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Techniques to produce hybrid between *Cicer arietinum* L. x *C. pinnatifidum* Jaub.

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Abstract

Androgenesis has not been reported in chickpea (*Cicer arietinum* L.). Interspecific hybridization between *C. arietinum* and *C. pinnatifidum* was possible by hormone-aided pollinations, embryo rescue and tissue culture techniques to save aborting hybrid embryos. Since the hybrids did not have a good root system, hybrid shoots were grafted to cultivated chickpea stocks. By this method it was possible to transfer hybrids to soil. Hybrid plants were fragile and were maintained in a growth room. Hybrid plants flowered only when the cytokinin zeatin (1 mg/l) was added to the sterilized tap water used to water the plants. Flowers were pale violet and cleistogamous. All of the components of the flower were present, although the anthers did not dehise. Anthers squashed in acetocarmine revealed from 4-10 divisions in many of the microspores. Induction of androgenesis is believed to be due to wide hybridization between *C. arietinum* and *C. pinnatifidum.*

Key words: Androgenesis, *Cicer arietinum, Cicer pinnatifidum,* cleft graft, interspecific hybridization

Introduction

The conventional methodology of producing pure breeding lines requires at least 6 to 8 years of selfing to achieve a satisfactory level of homozygosity. In comparison, haploid plants can produce pure lines in a single generation by doubling the chromosome number following colchicine treatment. Doubled haploids are equivalent to inbred lines, with 100% homozygosity and normal fertility. Haploid embryos in plants have been described to occur naturally in about 100 species of angiosperms, and have been documented in detail [1]. However, haploid plants occur rarely in nature.

There are three principle methods for haploid production: 1) parthenogenesis, 2) wide crossing with chromosome elimination, and 3) haploid plants produced from anther/ovule culture. In parthenogenesis, haploids arise from both unfertilized egg and male gametes. Gynogenic haploids arise as a result of stimulation of the unfertilized egg. In a few cases, offspring resembled the male parent and were thought to have originated from the pollen [2, 3]. The doubled haploid method used in barley is an example of preferential chromosome elimination. In this method, a cross is made between cultivated barley (*Hordeum vulgare*) and *H. bulbosum.* During embryo development, the chromosomes of *H. bulbosum* are gradually eliminated resulting in haploid plants [4]. The chromosome elimination phenomenon is quite prevalent among wide crosses between wheat and *H. bulbosum* [5] or maize [6, 7]. A more recent procedure to produce haploid plants is by anther culture/microspore culture [8-10]. The culture of anthers or microspores gives rise to haploid plants whose chromosomes can be doubled by suitable treatment to produce homozygous diploid plants.

In chickpea (*Cicer arietinum* L.), procedures for developing haploid plants have not been reported, and induction of androgenesis by anther culture is of a very low frequency (Mallikarjuna, unpublished data). Androgenesis was observed in the wide cross *C. arietinum* x *C. pinnatifidum*. *Cicer pinnatifidum* is a wild species in the incompatible gene pool of chickpea and many chickpea scientists prefer placing *C. pinnatifidum* in the tertiary gene pool [11]. Although there are reports on production of hybrids between *C. arietinum* and *C. pinnatifidum* [13-15], it is still not a routine process and hybrids have only been obtained after grafting hybrid shoots to chickpea stocks. We report here our results in the interspecific hybridization between *C. arietinum* and *C. pinnatifidum,* the methods to obtain hybrid plants

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by grafting hybrid shoots to chickpea stocks, and the induction of androgenesis in the anthers from the hybrid plants.

Materials and methods

Plant material

All experiments were carried out at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Andhra Pradesh, India. Seeds of the chickpea cultivars ICCV 10, ICCV 2, ICCV 92318, JG 11 and KAK-2; stable interspecific derivatives of *C. arietinum* x *C. reticulatum* (CB 611) and *C. arietinum* x *C. echinospermum* (CB 441); wild species *C. pinnatifidum* accessions ICCW 37 (ICC 17152) and ICCW 38 (ICC 17153) from Turkey, IG 69999 and IG 73077 (ILWC 248) from Lebanon and *C. reticulatum* accession ICC 17121(ICCW 6) and *C. echinospermum* accession ICC 17159 (ICCW 44) from Turkey were raised in the screenhouse and in the field. In the screenhouse, the seeds were sown in plastic pots (30 cm diameter, 30 cm deep) filled with a steam sterilized potting mixture consisting of black soil, sand, and farm yard manure (4:1: 1). The seeds were treated with thiram (2 g per kg of seed) and sown 5-7 cm deep in the soil and watered immediately. After germination, the plants were watered as needed. The screenhouse was cooled by desert coolers to maintain the temperature of 25 ± 3°C, and relative humidity >65%. Additional lighting was provided to induce flowering and pod set.

Chickpea cultivars were grown in the field during the 2002-2005 post-rainy season. Normal agronomic practices were followed for raising the crop (basal fertilizer, 100 N:60 P:40 K). The field was irrigated immediately after sowing, and at intervals of one month thereafter. The wild species on germination in the screen house were transferred to the field as 15 days old seedlings and transplanted on the hills 6-8 cm deep

Wide hybridization

Crosses were carried out by emasculating the flower buds of chickpea cultivars as well as the hybrids (CB 611 and CB 441), the wild species *C. reticulatum* (ICCW 6) and *C. echinospermum* (ICCW 44) (Table 1), followed by pollination with fresh pollen of *C. pinnatifidum* accessions between 08:00 and 10:00 hours. A mixture of growth regulators [gibberellic acid (75 mg/l) + naphthalene-1-acetic acid (10 mg/l) + kinetin (10 mg/l)] was applied to the base of the pollinated pistil to prevent pre-mature pod abscission. Pods from self pollinations were removed to facilitate the development of pods from cross pollinations. Pods from cross pollinations, which began to turn yellow (18-22 days), were harvested and ovules/immature seeds were cultured following *in vitro* culture practices on the ovule culture medium standardized for chickpea. Ovule culture medium consisted of ML-6 basal salts [16] with 0.25 mg/l indole acetic acid and 1 mg/l zeatin [13]. After 3-4 weeks of ovule culture, embryos emerged out of the ovule. Hybrid seedlings were transferred to longer culture tubes (25 x 200 mm), retaining the ovule culture medium.

Grafting hybrid shoots to chickpea stocks

Well grown hybrid shoots were used as scions and grafted on cultivated chickpea stocks. The cleft method of grafting was used (Fig. IB-g). Cultivated chickpea plants (ICCV 10, ICCV 92318 and ICCV 2) were grown in small pots with sterilized sand. Fifteen days old seedlings were used as stocks for grafts. The root stock plants were cut 2-3 cm above the soil, and lateral buds/ branches were removed. A 5 mm long plastic tubing with a diameter of 2.5-3 mm was slid over the root stock and a vertical slit was made across the diameter of the root stock. The scions for grafting consisted of 6 to 8 cm hybrid shoots whose cut end was scraped to expose the cells of the sub-epidermal and cortical regions of the scion. The scion was carefully inserted into the cut end of the root-stock and the plastic tubing was slid over the joint to immobilize the scion. A 250 ml beaker was inverted over the grafted plant to maintain high humidity. Grafted plants were maintained in a growth chamber with controlled conditions of temperature at 22°C (14 hr day) and 15°C (10 hr night) with relative humidity of 70 to 75 %. After a month of grafting, well established grafts were carefully transferred to 8" x 8" pots with sand and soil (1:1). Grafted plants were watered with sterilized tap water every alternate day.

Pollen fertility analysis

Pollen fertility analysis and the number of androgenic (multicellular) microspores formed were calculated by squashing the anthers in 2% aceto-carmine. The proportion of androgenic microspores was estimated as p = k/n, where k is the number of androgenic microspores and n is the number of normal microspores. Statistical analysis of these proportions was also carried out using equations (1) and (2). Pollen grains, which followed the normal microsporogenesis, were counted as normal pollen grains and/or microspores and those which had abnormal divisions in the microspores, which led to the formation of multicellular grains were counted as androgenic microspores.

Statistical analysis

The proportion (p) of pods formed was estimated as p = k/n where k is the number of pods and n is the number of pollinations. The value of p varied depending on the cultivated and wild species used in the crossing program. A 95% confidence interval (CI) was estimated [17] for each female's p as follows:

$$P \pm [1.96 \text{ sqrt}\{p(1-p)/n) + \{1/(2n)\}] \quad (1)$$

The CI estimated as above served two purposes. First, it gave an idea of the likely range of estimates of p had we conducted a large number of such experiments under similar conditions. Second, two females could be judged to be different in their true p if the two corresponding CIs did not overlap.

To test whether different females, when crossed to the same male, produced the same proportion p of pods, the following χ^2 test of homogeneity of proportions was carried out [17].

$$\chi^2 = (\Sigma_i\, p_i\, k_i)/\{P(1-P)\} \text{ with df} = f-1 \quad I = 1, 2, ..., f \quad (2)$$

where p_i and k_i are respectively the value of p and k for the i-th female, f is the number of females, and P is the average p value across all f females calculated as $P = S_i\, k_i / S_i\, n_i$.

Results and discussion

Crosses with C. pinnatifidum accession ICCW 38

The highest frequency of pod formation (67%) was observed with the cross ICCV 2 x ICCW 38 (Table 1; Figs. 1A-a&b) in which 7 ovules were cultured, with 5 ovules germinating. Although this was the cross where highest number (%) of ovules germinated, it was not possible to recover any hybrid plants as none of the ovules grew beyond 5-6 cm of growth. In the cross ICCV 10 x ICCW 38, 40 pods (33%) were obtained from 120 pollinations. Microscopic observation of hybrid ovules revealed that hybrid embryos aborted at cotyledonary stage of development (Fig. 1A-c). Embryo culture/rescue was necessary to obtain hybrid plants. Sixteen ovules were cultured, of which 6 (38%) germinated and formed hybrid plants with a robust shoot and stunted root system (Figs. 1A-d&e; Fig. 1B-f). Hybrid plants were maintained *in vitro* and the shoots were used in the grafting experiment (Table 2). One hundred and eleven pollinations formed 56 pods (50%) in the cross ICCV 92318 x ICCW 38 and 12 ovules were cultured, of which 4 germinated. None of the hybrid plants had a robust root system, hence were maintained *in vitro.* Less than 35% of the crosses with the wild species *C. reticulatum* (ICCW 6) and *C. echinospermum* (ICCW 44) with ICCW 38 or with their stable hybrids (C. *arietinum* x *C. reticulatum*-CB611 and *C. arietinum* x *C. echinospermum*-CB 441) with ICCW 38, formed pods, and did not show much variation amongst the crosses (Table 1). One ovule from the cross CB 611 x ICCW 38 and 4 ovules from the cross CB 411 x ICCW 38 germinated *in vitro.* Multiple shoots were maintained *in vitro* as all of them had stunted root systems. In the crosses with *C. reticulatum* and *C. echinospermum* as the female parent, there were not many ovules large enough for culture and the cultured solitary ovule from the cross *C. echinospermum* x ICCW 38 did not germinate *in vitro.* Upper and lower limits were calculated at 95% confidence intervals for the proportion of pods formed between ICCW 38 and different cultivars of chickpea (Table 1). Statistical analysis showed that there was not much variation between different cultivars with respect to pod formation except for crosses with ICCV2 and ICCV 92318, where the proportion of pods formed was higher compared to the rest of the cultivars.

Crosses with C. pinnatifidum accession ICCW 37

Pod formation in the crosses with ICCW 37 varied from 9 to 57 in number, and did not exceed 30% irrespective of the female parent used in the crossing program but the number of ovules cultured were large. Twenty one ovules were cultured from the cross ICCV 92318 x ICCW 37 and 19 ovules were cultured from the cross ICCV 10 x ICCW 37. A maximum of 8 (38%) ovules (ICCV 92318 x ICCW 37) germinated to form multiple shoots. All the shoots had a stunted root system and were maintained *in vitro.* Six ovules germinated from the cross ICCV 10 x ICCW 37, and none of the shoots had a good root system. Crosses with wild *Cicer, C. reticulatum* and *C. echinospermum* yielded 19% and 16% pod formation, but none of the pods had ovules suitable for culture. Percent pod formation was 25% and 29% in the crosses with CB 611 and CB 441, and five hybrid plants were obtained from each cross.

Crosses with C. pinnatifidum accession ILWC 248

In the cross KAK-2 x ILWC 248, a maximum of 48% pod formation was observed. In the crosses with other cultivars pod formation did not exceed 12% (Table 1). In spite of culturing a maximum of 54 ovules from the cross KAK-2 x ILWC 248, only 4 ovules responded to the culture medium and formed hybrid plants. Upper and lower limits calculated at 95% confidence intervals for the proportion of pods formed from the crosses

involving ILWC 248 with different female parents showed that there was not much variation except for the cross KAK-2 x ILWC 248 (Table 1).

Crosses with C. pinnatifidum accession IG 69999

Cicer pinnatifidum accession IG 69999 was crossed with two cultivars of chickpea and there was a difference in the proportion of pods between the two cultivars (Table 1). Although a large number of ovules from both the crosses were cultured, many of them either did not germinate *in vitro,* or had only the emergence of a stunted radicle. Two of the ovules which germinated from the cross KAK-2 x IG 69999, gave rise to multiple shoots. Shoots were fragile and none of them were suitable for grafting experiment. None of the plants had a robust root system and were not transferred to soil. Irrespective of the *C. pinnatifidum* pollen donor or the female parent used in the crossing program, hybrid shoots obtained from ovule culture were initially pale green to yellow in color, but upon continuous culture in the ovule culture medium gave rise to semi-green shoots. Every 3 weeks, shoots were transferred to fresh ovule culture medium. Transfer to fresh medium induced new shoots and some of the pale yellow shoots turned green.

Grafting hybrid shoots to chickpea stocks

A total of 55 hybrid seedlings were obtained in the cross *C. arietinum* x *C. pinnatifidum* as a result of germinating hybrid immature ovules *in vitro* (Fig. 1A-d&e). The majority of the seedlings developed multiple shoots with a stunted root system. A few hybrids had fragile root systems that did not support the transfer of plants to soil. As an alternative, selected hybrid shoots from the seedlings, which were more than 6 to 8 cm in length with at least 3 to 4 leaves, were grafted to *C. arietinum*

Table 1. Interspecific hybridization between *C. arietinum* and *C. pinnatifidum* (2002-2005)

Crosses	No. of plants	No. of pollinations (n)	No.of pods (k)	Proportion of pods formed (p=k/n)	95% CI for p		No. of ovules	No. of ovules germinated (%)
					Lower limit	Upper limit		
ICCW 10 x ICCW 38	5	120	40	0.33	0.241	0.426	16	6(38)
ICCV 2 x ICCW 38	5	113	76	0.67	0.577	0.768	7	5(71)
ICCV 92318 x ICCW 38	5	111	56	0.50	0.403	0.606	12	4(33)
CB 611 x ICCW 38	5	159	32	0.20	0.133	0.270	6	1(17)
CB 441 x ICCW 38	5	190	43	0.23	0.162	0.291	11	4(36)
ICCW 6 x ICCW 38	5	79	25	0.32	0.201	0.431	0	0
ICCW 44 x ICCW 38	5	48	12	0.25	0.107	0.393	1	0
ICCV 10 x ICCW 37	5	156	35	0.22	0.153	0.296	19	6(32)
ICCV 2 x ICCW 37	5	250	24	0.10	0.056	0.136	8	1(13)
ICCV 92318 x ICCW 37	5	195	50	0.26	0.190	0.323	21	8(38)
CB 611 x ICCW 37	5	179	44	0.25	0.177	0.314	25	5(20)
CB 441 x ICCW 37	5	198	57	0.29	0.220	0.356	20	5(25)
ICCW 6 x ICCW 37	5	63	12	0.19	0.078	0.303	0	0
ICCW 44 x ICCW 37	5	58	9	0.16	0.045	0.265	0	0
ICCV 10 x IL WC 248	5	250	21	0.08	0.046	0.122	7	1(14)
ICCV 2 x ILWC 248	5	333	39	0.12	0.080	0.155	7	0
JG 11 x ILWC 248	5	365	40	0.11	0.075	0.144	20	2(10)
KAK-2 x ILWC 248	5	405	196	0.48	0.433	0.535	54	4(8)
CB 613 x ILWC 248	5	160	30	0.19	0.121	0.254	22	0
CB 441 x ILWC 248	5	254	49	0.19	0.141	0.245	17	3(18)
JG 11 x IG 69999	5	175	28	0.16	0.100	0.220	20	3(15)
KAK-2 x IG 69999	5	396	137	0.35	0.297	0.395	38	2(5)

CI = confidence interval.

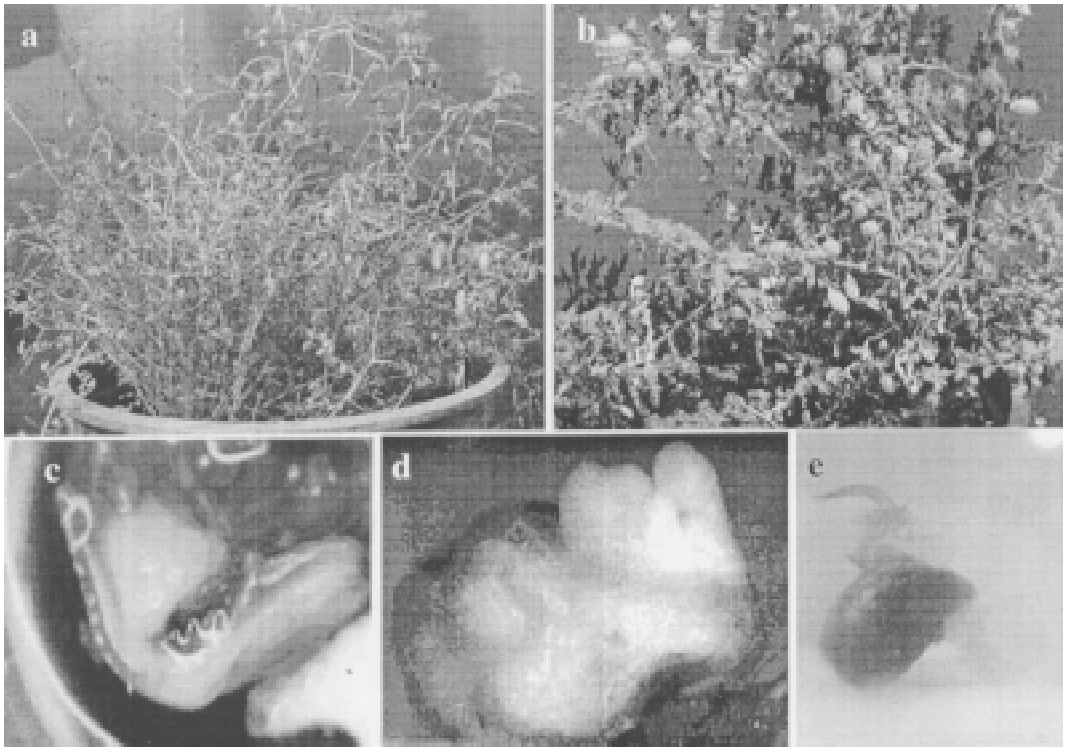

Fig. 1A. a: Wild species *Cicer pinnatifidum*; b: Cultivated chickpea; c: Aborting embryo after 22 days of pollination; d: Hybdid embryo growing out of immature seed; e: Germinating embryo with plumule and callusing radicle

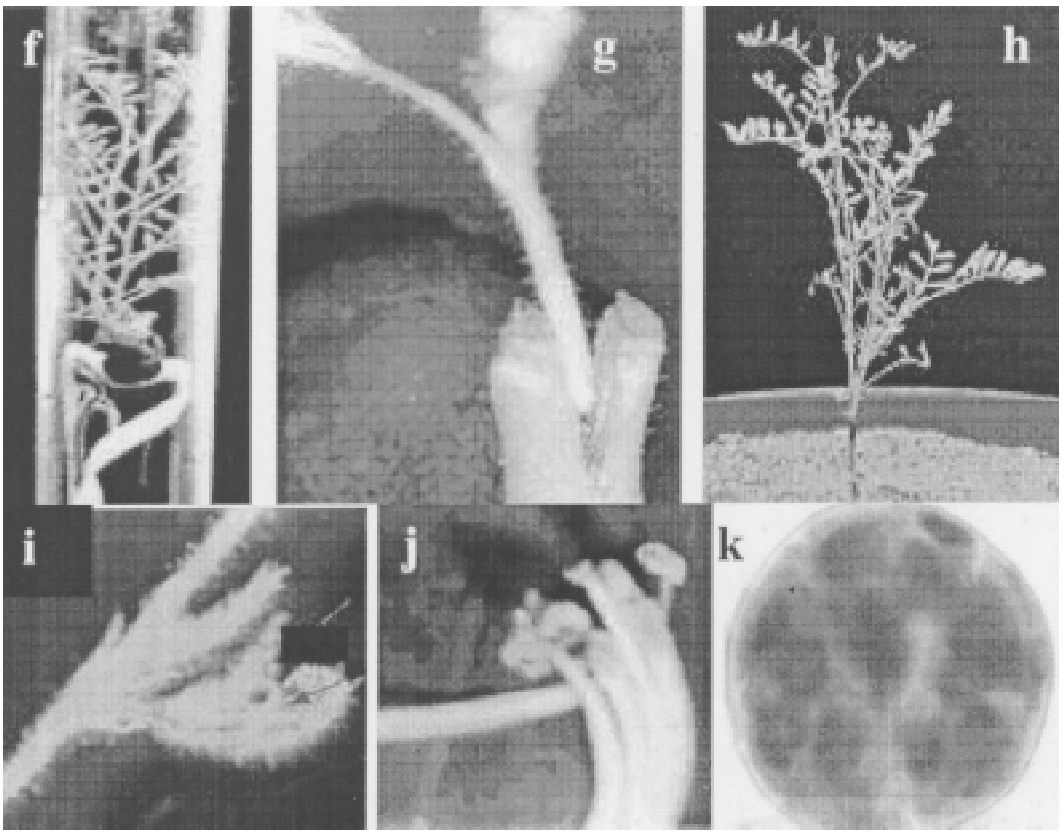

Fig. 1B. f: Seedling growing *in vitro*. Note the blunt radicle; g: Hybrid shoot grafted to chickpea stock; h: Grafted plant growing in soil; i: Arrow points at the floral bud; j: Anthers and stigma exposed; k: Androgenic pollen

stocks (Fig. 1B-f&g). As a result, it was possible to obtain 25 hybrid plants between *C. arietinum* cultivars and C. *pinnatifidum* accessions (Table 2; Fig. 1B-h). Sixty percent of the grafted shoots from the cross ICCV 10 x ICCW 37, survived. In the rest of the crosses, the percent success was less than 55%. None of the grafts from the cross KAK-2 x ILWC 248 survived to form hybrid plants. Hybrid plants in general were robust in growth but the branches were fragile (Fig. 1B-i) and were maintained in the growth chamber. Flower buds were formed only when the tap water used to water the hybrid plants was supplemented with zeatin (1 mg/l). New floral buds did not form when zeatin was removed or replaced with kinetin. Flowers were semi-violet to white with well-developed floral partsand were cleistogamous. Anthesis was not observed (Fig. 1B-j) but mature pollen grains formed in the anthers. Chickpea cultivars KAK-2 and ICCV 10 were grown and zeatin (1 mg/l) included in the tap water used water the seedlings. None of the seedlings had multicellular microspores in their anthers.

Table 2. Grafting hybrid shoots to chickpea stocks

Cross	Total no. of grafts	No. survived
ICCV 92318 x ICCW 37	10	4
ICCV 10 x ICCW 37	10	6
ICCV 10 x ICCW 38	10	4
ICCV 92318 x ICCW 38	10	5
CB 441 x ICCW 37	5	1
CB 441 x ICCW 38	4	1
ICCV 10 x ILWC 248	10	4
KAK-2 x ILWC 248	2	0

Induction of androgenesis in hybrid plants: Fourteen hybrid plants were selected for pollen fertility analysis. Anthers were squashed in acetocarmine and divisions were observed in some of the microspores (Fig. 1B-k). The number of divisions varied from 2 to 10. The number of microspores and/or pollen grains in an anther varied from 11 to 151 compared to more than 500 pollen grains in cultivated chickpea. The number of pollen grains that had undergone microsporogenesis or the induction of androgenesis varied from 0-100% (Table 3). Plants 3, 8, 11 and 12 did not have any

Table 3. Androgenic response in *C. arietinumx C. pinnatifidum* hybrids.

Plant no.	Cross	Total micro-spore(n)	No. of non-androgenic microspore		No. of andro-genic micro-spore (k)	Propor-tion of andro-genic micro spore (p=k/n)	95% CI for P		Max. no. of cells in a microspore
			fertile	sterile			Lower limit	Upper limit	
15	ICCV 92318 x ICCW 37	65	10	52	3	0.05	0.0000	0.1107	2-4
16	ICCV 10 x ICCW 37	11	0	0	11	1.00	0.9545	1.0000	8-10
2	CB 441 x ICCW 37	122	18	91	13	0.11	0.0504	0.1696	3-4
3	ICCV 10 x ICCW 37	73	9	64	0	0.00	0.0000	0.0068	-
4	ICCV x ICCW 37	46	0	18	28	0.61	0.4582	0.7618	2-4
5	ICCV 10 x ICCW 37	28	3	20	5	0.18	0.0198	0.3402	4-6
10	ICCV 92318 x ICCW 37	31	0	12	19	0.61	0.4222	0.7978	2-4
6	ICCV 10 x ICCW 38	27	1	11	15	0.56	0.3542	0.7658	2-4
7	ICCW 10 x ICCW 38	83	8	43	32	0.39	0.2790	0.5010	2-4
8	ICCV 92318 x ICCW 38	86	12	74	0	0.00	0.0000	0.0058	-
9	ICCV 92318 x ICCW 38	151	21	122	8	0.05	0.0119	0.0881	4-6
12	ICCV 441 x ICCW 38	74	17	57	0	0.00	0.0000	0.0068	-
13	ICCV 10 x ICCW 38	43	4	32	7	0.16	0.0388	0.2812	2-4
14	ICCV 92318 x ICCW 38	16	0	0	16	1.00	0.9688	1.0000	8-10
11	ICCV 10 x ILWC 248	35	4	31	0	0.00	0.0000	0.0143	-
1	ICCV 10 x IL WC 248	57	0	43	14	0.25	0.1288	0.3712	3-4

CI = 95% confidence intervals

androgenic pollen grains, with all of the microspores following the normal microsporogenesis and producing mature pollen grains. In plants 14 and 16, all of the pollen grains were androgenic, or in other words had multicellular microspores. The number of cells in a multicellular microspore varied from 8-10, unlike the 2-6 cells found in multicellular microspores in other hybrid plants which had androgenic microspores. Upper and lower limits were calculated for the proportion of androgenic microspores at 95% confidence limit. The limits gave a picture on how much the proportion varied for the hybrid plants. Large variation was observed in the proportion of androgenic microspores obtained, and were found to be significantly different from each other (Table 3).

Pollen fertility: Pollen fertility in the hybrids did not exceed 23%, and some of the hybrid plants did not have any fertile grains (Table 3). Although percent sterility in the hybrids was more than pollen fertility, the presence of few fertile grains shows that it may be possible to advance the generation.

The choice of female parent used in the crossing program was important to obtain hybrid plants in large numbers, although this was not correlated with high percentage of pods set. Some of the crosses with high pod set (e.g. ICCV 2 x ICCW 38) did not produce any hybrid plants, whereas some with low pod set (e.g., ICCV 10 x ICCW 37 and ICCV 92318 x ICCW 37) produced higher numbers of hybrid plants.

In ovule embryo culture was standardized for the cross *C. arietinum* x *C. pinnatifidum* [13], and was used to save aborting embryos from crosses involving different cultivars of cultivated chickpea and different accessions of *C. pinnatifidum.* Hybrid seedlings were initially albino, which upon continuous culture in the ovule culture medium with zeatin as the source of cytokinin, turned semi-green. Seedlings grew with an extensive shoot system, but with a scanty to nil root system. None of the seedlings, which were directly transferred to soil, survived beyond 30 days. Badami *et al.* [12] studied the chloroplasts of albino and semi-green hybrid Seedlings by transmission electron microscopy and showed that the etioplasts of the albino plants had poorly developed thylakoids containing few and disorganized grana. Semi-green hybrids grown in the culture medium with zeatin, had improved chloroplast structure with grana being more regularly stacked with thylakoids, as seen in normal green chickpea plants. Light incubation conditions coupled with cytokinin in the culture medium has a very important role to play in the conversion of etioplasts to chloroplasts in *Arabidopsis thaliana* mutants.

Cicer pinnatifidum accessions used in the crossing experiments originated from different countries and an assortment of cultivars were used in the crossing program [13], but none of the accessions could produce mature seeds and the hybrids plants with normal chlorophyll pigmentation. This indicated that the interaction of the nuclear genome of *C. pinnatifidum* with the cytoplasmic genome of cultivated chickpea can give rise to defective chloroplasts. Based on the previous results [12, 13] and the present experiment, it can be concluded that *C. pinnatifidum* may not produce hybrids with normal chlorophyll pigment. Hopefully the fertile pollen grains present in the hybrid plants can be utilized to advance the generation and transfer useful characters present in *C. pinnatifidum* into cultivated chickpea.

The critical factor in obtaining hybrid plants between *C. arietinum* with *C. pinnatifidum* was the grafting technique used to obtain hybrid plants. Grafts were earlier used in chickpea to transfer diseases or obtain disease free plants [3]. Although hybrids seedlings were obtained in 1999, it was not possible to transfer them to soil as the seedlings had very fragile roots.

The breakthrough in obtaining fertile plants with floral buds was the identification of the essential cytokinin, zeatin, for induction and development of floral buds. Removing zeatin from the watering medium caused cessation of floral bud formation. Although cytokinins have been implicated in the development of leucoplasts into normal chloroplasts, this is the first report where a cytokinin was necessary for the initiation and development of floral buds in *C. arietinum* x *C. pinnatifidum* hybrid plants.

Haploids have been reported by parthenogenesis [2], wide-cross induced chromosome-elimination in wheat [7] and oat [18], and by anther/ovule culture [8-10]. There is no report in the literature on the induction of androgenesis as result of wide crosses. Interspecific hybridization between *C. arietinum* x *C. pinnatifidum* offers double benefit in allowing gene transfer through hybridization and induction of androgenesis in some of the pollen grains. A next step would be to explore more fully the feasibility of androgenesis from wide crosses for the rapid development of homozygous lines.

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