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Embryo excision and growth evaluation of chickpea (*Cicer arietinum*) on tissue culture medium

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Abstract
In present study efforts were made to improve to study the effects of different growth regulators with different concentration on excised embryo from overnight soaked mature seeds of Chickpea. For study of Chickpea, seeds were sterilized and soaked overnight aseptically and then used for explants isolation. For both explants we use the mature Chickpea seeds. These seeds used for isolation of embryonic axis and Cotyladory Node explants. Both explants inoculated on different concentrations of growth regulators. Among those MS media fortified with 3 mg/L BAP shows maximum shoot initiation. The mature embryonic axis explants of cv. Vijay (Phule G-81-1-1) showed maximum growth and development in all types of media for all types growth conditions. The MS media fortified with 0.3 mg/L IBA as growth regulators gave better results for root formation in both types of explants and in both cultivars. In MS media without growth regulators, very poor response had occurred.

Keywords: Chickpea, Regeneration, BAP, IBA. Mature embryos

1. Introduction
Chickpea (*Cicer arietinum* L.) is the foremost grain legume of India and Bangladesh, both in area planted and production. This crop is significant source of protein, phosphours, iron and certain water-soluble vitamins; the total amount of fat they contain is extremely unsaturated. Food legumes are important source of nutrients and provide supplementary protein to diets based on cereal grains and starchy foods. Protein provided mainly by the cotyledon, ranges in concentrations from about 17 to 40%. Protein content of chickpea can be improved by using tissue culture and genetic transformation technique. The tissue culture method is a novel approach, and the main idea is that cultivated cells are used as the selection units rather than whole plants (Butenko *et.al* 1979) [1]. The insertion of in vitro tissue culture techniques in a breeding program offers considerable opportunities for genetic improvement of plants by saving space and time required by conventional methods (Ortiz, 1998) [2]. The utilisation of biotechnology in plant breeding is largely dependent on callus induction and subsequent plant regeneration from various explant sources. The success in this process is affected predominantly by genotypes and the type of explant material (Ozgen *et al.,* 1996; Ozgen *et al.,* 1998) [3, 4].
Successful development of an embryo depends on many factors. As with most other processes, the plant genotype greatly influences success. Embryos of some species are easier to grow in culture than are others, and differences sometimes occur between closely related cultivars (Collins and Grosser, 1984; Rangan, 1984) [5, 6]. According to Pierik (1989) [7], there are in principle two types of embryo culture: culture of immature embryo and mature embryos.
Present investigation attempts to obtain to excise embryo from overnight soaked chickpea genotypes and check the effects of growth regulators on embryo germination. The cultivars Vijay (Phule G-81-1-1) and Digvijay (Phule G-9425-5)' which are considered to be good cultivars in the Maharashtra. However, no previous studies have been performed to standardize regeneration protocols using these two cultivars.

Material and Methods
The commercial Local cultivars of Chickpea 'Vijay (Phule G-81-1-1) and Digvijay (Phule G-9425-5)' were selected for the present study. Seeds were obtained from the 'Krishi Vigyan Kendra, Pokharni, Nanded'. Initially chickpea seeds were washed thoroughly with tap water in order to remove dust and other particles followed by washing with distilled water with 2-3 drops of tween-20 for 20 min. The seeds were rinsed with distilled water 8-9 times. Further sterilization was carried out inside the laminar air flow chamber. Seeds were treated with 70% ethanol for 2 min followed by 0.5% $HgCl_2$ solution for 5 min. Sterilized seeds were washed thoroughly with autoclaved distilled water for 4-5 times to overcome the poisonous effect of

$HgCl_2$. Finally, seeds were soaked in sterile water for overnight prior to inoculation in to MS medium.

After germination embryonic axis and cotyladory nodes were removed from the imbibed seed coat and placed on MS medium with different combinations of growth regulators as mentioned in Table 1 (Fig. 1). Plates were incubated at 25°C in dark. For the study of effect of growth regulators on regeneration, embryo genic part of the callus was cut into small pieces approximately 2-3mm in size and inoculated on MS fortified with different concentration of auxins and cytokinins and incubated at 25^0C in a 16h light and 8h dark photoperiod. Firstly inoculated the explants in MS media fortified with different concentration of BAP for shoot induction and multiplication studies. Small shoots (2-3cm) were sub-cultured on MS media fortified with different concentration of IBA for study effects on root regeneration with MS media without growth regulators as control.

Table 1: Concentrations of plant growth regulators fortified with MS culture media for preliminary experiments.

Sr. No.	Media	Growth regulators (mg/L)	
		BAP	IBA
1	MS0	-	-
2	MS1B	1.0	-
3	MS2B	2.0	-
4	MS3B	3.0	-
5	MS0.1I	-	0.1
6	MS0.2I	-	0.2
7	MS0.3I	-	0.3

Results and Discussion

The present investigation was carried out with an objective to excise embryo from overnight soaked chickpea genotypes and check the effect of growth regulators on embryo germination from different explants of Chickpea. For study of Chickpea seeds were sterilized, overnight soaked in sterile water and used for explants isolation. For the *in vitro* morphogenesis studies in Chickpea two separate experiments were conducted with embryonic axis and Cotyladory Node explants obtained from mature seeds. Explants of two genotypes *viz.* 'Vijay (Phule G-81-1-1) and Digvijay (Phule G-9425-5)' were cultured on different MS media fortified with different concentration of growth regulators. The media were selected only for the basic study of experiments conducted to screen effects of plant growth regulators for *in vitro* response. The basal MS medium was fortified with different combinations of BAP and IBA in varying concentrations with control. During present investigation, observations were recorded for shoot initiation and root initiation abilities.

Table 2: Observation on *in vitro* regeneration of Chickpea cultivars.

Morphological growth studied for	Media	Concentration of different growth regulators mg/L	No. of explants showing morphogenic growth/20 explants			
			Vijay		Dig vijay	
			EA	CN	EA	CN
Shoot initiation	MS0	Without GR (Control)	4	6	3	4
	MS1B	BAP 1 mg/L	14	12	13	10
	MS2B	BAP 2 mg/L	16	15	16	13
	MS3B	BAP 3 mg/L	19	16	17	15
Root initiation	MS0	Without GR (Control)	3	4	3	2
	MS0.1I	IBA 0.1 mg/L	14	12	13	10
	MS0.2I	IBA 0.2 mg/L	16	15	16	13
	MS0.3I	IBA 0.3 mg/L	18	16	17	15

Note:-EA-Mature Embryonic Axis, CN- Mature Cotyladory Node

Mature embryonic axis culture

The shoot initiation from mature embryonic axis cultures varied from 20-95%. Among two accessions, maximum shoot initiation was observed in cultivar Vijay (Phule G-81-1-1) (95%) followed by Digvijay (Phule G-9425-5) (85.0%). In terms of the culture media response to *in vitro* culture, the performance of culture media MS3B (95.0%) followed by MS2B (80.0%) was found to be most responding explants for shoot initiation. In control shoot initiation were very poor (20%).

The average mean roots formation from Embryonic axis cultures explants varied from 15-90%. Maximum root formation was observed for cv. Vijay (Phule G-81-1-1) (90%) on MS0.3I media followed by Digvijay (Phule G-9425-5) (85%).

Cotyladory node culture

Cotyladory Node explants were cultured on MS medium fortified with different combinations of growth regulators. After that, shoot initiation and root initiation were observed on different combination of MS medium. Cotyladory Nodes were observed to swell 5-10 days after plating. Shoot initiation was observed after 20-25 days of plating. Explants swellings started from the lower portion of explants. After that, initiated explants tissues developed distinct phenotypes *viz.* wet, rough, hard dense and glossy, greenish with different developmental potentials. After 40-45 days of inoculation, explants could be distinguished on the basis of their phenotypic appearances. Compact, light green swollen tissues with few or many dark green bead like structures and sometimes partially covered with thin layer of white loose structure were recognized live explants.

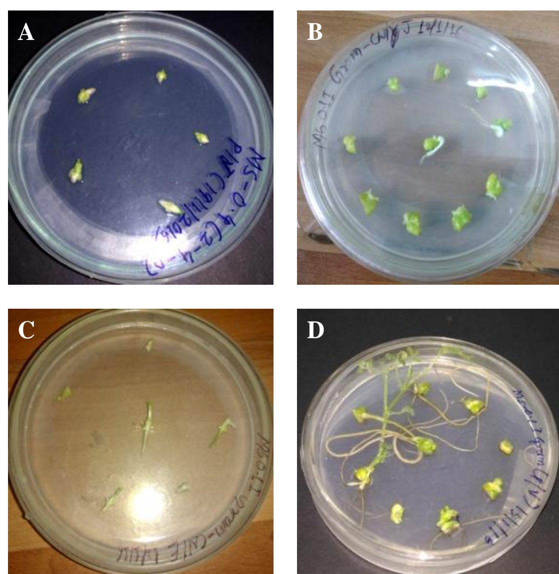
The shoot initiation from cotyladory node cultures varied from 20-80%. Among two accessions, maximum shoot initiation was observed in cultivar Vijay (Phule G-81-1-1) (80%) followed by Digvijay (Phule G-9425-5) (75.0%). In terms of the culture media response to *in vitro* culture, the performance of culture media MS3B (80.0%) followed by MS2B (75.0%) was found to be most responding for shoot initiation. In case of MS media without growth regulators

explants shows very less response to regeneration (30%) as compare to MS media fortified with growth regulators

The average mean roots formation from embryonic axis cultures explants varied from 10-80%. Maximum root formation was observed for cv. Vijay (Phule G-81-1-1) (80%) on MS0.3I media followed by Digvijay (Phule G-9425-5) (75%).

Here attempt has been made to develop a simple procedure to regenerate popular cultivars Chickpea 'Vijay (Phule G-81-1-1) and Digvijay (Phule G-9425-5)' with the use of BAP and IBA. All these different growth regulators were used for the various stages of the regeneration. This study revealed that Chickpea plants can be produced by using these growth regulators with its proper concentrations. These findings will helpful to perform the genetic transformation study in the Chickpea cultivars.

Plate I: (A - Gram Plant, B - Strerilization of chickpea seed, C - sterilized seed of chickpea, D - Explant ready for inoculation, E - Inoculated mature embryos and F - Inoculated mature cotyladory nodes)



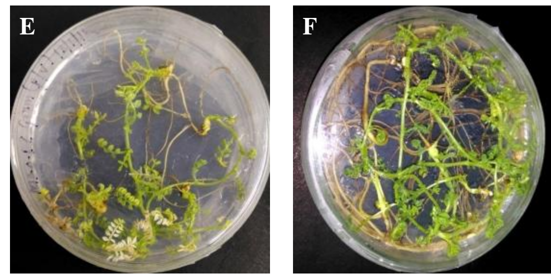


Plate II: (A - Callus initiation in embryonic axis, B - Callus Initiation in cotyladory node, C - Indirect shoot initiation in embryonic axis explants, D - Indirect shoot initiation in Cotyladory node explants, C - Indirect shoot and root development in embryonic axis explants, D - Indirect shoot and root development in cotyladory node explants)

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