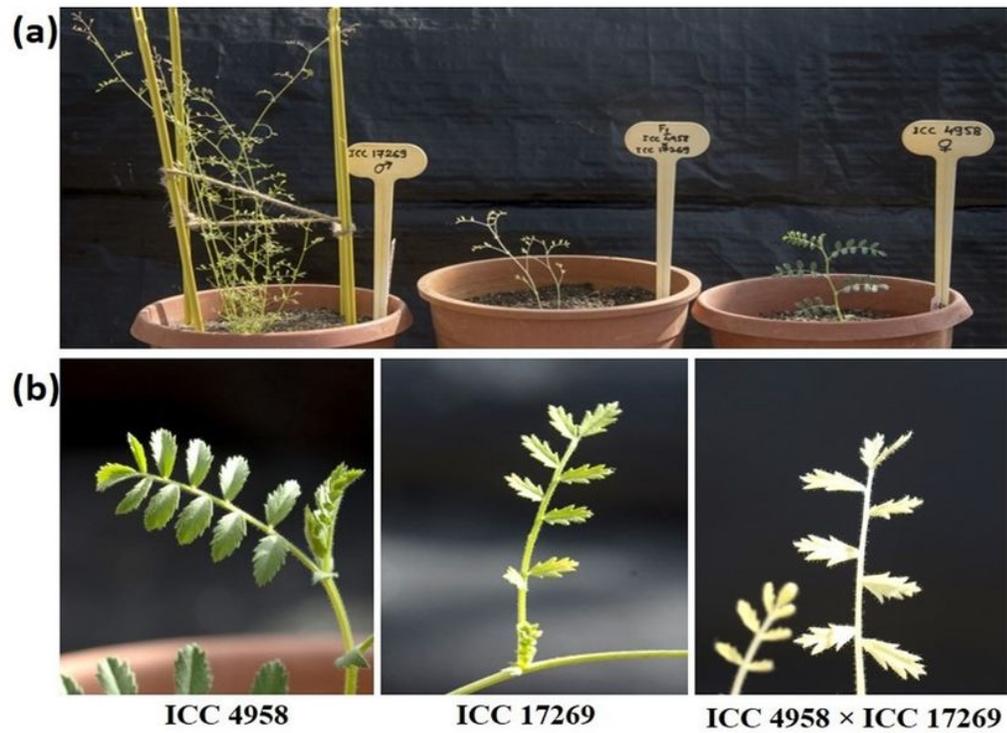


Figure 1

F1 plant generated from ICC 4958 × ICC 17269; a. *Cicer pinnatifidum* accession ICC 17269 (left), albino F1 plant (middle), and chickpea cultivar ICC 4958 (right); b. Leaf shape of cultivated parent ICC 4958 (left), wild parent ICC 17269 (middle), and F1 plant resembling wild parent (right).

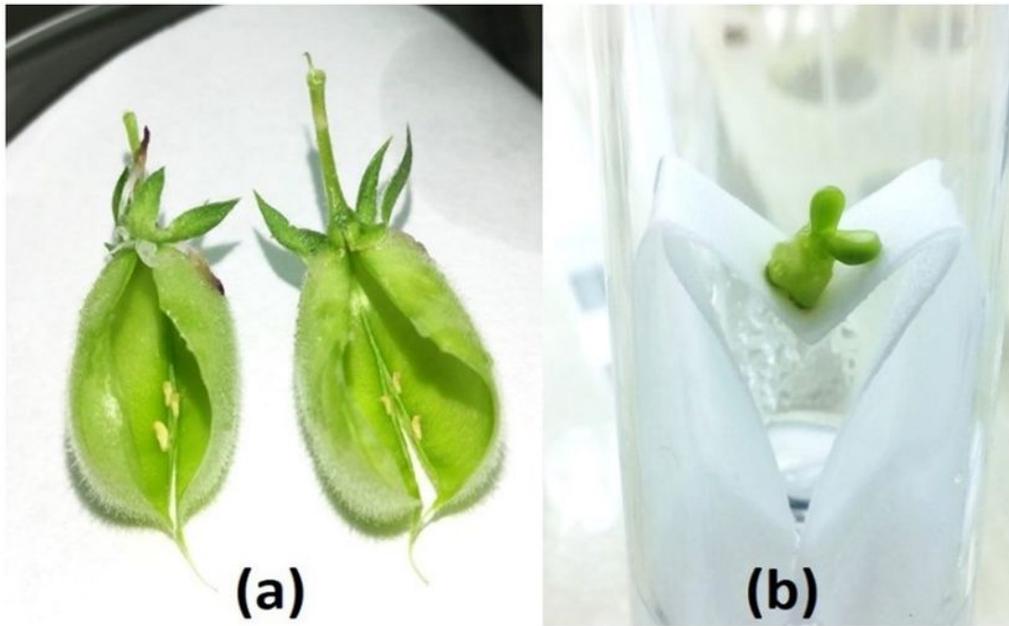


Figure 2

Ovule culture to rescue hybrid embryo from ICCV 96030 × ICC 17269; a. Well-developed pods containing ovules that failed to develop; b. Ovule cultured in liquid medium.