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Plant regeneration *via* somatic embryogenesis in chickpea (*Cicer arietinum* L.)

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Abstract

Efficient plant regeneration *via* somatic embryogenesis has been developed in chickpea from embryonic axes on MS medium supplemented wth 1.25 mg/l 2, 4-D and 0.25 mg/l, kinetin induced callusing after 7 days of culture. On the same medium, 7 days old callus masses started differentiating with high efficiency of globular structures during dark incubation. 1/2 MS salts + B_5 vitamins + 40 g/l, sucrose was found suitable for embryo maturation. The well formed embryos germinated into plantlets on MS salts + B_5 vitamins + 1.25 mg/l IBA + 2.0 mg/l BAP. Normal plants were transferred to soil.

Key words: Chickpea, *in vitro* regeneration, somatic embryogenesis, explants

Introduction

A basic requirement for exploiting biotechnology for crop improvement is the availability of reliable *in vitro* regeneration protocol. In grain legumes, somatic embryogenesis has been reported in cultures initiated from complex explant such as shoot apex [1, 2] and immature embryo [3]. Regeneration from leaf explants *via* somatic embryogenesis has been reported only in two-grain legumes i.e. chickpea [4-6] and peanut [7] but the frequency of plantlet formation was low and the protocol served only a limited purpose. Therefore, an attempt has been made to develop an efficient and reliable protocol for whole plant regeneration via somatic embryogenesis from embryonic axes.

Materials and methods

Three genotypes of chickpea *viz*, C 235, K 850 and BG 256 with four combinations of growth regulators were used as the experimental material. Seeds were immersed in 50 ml of sterile water with two drops of liquid detergent (Tween 20) for 15 minutes. Explants were surface fertilized with 10% sodium hypochlorite solution for 15 minutes followed by 70% ethyl alcohol for 2 minutes. Embryonic axes were isolated from seeds and cultured on MS medium supplemented with 2, 4-D at concentrations of 0.5, 1.25, 1.4 and kinetin at 0.01, 0.25, 0.5 mg/l and GA_3 at 0.5 mg/l. The medium was adjusted to pH 5.8, 0.8% agar was added and autoclaved at 15 psi for 15 minutes. Cultures were incubated in the dark at 25 ± 1°C for 4 weeks. The experiment was repeated thrice.

After four weeks on induction medium, calli from the preliminary experiment were transferred to 4 maturation medium containing MS salts + B_5 vitamins; 1/2 MS salts + B_5 vitamins; MS salts + B_5 vitamins supplemented with 0.125 mg/l IBA, 0.5 mg/l NAA and 0.5 and 2.0 mg/l BAP.

Fully developed embryos were transferred to (i) MS medium with 0.125 mg/l IBA and 2.0 mg/l BAP (ii) MS medium with 0.5 mg/l NAA and 0.5 mg/l BAP and (iii) basal B_5 medium. Medium, which promoted the initiation of the root and shoot primordia, was identified at the best germination medium. The well developed plantlets were transferred to pot.

Results and discussion

The basic three-step procedure of embryogenesis (consisting of embryo induction in dark on MS medium with 2, 4-D and cytokinin, embryo maturation on basal and cytokinin rich medium under light and germination of embryo) has been found useful.

The creamy white callus (100%) was observed on MS medium containing 1.25 mg/l 2,4-D + 0.25 mg/l kinetin whereas whitish callus was observed on MS + 0.5 mg/l 2,4-D (45.0-48.0%) (Fig. 1a) and 1.4 mg/l, 2, 4-D + 0.25 mg/l kinetin (25.0-30.0%). Medium with 1.25 mg/l, 2, 4-D and 0.25 mg/l, kinetin (Table 1) was found to be the best for embryo induction (28.56-30.83 av. no. of somatic embryo/callus) (Fig. 1b). Barna and Wakhlu [5], however, found that 2, 4-D supplied singly at ~ 5.2 mg/l, was the best for induction of embryos from immature leaflets of chickpea and that the addition of kinetin or BAP to induction medium reduced embryo formation.

Few combinations has been developed for maturaton of embryos. Half strength MS salts + B_5

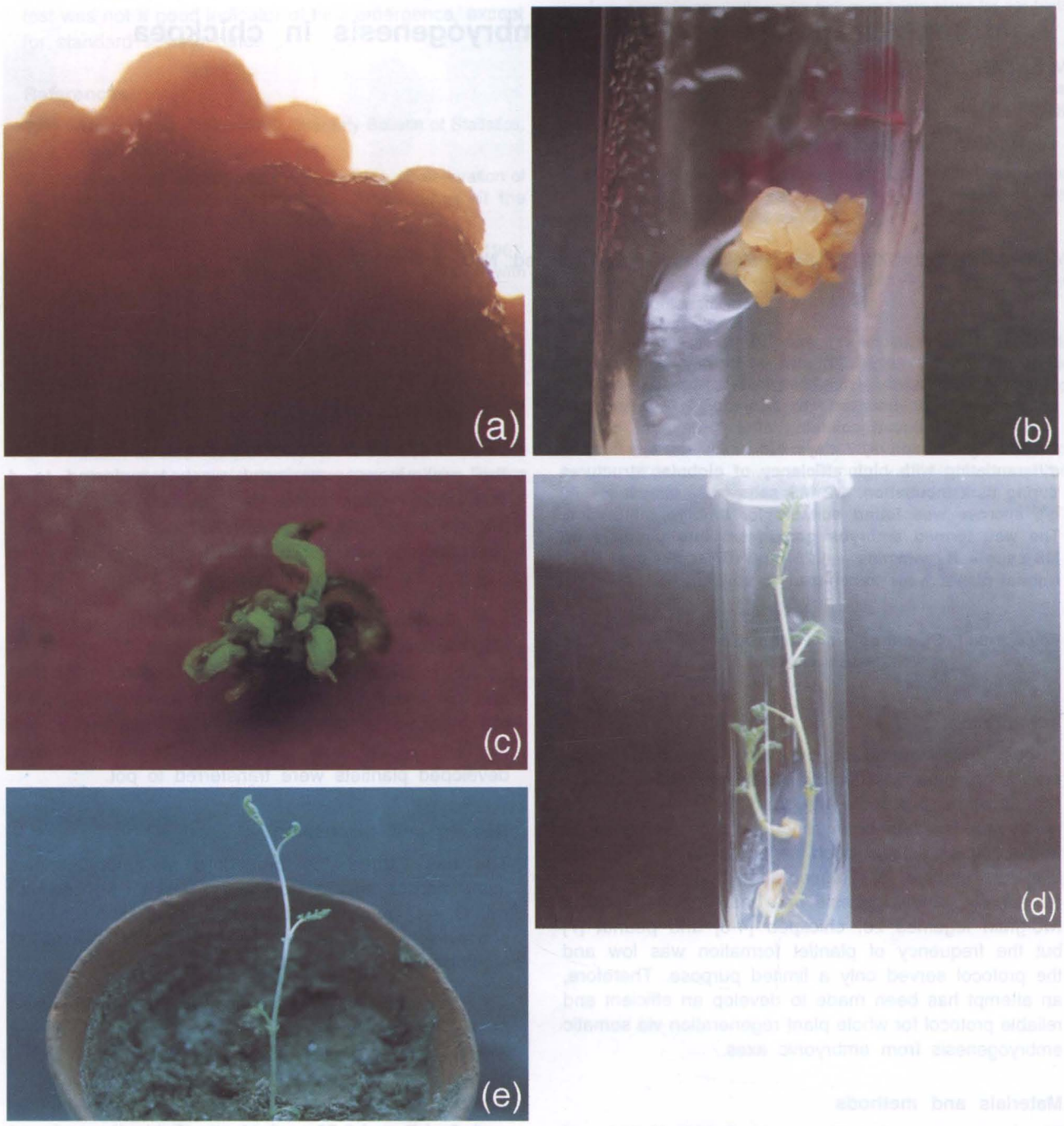

Fig. 1. (a) Globular stage of somatic embryo induce from creamy callus on MS + 1.25 mg/l 2, 4-D + 0.25 mg/l Kinetin; (b) Different stage of somatic embryo induced from callus on MS + 1.25 mg/l 2, 4-D + 0.25 mg/l Kinetin; (c) Mature somatic embryo on MS salt + B_5 vitamin; (d) Germination from somatic embryo; (e) Plantlet transferred to pot

vitamins was the best medium for maturation of embryos (21.33 av. no. of somatic embryo/callus) (Fig. 1c). Medium with higher dose of BAP (2 mg/l) promoted recallusing of the embryos. Medium which contains lower dose of BAP (0.5 mg/l) was not good for embryo maturation. Indirect somatic embryogenesis takes four weeks for callus induction and subsequent 10 days for induction of globular shape embryo followed by heart, torpedo and cotyledon shape. Most often torpedo shape structure is not visible as it appeared for short period. These results agree with the observation made by Barna and Wakhlu [5].

Out of three genotypes, K 850 induced maximum frequency (60.53%) of callus followed by C 235 (58.46%) and BG 256 (56.56%). However, all three genotypes showed comparable response in terms of induction of somatic embryos expressed as range, av. no. of somatic embryo/explant and frequency. Regarding maturation of somatic embryos, C 235 gave maximum (70.56%) response followed by BG 256 (63.54%) and K 850 (58.82%). Besides, widestrange (5-23) for maturation of somatic embryos was obtained with K 850 and BG 256. Whereas, C 235 showed comparatively lower range towards higher values.

For germination of the embryos, MS salts + B_5 vitamins supplemented with 0.125 mg/l IBA and 2.0 mg/l BAP was found suitable. Germination of embryos was characterized by simultaneous production of root and shoot systems. The complete plantlets were elongated on basal MS medium (Fig. 1d).

Hardening process, which takes nearly 30d, was critical to successful transfer of plants to soil (Fig. 1e). This protocol has several distinct advantages (i) The conversion of plantlets is as high as 50% and more than 25 complete plantlets could be obtained for 100 explants, (ii) the protocol is not genotype specific (Table 1).

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Table 1. Induction of callus and embryogenic response from embryonic axes

Genotype	Media with concentration of growth regulators	No. of explant	Callus induction (%)	Induction of somatic embryo Av. no. of somatic embryo ± SE	Range
C 235	1. MS + 2,4-D (0.5)	200	48	0.83 ±1.28	2-5
	2. MS + 2,4-D (1.25) + kinetin (0.25)	200	100	28.56 ±4.75	25-30
	3. MS + 2,4-D (1.4) + kinetin (0.25)	200	30	09.20 ±1.00	8-20
	4. MS + kinetin (0.5) GA_3 (0.5)	200	00	00.00	0-0
K 850	1. MS + 2,4-D (0.5)	200	45	08.00 ±1.20	2-5
	2. MS + 2,4-D (1.25) + kinetin (0.25)	200	100	28.61 ±3.82	25-30
	3. MS + 2,4-D (1.4) + kinetin (0.25)	200	25	9.23 ±1.20	8-20
	4. MS + kinetin (0.5) GA_3 (0.5)	200	00	00.00	0-0
BG 256	1. MS + 2,4-D (0.5)	200	45	08.20 ±1.23	2-5
	2. MS + 2,4-D (1.25) + kinetin (0.25)	200	100	30.83 ±3.69	25-30
	3. MS + 2,4-D (1.4) + kinetin (0.25)	200	30	08.90 ±1.31	8-20
	4. MS + kinetin (0.5) GA_3 (0.5)	200	00	00.00	0-0