Induction of Androgenesis as a Consequence of Wide Crossing in Chickpea

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The value of haploids in genetics and plant breeding has been known for a long time. Natural haploid embryos and plants have been described in about 100 species of angiosperms, and documented in detail by Kimber and Riley (1963). However, haploids occur rarely in nature. Doubled haploids are equivalent to inbred lines, with normal fertility, retaining the advantage of homozygosity, which by conventional program of producing pure lines would require 6–7 generations of selfing to achieve a satisfactory level of homozygosity.

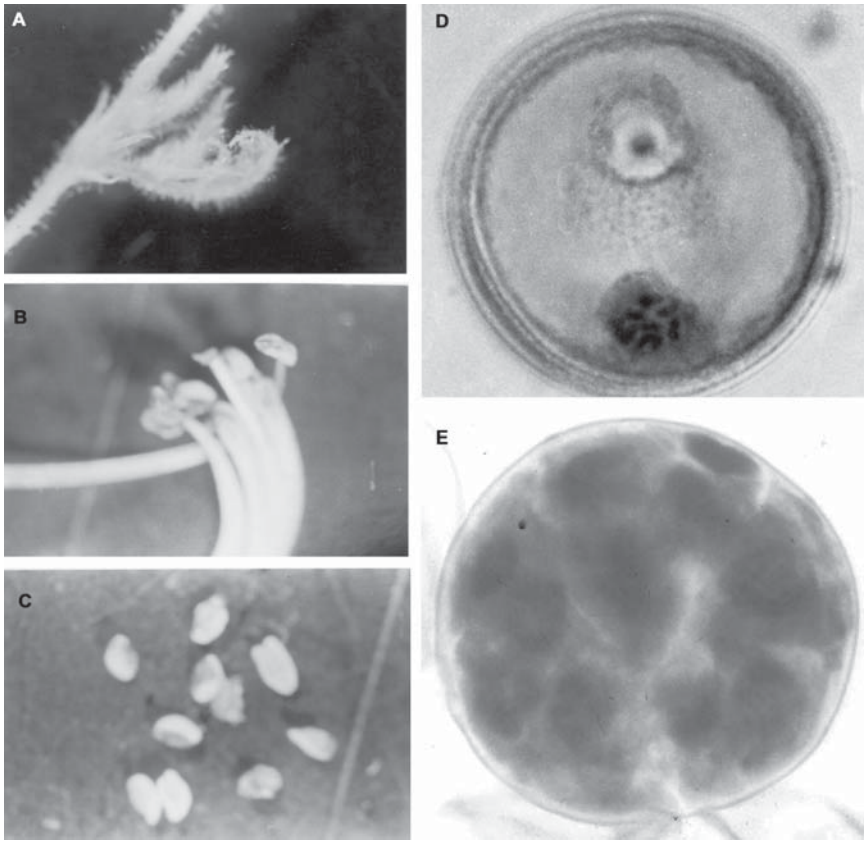
Three principal methods of haploid production include 1. parthenogenesis, 2. wide crosses – chromosome elimination, and 3. haploid plants from anther/ovule culture. In the first method of haploid production, haploids arise from both an unfertilized egg and from a male gamete. Gynogenetic haploids arise as a result of stimulation of the unfertilized egg, and in a few cases the offsprings resembled the male parent and hence were thought to have originated from the pollen (Clausen and Laments 1929; Kostoff 1929; Rhoades 1948). The doubled haploid method used in barley, is an example of preferential chromosome elimination in the cross between barley and *Hordeum bulbosum*, where the chromosomes of *H. bulbosum* were gradually eliminated. In that 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 (Subrahmanyam and Kasha 1973). The chromosome elimination phenomenon is quite prevalent among wide crosses between wheat and *H. bulbosum* as well (Barclay 1975). A more recent procedure to produce haploid plants is by anther culture/microspore culture (Maheshwari 1996; Guha and Maheshwari 1966; Melchers 1972). The culture of anthers or microspores gives rise to haploid plants whose chromosomes can be doubled by suitable treatment to produce homozygous diploid plants. Later Rangan (1994) and Keller and Korzun (1996) reported parthenogenesis of the egg in culture.

Chickpea procedures for developing haploid plants have not been reported, and induction of androgenesis by anther culture is of a very low frequency (Mallikarjuna, personal observation). Androgenesis was observed in a wide cross of *Cicer arietinum* x *C. pinnatifidum*. Hybrids between *C. arietinum* x *C. pinnatifidum* were obtained after rescuing the hybrid embryos in vitro. The hybrids were initially devoid of any chlorophyll pigment and were albinos. Upon continuous culture in a zeatin-rich medium and in the presence of light, the hybrids turned semi-green (Mallikarjuna 1999). Hybrid shoots were grafted to chickpea root stocks to obtain hybrid plants. None of the hybrid plants flowered. When the nutrient solution with zeatin (1 mg/L) was added, flower buds were observed on the hybrid plants. Flower buds were fragile, albino to semi-green, but with normal morphology (Fig. 1A). Anthers (Figs. 1B and 1C) were squashed in acetocarmine and divisions were observed in some of the microspores (Fig. 1E). The number of divisions varied from 4–6. Adding nutrient solution with zeatin (1 mg/L) to in vivo grown chickpea plants did not induce divisions in the microspores.

A total of 16 hybrid plants were obtained. The number of microspores/pollen grains in an anther varied from 11–151 compared to more than 500 pollen grains in cultivated chickpea. The number of pollen grains, which had undergone microsporogenesis and induction of androgenesis, varied from plant to plant. Percent androgenic pollen grains varied from 0–100%. Plant no. 8, 11 and 12 (Table 1) did not have any androgenic pollen grains, whereas in plant no. 14 and 16, all the pollen grains were androgenic, or in other words had multicellular microspores. The number of cells in multicellular microspores in plant no. 14 and 16 varied from 8–10 (Fig. 1E) unlike 4–6 cells in multicellular microspores in other hybrid plants which had androgenic microspores.

This is the first report in literature wherein multicellular microspores have been consistently produced as a result of wide crossing. Wide crosses are not only important in gene transfer from wild species but also in the production of haploid plants by *in vitro* culture of anthers with multicellular microspores.

Next logical step would be to explore the feasibility of androgenesis from wide crosses, for rapid development of homozygous lines.



A Fragile buds from the cross *C. arietinum* x *C. pinnatifidum*.
B & C anther bundle and anthers from the cross *C. areitinum* x *C. pinnatifidum*.
D A normal pollen grain undergoing the microsporogenesis.
E A multicellular pollen grain from the hybrid.

Table 1. Androgenic response in interspecifc incompatible cross *Cicer arietinum* x *C. pinnatifidum*.

Plant No.	Total microspores	No. Normal microspores	No. Androgenic microspores (%)	Maximum no. of cells in a microspore
1	57	43	14 (25)	3–4
2	122	109	13 (11)	3–4
3	73	73	0	
4	46	18	28 (61)	2–4
5	28	23	5 (18)	4–6
6	27	12	15 (56)	2–4
7	83	51	32 (39)	2–4
8	86	86	0	
9	151	143	8 (5)	4–6
10	31	12	19 (61)	2–4
11	35	35	0	
12	74	74	0	
13	43	36	7 (16)	2–4
14	16	0	16 (100)	8–10
15	65	62	3 (5)	
16	11	0	11 (100)	8–10

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