An optimal protocol for in vitro regeneration, efficient rooting and stable transplantation of chickpea

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ABSTRACT

A rapid, reproducible and efficient regeneration method was developed for chickpea (Cicer arietinum L.) using single cotyledon with half embryonal axis as explants. MS medium supplemented with 4 μM TDZ, 10 μM 2-iP and 2 μM kinetin induced 50-100 adventitious buds/shoots after 14 days of culture and elongated on MS medium supplemented with 5 μM 2-iP and 2 μM kinetin. Healthy, strong and 100 % rooting was achieved by exposing cut ends of the shoots to 10 sec pulse treatment with 100 μmoles/ml IBA followed by their transfer to liquid MS basal medium within 10-14 d. 2-3 cm long shoots were most suitable for rooting. Potting-mixture with good aeration and lesser capacity to retain water was most suitable for achieving successful establishment of chickpea plantlets. Garden soil mixed with sand (gravel) and bio-manure in the ratio of 1:1:1 is most suitable for achieving cent percent transplantation success. Cent percent of plantlets got acclimatized, survived in the pots and showed normal growth, development, flowering followed by podding and seeds setting. Harvesting of seeds was done after the pods were fully matured and dry. In this communication, we have demonstrated for the first time that shoot length, pulse treatment of cut ends of shoots with 100 μmoles/ml IBA and aeration of potting mixture are key factors for rapid micro-propagation and successful establishment of chickpea. [Physiol. Mol. Biol. Plants 2008; 14(4) : 329-335] E-mail : ppsaradhi@gmail.com

Key words : Cicer arietinum, Chickpea, Regeneration, Rooting, Transplantation, IBA

INTRODUCTION

Chickpea is the world’s third most important pulse crop and India accounts for 75 % of the world’s production. Chickpea is good as a source of carbohydrate (48.2-67.6 %), protein (12.4-31.5 %), fat (6 %) and nutritionally important minerals. Among the legumes chickpea is the best hypocholesteremic agent, followed by black gram and green gram. Direct shoot organogenesis and establishment of plantlets from different explants of chickpea was reported earlier (Polisetty et al., 1996, 1997; Paul et al., 2000; Rizvi and Singh, 2000; Chauhan et al., 2003; Jayanand et al., 2003; Chakraborti et al., 2006). Plantlets were developed through direct somatic embryogenesis and through callus from different explants of chickpea (Barna and Wakhlu, 1993; Sagare et al., 1993; Suhasini et al., 1994; Kumar et al., 1994, 1995; Rizvi and Singh, 2000; Chauhan et al., 2002; Kar et al., 1996, 1997; Kiran et al., 2005).

Inspite of several reports of successful regeneration, chickpea is widely considered to be highly recalcitrant (Shri and Davis, 1992; Vani and Reddy, 1996; Rizvi and Singh, 2000; Polowick et al., 2004). Surprisingly, majority of published/reported chickpea regeneration protocols often are either not repeatable or work only in certain research laboratories, making researchers to believe that chickpea regeneration is highly recalcitrant. Two major hurdles that limit in vitro regeneration of chickpea are (i) induction and development of strong root system; and (ii) establishment of in vitro raised plantlets in pots. In order to escape from these hurdles, researchers have preferred to go for grafting (Krishnamurthy et al., 2000; Sarmah et al., 2004; Senthil et al., 2004; Tewari-Singh et al., 2004; Sanyal et al., 2005). In general, grafting is tedious and time consuming requiring special skills. Moreover, grafting besides requiring additional seed lot, also might promote emergence of shoots/branches from axillary buds that are often retained in the axils of cotyledons and the later might dominate over the grafted shoots.

Therefore, present investigations were carried to fine-tune the protocol for achieving strong root system and
successful establishment of the in vitro raised plantlets. In this communication we have been successful in furnishing a perfect protocol for in vitro production of plantlets with strong root system and their successful establishment.

**MATERIAL AND METHODS**

The seeds of chickpea (*Cicer arietinum* L.) cv. PG 114 (gifted by Dr. S.S. Yadav and Dr. Jitender Kumar, Indian Agricultural Research Institute, New-Delhi, India) were treated with 0.2 % cetrimide for 5 min, rinsed thoroughly with double distilled water followed by treatment with 70 % ethanol for 3 min and 0.1 % mercuric chloride solution for 5 min. Finally the seeds were washed 5-6 times with sterile double distilled water under aseptic conditions prior to soaking overnight. Fourteen hour imbibed seeds were de-coated (Figure 1A) and germinated on shoot induction medium (SIM), consisting of MS medium supplemented with 4 μM TDZ, 10 μM 2-iP and 2 μM kinetin, for 6 d. Explants consisting of single cotyledon with half embryonal axis with plumular and radicular ends were excised from resulting 6 d old seedlings (Figure 1B) were inoculated on SIM for further duration of 6 d. The explants were subsequently transferred to MS basal medium (i.e. devoid of growth regulators) for 10-15 d.

**Elongation of Shoots**

The adventitious buds/multiple shoot induced from the explants were excised from the bunch without any callus or globular structures and cultured on the shoot elongation medium (SEM), consisting of MS medium supplemented with 5 μM 2-iP and 2 μM kinetin, for 10 d. They were then routinely sub-cultured at an interval of 10-15 d on SEM.

**Rooting**

Two basic approaches were used for rooting elongated shoots varying in length from 3 to 8 cm in length based on several preliminary experiments in our laboratory. In the first approach, 3 to 8 cm long shoots were transferred to either semi-solid or onto Whatman filter paper bridges in liquid MS medium supplemented with 5 nmoles/ml (5 μM) IBA. In the second approach, the cut end of shoots were exposed to 10 sec pulse treatment with 100 μmoles/ml (100 mM) IBA and were transferred onto Whatman filter paper bridges in liquid MS basal medium.

All the cultures were maintained at 25±2 °C with a light/dark cycle of 16/8 h. White fluorescent light with an intensity of 120 μmol m⁻² s⁻¹ was used for illumination.

**Transplantation**

The plantlets were carefully taken out from tubes, roots were thoroughly washed with tap water and transferred to small earthen pots (~8 cm diameter and 10 cm height) filled with potting mixture consisting of garden soil, sand and bio-manure (Khadi and Village Commission, Govt. of India) in equal proportion. Each pot with plantlet was initially covered with transparent polythene bag for 6 d. However, after 3 d the corners of the polythene bags were cut. Subsequently, the polythene bags were removed. After allowing the plants to grow further for another 6 d, the plants were carefully transferred to large earthen pots (30 cm diameter x 37.5 cm height) having garden soil, sand and bi-manure in equal proportion. Cent percent of plants got acclimatized, survived in the pots and showed normal growth, development, flowering followed by seed setting. Harvesting of seeds was done after pods were fully dried.

**Fig. 1.** Regeneration of chickpea PG-114. A. Seeds de-coated after 14 h imbibition, on shoot induction medium (SIM) (MS medium supplemented with 4 μM, 10 μM 2-iP and 2 μM Kin); B. Chickpea seedlings (6 d after incubation of de-coated seeds on SIM) used for obtaining explants for regeneration and transformation; C. Swollen embryonal axis attached to cotyledon showing the initiation of shoot induction (6 d after incubating the explants on SIM); D. Two week old culture of embryonal axis with single cotyledon, showing the emergence of large number of adventitious buds (15 d after incubating 6 d old cultures from SIM on MS medium); E. Shoots on shoot elongation medium (MS medium with 5 μM 2-iP and 2 μM kinetin) (14 d after transfer of shoots from SIM).