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Cell Biology

A Protocol for Micropropagation of *Cicer* spp

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Micropropagation refers to rapid in vitro asexual or vegetative propagation. Multiple shoot production and plantlet regeneration from meristems (Kartha et al. 1981) and shoot apices (Anil Neelam et al. 1986) of young chickpea (*Cicer arietinum* L.) seedlings grown aseptically have been reported. We developed a technique for micropropagation of chickpea and wild *Cicer* spp using shoot tips from aseptically-raised seedlings and greenhouse-grown plants.

Multiple shoot production. Shoot tips from 15-day old seedlings of four chickpea genotypes L 550, K 850, ICCV 6 (ICCC 32), and ICC 12237 raised on modified MS (MMS) medium were used as explants. The MMS medium contained the constituents of MS medium (Murashige and Skoog 1962) but with vitamins as used in B5 medium (Gamborg et al. 1968). The media also contained 0.9% agar and were adjusted to pH 5.6. The cultures were incubated at 25° ±3°C under fluorescent light of 16-h daylength. The explants were cultured on two media—MMS and modified L-6 (ML-6) (Kumar et al. 1988). It contained 440 mg L^{-1} $CaCl_2.2H_2O$, 170 mg L^{-1} $MgSO_4.7H_2O$, 50 mg L^{-1} (Ethylenediaminetetra acetic acid ferric monosodium salt) (EDTA FeNa), and all other constituents as in L-6 medium. The two media were supplemented with seven (S1 to S7) hormone combinations (Table 1). All the four genotypes tested produced multiple shoots (Fig. 1). ICC 12237 and K 850 produced more shoots explant^{-1} than L 550 and ICCV 6 (Table 1). Shoot tips of L 550, ICCV 6, and ICC 12237 produced maximum shoots explant^{-1} on S3 medium. Subculturing the shoots at 6-week intervals on S3 medium resulted in successful multiplication. A total of 134 shoots were produced from a single explant of K 850 after three transfers (Table 2).

Figure 1. Multiple shoots produced from a shoot tip explant of chickpea genotype K 850 on S3 medium.

The S3 medium was also successfully used to propagate greenhouse plants. Shoot tips from 30-day old plants raised in the greenhouse were surface sterilized with 10% Clorox® (5.25% sodium hypochlorite), using Tween 20® (polyoxyethylenesorbitan monolaurate) as a surfactant, for 10 min. Explants from six chickpea genotypes (L 550, K 850, ICCV 6, ICC 12237, ICCC 42, and C 235)

Table 1. Number of shoots produced[1] explant^{-1} from shoot tips[2] of four chickpea genotypes (L 550, K 850, ICCV 6, and ICC 12237) on different media.

	L 550		K 850		ICCV 6		ICC 12237[5]	
Medium[3]	Ave[4]	Range	Ave[4]	Range	Ave[4]	Range	Ave[4]	Range
MMS + 2.0 mg L^{-1} BA + 0.5 mg L^{-1} IAA (S1)	5.0	3-7	6.2	2-10	5.3	3-10	4.8	4-6
MMS + 2.0 mg L^{-1} ZEA + 0.5 mg L^{-1} IAA (S2)	1.3	1-3	3.8	1-7	1.8	1-3	3.2	1-7
ML-6 + 2.0 mg L^{-1} BA + 0.5 mg L^{-1} IAA (S3)	5.8	3-9	4.9	2-9	5.5	3-9	8.8	8-10
ML-6 + 2.0 mg L^{-1} ZEA + 0.5 mg L^{-1} IAA (S4)	1.4	1-2	2.9	1-5	2.2	2-3	1.8	2-3
MMS + 0.5 mg L^{-1} BA + 0.1 mg L^{-1} IAA (S5)	3.1	1-7	na[6]	na	1.2	1-2	1.7	1-2
ML-6 + 0.5 mg L^{-1} BA + 0.1 mg L^{-1} NAA (S6)	4.0	2-6	5.3	2-7	4.1	1-9	6.0	1-10
MMS + 0.5 mg L^{-1} ZEA + 0.1 mg L^{-1} IAA (S7)	1.1	1-2	1.1	1-2	1.2	1-2	1.1	1-2

1. Ten explants genotype^{-1} were cultured.
2. Shoot tips from 15-day old aseptically raised seedlings.
3. MMS = modified MS medium, ML-6 = modified L-6 medium, BA = benzyladenine, ZEA = zeatin, IAA = indole acetic acid, NAA = napthalene acetic acid.
4. Average number of shoots produced explant^{-1} recorded 6 weeks after culturing.
5. Five explants of this genotype were tested on S1, S2, S3, and S4 only.
6. na = data not available.

Table 2. Multiple shoot production from one shoot tip explant of four chickpea genotypes by frequent subculturing[1].

	Initial culture		First transfer		Second transfer		Third transfer	
			Number of shoots		Number of shoots		Number of shoots	
Genotype	Initial explant[2]	Number of shoots produced	Cultured	Produced	Cultured	Produced	Cultured	Produced
L 550	1	7	7	17	11	c[3]	-[4]	-
K 850	1	8	8	33	27	51	33	134
ICCV 6	1	6	6	7	3	c	-	-
ICC 12237	1	10	10	23	12	c	-	-

1. Shoots were subcultured at 6-week intervals on S3 medium (ML-6 + 2.0 mg L^{-1} BA + 0.5 mg L^{-1} IAA).
2. Shoot tips from 15-day old aseptically-raised seedlings.
3. c = shoots produced callus or clusters of leaflets.
4. - = subculturing was discontinued.

and four wild *Cicer* spp (*C. bijugum* No. 201, *C. cuneatum* SL 157, *C. judaicum* No. 185, and *C. pinnatifidum* No. 188) were cultured on S3 medium. *Cicer cuneatum* produced on an average 10 shoots explant^{-1} and gave the best multiplication response.

Root induction. Shoot tips from aseptically-raised and greenhouse-grown plants of 13 chickpea and wild *Cicer* spp were used as explants. The basal media used were: (1) MMS containing half strength major salts, and (2) rooting medium (RM) (Kumar, A.S., University of Hawaii, personal communication 1987). The media were supplemented with various concentrations of naphthalene acetic acid (NAA), indole butyric acid (IBA), and indole acetic acid (IAA) alone or in different combinations. All the genotypes tested, except *C. bijugum* produced roots at varying frequencies in different media. RM was observed to be a better rooting medium than half strength MMS. Shoot tips cultured on media supplemented with NAA and IBA, alone or in combination, produced roots. However, IBA-supplemented medium induced normal roots while NAA-supplemented medium induced thick

roots. RM supplemented with 0.2 or 0.5 mg L^{-1} IBA showed the best response.

Acclimatization. The plantlets produced from shoot-tip cultures were transferred to 6-cm square plastic pots containing sterile sand and placed in a growth chamber at 25°±3°C under fluorescent light with 16-h daylength. The plants that survived and established were transferred to 15-cm diameter plastic pots containing sterile sand + Vertisol (3:1) mixture and maintained in the greenhouse till maturity. Normal pods and seeds were produced from such micropropagated plants.

Based on our experiments, we summarize the following protocol for micropropagation of chickpea and wild *Cicer* spp.

1. Shoot tips (1-3-cm long) from young plants in the vegetative stage are sterilized with 10% Clorox® (using Tween 20®) as a surfactant) for 10 min, and used as explants.
2. The explants are cultured on S3 medium for multiple shoot production.
3. The shoots produced are excised individually and cultured on RM containing 0.2 or 0.5 mg L^{-1} IBA for root induction.
4. The plantlets are transferred to sterile sand.
5. After acclimatization, the plants are transplanted to sand + soil medium and maintained in the greenhouse.

The system developed will be useful, especially for disease resistance, to propagate elite chickpea materials selected in the field and also in greenhouse screenings, We have used the technique successfully to propagate interspecific *Cicer* hybrids.

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Entomology

An Analysis of HCH and Endosulfan Residues in Chickpea Seed

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The chickpea pod borer, *Helicoverpa* (*Heliothis*) *armigera* (Hübner) is effectively controlled by many pesticides. Endosulfan and HCH [Hexachloro cyclohexane or benzene hexachloride (BHC)] are widely used by farmers because they are readily available and cheaper than organophosphorus and pyrethroid insecticides. As both insecticides are persistent chlorinated hydrocarbons, their evaluation for potential residues in harvested seed was considered to be important.

The experiment was conducted on cv PG 5 (Viswas) in a randomized-block design with three replications during the postrainy season of 1987 (sowing date 20 Nov 1987). HCH (BHC 50 WP) and endosulfan (Endocel® 35 EC) were applied at the recommended and double the recommended spray concentrations using a knapsack sprayer. The recommended spray concentration was 0.2% HCH and 0.05% endosulfan. For each chemical treatment, two sprays were applied at 2-week intervals after the initiation of the first pod-formation stage (9 Feb 1988).

The crop was harvested on 14 Mar 1988 and the seed from each treatment subjected to residue analysis. Residues were determined by gas-liquid chromatography equipped with a high-temperature electron capture detector (300°C). The stainless steel column (1.8 m × 2 mm) was loaded with 1.5% OV-17 + 1.95% OV-210 on Chromosorb® WHP (60/80 mesh). The carrier gas was nitrogen at a flow rate of 60 mL min^{-1}. The working temperature for the injector port was 210°C and for the column 190°C. Prior calibration showed that the method was sensitive to 0.01 ppm of insecticide with a mean recovery of 90.6% (HCH) and 89.6% (endosulfan).

The data given in Table 1 clearly indicate that the residues of both the pesticides were below the detectable