Indian Journal of Agricultural Sciences **84** (1): 167–9, January 2014/Short Communications

A protocol for efficient callus induction from hypocotyls explant in chickpea (*Cicer arietinum*)

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Received: 8 February 2012; Revised accepted: 11 December 2013

Key words : Callus, Chickpea, Hypocotyls

Chickpea (*Cicer arietinum* L.) is the third most important food legume crop grown over 45 countries across five continents. It ranks first in the Indian subcontinent and Mediterranean basin (Pande *et al.* 2006). It is one of the major dietary proteins source for vegetarian population in developing countries. Chickpea is not only an important source of human food and animal feed, but it also maintains soil fertility through biological nitrogen fixation and contributes to the sustainability of cropping systems in cereal-legumes rotation (Singh *et al*. 2003). The yield potential, productivity and nutritional value of chickpea are affecting by a number of biotic and abiotic stresses from seedling to harvesting stages (Haware 1998). Genetic improvement of this crop, especially cultivars resistant to biotic and abiotic stresses as well as better protein quality and quantity using conventional breeding methods have been found to be slow because of narrow genetic base of crop and presence of strong sexual incompatibility barriers with its wild relatives (Robertson *et al*. 1997, Mallikarjuna and Muehlbauer 2011). Moreover, these methods are laborious, time consuming and need a large experimental area. Plant tissue culture techniques have been used extensively as alternative tools to accelerate the breeding programme in many crop species (Brown 1995, Jain 2001). Thus, there is need to widen the genetic base and incorporate desirable characters. There is an urgent need to use transgenic technologies for improvement of this crop. Callus induction in any crop is a pre- requisite for its utilization in tissue culture based techniques like somaclonal variation, screening of calli against biotic or abiotic stress, regeneration of plant and transgenic plant development. Callus inductions from cotyledon, epicotyls, leaf and meristem explants were observed in chickpea by Rao and Chopra (1987) using MS medium (Murashige and Skoog 1962) with different concentrations of growth regulators. Callus induction depends on composition of nutritive media, growth regulators and other organic additives. It also depends on choice of explants and its age. The objective of this study, to develop a protocol for efficient callus induction in chickpea genotypes derived from hypocotyls explant using MS medium and its combinations with different growth regulators.

Seeds of four chickpea genotypes/varieties comprising three desi types like E100 Y (M), Gaurav and Pb-7; and one Kabuli type- L-550 were taken for the study. Hundred seeds of these genotypes/varieties of chickpea were taken in a beaker and soaked for 10 minutes in fresh water containing 4 drops of teepol (a liquid detergent, manufacture by Aaykay Detergents and Chemicals, Ludhiana) followed by washing with running tap water. Then, seeds were surface sterilized by immersing in 0.1 % mercuric chloride ($HgCl_2$) solution for 10 minutes, which were subsequently rinsed thoroughly for 4 times with double distilled sterilized water to eliminate the traces of mercuric chloride under aseptic conditions. Sterilized seeds were soaked in sterilized distilled water for 20 hr and then, 20 seeds were placed on each flask (250 ml) containing 50 ml sterilized sucrose-agar medium (2% sucrose and 0.8% agar-agar dissolved in distilled water) and kept in the culture room where automatic control temperature (25ºC) and light (16 hr white fluorescent light at 1500 Lux and 8 hr dark conditions). Hypocotyl segments measuring 5-6 mm were excised from 7 days old seedlings and implanted on Petri plate (9 cm diameter) containing 25 ml solidified MS basal medium supplemented with different concentrations of plant growth regulators, i.e. auxins alone and in combination with cytokinin. Six hypocotyls segments were kept in each Petri plate. Each treatment contained 10 replications and the experiment was designed in CRD (completely randomized design). Inoculated Plates were placed at 25±1°C in automatic

Based on a part of M Sc thesis of the first author submitted to CCSHAU, Hisar in 2009

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Table 1 Callus induction in different chickpea genotypes from explant hypocotyls

MS basal medium with auxins alone or in combination with cytokinins	Morphological characters of callus	Callus Induction (%)			
		Chickpea genotypes			
		E100 Y(M)	Gaurav	Pb-7	L- 550
Only MS basal	No callus induction rooting and shooting occurs from hypocotyl	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
With auxin					
2,4-D (1.0 mg/L)	Pale yellowish green and fragile calli; callus induction from one end of hypocotyls	71.66 ± 8.05	61.66 ± 8.04	53.33 ± 10.53	61.66 ± 8.04
2,4-D (2.0 mg/L)	Pale yellowish green and fragile calli; callus induction from one end of hypocotyls	100.00 ± 0.0	100.0±0.0	100.0±0.0	100.0 ± 0.0
2,4-D (3.0 mg L^{-1})	Pale yellowish green and fragile calli; callus induction from both end of hypocotyls	79.99 ± 7.02	68.32 ± 5.27	76.66 ± 8.60	76.66 ± 8.60
With auxins and *Cytokinin*					
IAA (2.5 mg/L) + BAP (0.5 mg/L)	Dark green and compact calli; callus induction from both end of hypocotyls	100.00 ± 0.0	100.00 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
IAA (3.0 mg/L) + BAP (0.5 mg/L)	Dark green and compact calli; callus induction from one end of hypocotyls	51.66 ± 9.45	43.33 ± 8.60	18.32 ± 5.027	18.32 ± 5.27
NAA (3.0 mg/L) + BAP (3.0 mg/L)	Yellowish- green and fragile calli; callus induction from both end of hypocotyls	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0
LSD (0.05)	*Media – 2.28*	*Genotypes- 1.72*		*Interaction (Media × Genotypes)- 4.56*	

Individual value indicates mean of 10 replications ± standard deviation MS basal (Murashige and Skoog); 2, 4-D: 2, 4 Dichlorophenoxyacetic acid; BAP: Benzylamino purine; IAA: Indole-3-acetic acid; NAA: Naphtalenacetic acid

regulated culture room for one month under 16 hr fluorescent light (1500 Lux) and 8 hr dark conditions for callus induction (Naz *et al*. 2008) and observation of percent callus induction and morphological characters of callus were recorded.

Among different combinations of growth regulators, MS basal medium supplemented with IAA (2.5 mg/L) + BAP (0.5 mg/L); 2, 4-D (2 mg/L) and NAA (3 mg/L) + BAP (3 mg/L) induced significant higher callus induction (100 %) in all genotypes of chickpea E100 Y (M), Gaurav and Pb-7 and L-550 from hypocotyls explant (Table 1). Callus induction from hypocotyl segments were initiated as surface swelling after 3-4 days of inoculation on medium and with the appearance of dividing cells at both ends of hypocotyls segments followed by division over entire surface. The calli colour and their structure were depended on using different types and combinations of growth regulators. Application of 2, 4-D as growth regulator induced pale yellowish green calli with fragile structure, whereas combined application of IAA and BAP induced green and compact calli, whereas NAA and BAP combination induced yellowish-green and fragile calli in all genotypes of chickpea (Fig 1). MS basal medium without any growth regulator did not induced calli but promoted rooting and shooting in hypocotyls of all genotypes of chickpea (Fig 1b). Callus induction in chickpea has also found by other workers using different explants and MS medium supplemented with different concentrations of growth

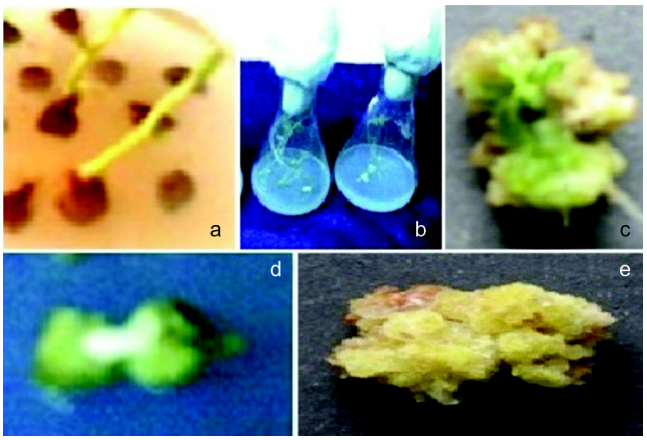

Fig 1 a) seedling; b) shooting and rooting on MS basal medium from hypocotyls; c) callus induction on MS basal + 2,4-D (2.0 mg/L); d) callus induction on MS basal + IAA (2.5 mg/L) + BAP (0.5 mg/L); e) callus induction on MS basal + NAA (3.0 mg/L) + BAP (3.0 mg/L) in chickpea genotype E100 y (m)

regulators, viz. NAA and BAP (Rao and Chopra 1987, Huda *et al.* 2003, Gawande *et al*. 2007) that supported our present investigation.

SUMMARY

Callus induction in any crop is a pre-requisite for its utilization in tissue culture based techniques like somaclonal variation, screening of calli against biotic or abiotic stress, regeneration of plant and transgenic plant development. In present investigation an attempt was made to develop a protocol for efficient callus induction from hypocotyl explants in chickpea crop. Among different combinations of growth regulators- auxin alone and in combination with cytokinin used for callus induction, MS basal medium supplemented with IAA (2.5 mg/L) + BAP (0.5 mg/L), 2, 4-D (2 mg/L) and NAA (3 mg/L) + BAP (3 mg/L) were induced significantly higher callus induction (100%) in all genotypes of chickpea E100 Y (M), Gaurav and Pb-7) and (L-550) from hypocotyls explant.

ACKNOWLEDGEMENT

Authors are grateful to Department of Genetics and Plant Breeding, College of Agriculture, CCSHAU, Hisar for providing culture room facilities in our present investigation.

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