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Studies on regeneration potential of callus in chickpea cv. ICCV - 2

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For callus induction studies explants i.e., shoot tips hypocotyls and roots were excised from six days aseptically grown seedlings. NAA 2mg/l +BAP 2mg/l was found best among various combinations used for induction of callus. For differentiation of callus BAP 1mg/l + NAA 0.5mg/l treatment combination was found to be the best that resulted in maximum number of shoot lets, for differentiation followed by profuse rooting to the regenerated shoots. For the completely developed plantlets, the maximum survival was observed with the hardening treatment where in the plants were directly transferred into polybags were kept in mist chamber.

Key words : Callus, Chickpea, BAP, NAA.

INTRODUCTION

Chickpea (*Cicer arielinum* L.) is one of the most important pulse crops of India by virtue of its average and production. It accounts for 33 per cent of area and 40 per cent of production. Interspecific hybridization has tremendous potential for breaking yield barriers and broadening genetic base of Chickpea (Sharma *et al.*, 1979). The problem of sexual incompatibility coning in the way of development of interspecific hybrids could be resolved by using tissue culture techniques and that is to go for synthesis of Somatic hybrids which is not difficult when there are well developed conditions for induction of callus and its differentiation to develop whole plants. So unless regeneration protocol in Chickpea becomes available, the fruit of innovative plant biotechnological approaches cannot be reaped.

Thus for media standardization for callus induction from a suitable explants the present investigation Callus induction in Chickpea (*Cicer arietinum* L.) was undertaken.

Conventional breeding methods seem inadequate in genetic improvement of chickpea due to narrow genetic base and sexual incompatibility existing between the relatives of Cicer species. Plant tissue culture techniques such as embryo rescue, protoplast fusion, etc could assist the convention breeding approaches made in the improvement of Chickpea. Also through tissue culture, useful variability could be created and exploited which would enrich the general and could open the mew avenues for improvement in Chickpea .

For efficient utilization of improvement in Chickpea techniques like embryo rescue, protoplast fusion, in vitro pollination & test tube fertilization in any crop, in vitro regeneration of that Species is a prerequisite.

MATERIALS AND METHODS

The experimental material comprised of one Chickpea cultivars ICCV-2 which is main commercial cultivars of Maharashtra state. The study was conducted in Tissue culture Laboratory, Department of Botany, Dr.PDKV, Akola.

Glasswares were first washed in sterile distilled water with soda water solution for two hours then they were rinsed with tap water and dipped in dilute nitric acid overnight. After drying they were sterilized. MS medial was prepared and various composition of MS & B5 medium were prepared alone with supplements of NAA, BAP, KIN. Inoculation was done *in vitro* conditions by growing the seedlings aseptically for induction of Callus. The effect of different treatment combinations on induction of fresh & dry weights of calli produced 25 days after culturing or explants. The calli obtained from shoot tip, hypocotyls & root explant were transferred on differentation media for regeneration. It was followed by rooting on and the plantlets were hardened ultimately.

RESULTS AND DISCUSSION

The calli obtained from shoot tip explant were transferred on differentiation media. B_5 and MS basal media supplemented with different levels of auxins and cytokinins in combination were used for regeneration of callus. After 12-15 days of culturing of calli in

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Effect of different treatments on regeneration of callus

Treatment (mg/l)	Shoot tip	Hypocotyl	Root
MS + 1BAP + 0.5 NAA	5.7 + 0.27	5.9 + 0.42	----------
MS + 4 BAP + 1.5 NAA	----------	2.6 + 0.09	----------
MS + 4 BAP + 2.0 NAA	1.9 + 0.07	----------	----------
MS + 1 KIN + 1.5 NAA	----------	----------	----------
MS + 4 KIN + 2.0 NAA	----------	----------	----------
B5 + 1 BAP + 0.5 NAA	6.9 + 0.39	7.3 +0.51	1.91 + 0.41
B5 + 4 BAP + 2.0 NAA	1.2 + 0.06	1.9 + 0.28	----------
B5 + 1 KIN + 0.5 NAA	1.7 + 0.02	----------	----------

differentiation media, regeneration was initiated. The effect of different treatments on regeneration of callus was recorded in terms of number of shoots produced from the callus 25 days after inoculation.

The data revealed that in chaffa, the maximum number of shoots produced were 6.9 shoots from shoot tip callus with B5 medium supplemented with the treatment BAP 1mg/l + NAA 0.5 mg/l followed by 5.7 shoots from, shoot tip callus with MS medium supplemented with BAP 1mg/l + NAA 0.5 mg/l. which was followed by 1.9 shoots obtained on MS medium supplemented with BAP 4mg/l + NAA 2.0 mg/l. It was followed by 1.7 shoots which were obtained on B_5 medium supplemented with KIN 1mg/l + NAA 0.5 mg/l. 1.5 shoots from shoot tip callus were obtained on MS medium supplemented with KIN 1mg/l+NAA 0.5 mg/l. The least number of multiple shoots i.e., 1.2 shoots were obtained on B_5 medium supplemented with BAP 4mg/l + NAA 2.0 mg/l.

When hypocotyls was used as explants highest number of multiple shoots i.e., 7.3 + 0.51 were obtained on B_5 medium supplemented with BAP 1mg/l + NAA 0.5 1mg/l. It was followed by 5.9 shoots obtained on MS medium supplemented with BAP 1mg/l + NAA 0.5 mg/l.

It was followed by 2.6 shoots obtained on MS medium supplemented with BAP 4mg/l + NAA 2.0 mg/l. The least number of multiple shoots *i.e.*,1.9 were obtained on B_5 medium supplemented with BAP 4mg/l + NAA 2.0 mg/l.

Root explants showed highest number of multiple shoots 1.91 on B_5 medium supplemented with BAP 1mg/

Effect of different treatments on regeneration of callus var. ICCV-2

Treatment (mg/l	Average number of multiple shoots		
	Explant		
	Shoot tip	Hypocotyl	Root
1 MS + 1BAP + 0.5 NAA	2.9 + 0.46	4.3 + 0.35	----------
2 MS + 2 BAP + 1.5 NAA	1.7 + 0.32	3.2 + 0.16	----------
3 MS + 4 BAP + 2.0 NAA	2.3 + 0.28	1.6 + 0.2	----------
4 MS + 1 KIN + 0.5 NAA	2.6 + 0.12	----------	----------
5 MS + 2 KIN + 1.5 NAA	----------	----------	----------
6 MS + 4 KIN + 2.0 NAA	----------	----------	----------
7 B5 + 1 BAP + 0.5 NAA	5.4 + 0.31	2.3 + 0.51	2.3 + 0.41
8 B5 + 2 BAP + 1.5 NAA	3.2 + 0.32	1.7 + 0.59	----------
9 B5 + 4 BAP + 2.0 NAA	1.6 + 0.02	1.2 + 0.38	----------
10 B5 + 1 KIN + 0.5 NAA	1.9 + 0.23	----------	----------
11 B5 + 2 KIN + 1.5 NAA	----------	----------	----------
12 B5 + 4 KIN + 2.0 NAA	----------	----------	----------

l + NAA 0.5 mg/l.

In ICCV-2 cultivars, the maximum number of shoots obtained were 5.4 from shoot tip callus in B_5 medium supplemented with the combination of BAP 1mg/l + NAA 0.5 mg/l, followed by 4.3 from hypocotyls callus in MS medium where in BAP 1mg/l + NAA 0.5mg/l combinations was supplemented to it.

When the regeneration potential of callus resulted from various explants was compared, it was observed that the shoot tip callus, the maximum number of shootlets obtained were 5.4 with the combination of BAP 1mg/l +6 NAA 0.5 mg/l, followed by 3.2 with the treatment where in B_5 medium was fortified with the combination of BAP 2mg/l + NAA 1.5 mg/l. In case of hypocotyls calli the maximum number of shootlets obtained were 4.3 followed by 3.2 recorded on MS medium fortified with the combination of BAP 1 mg/l + NAA 0.5 mg/l and BAP 2 mg/l + NAA 1.5 mg/l respectively. While the root calli failed to regenerate with all regeneration treatments tried, except on B_5 medium resulted in 2.3 multiple shoots.

It is indicated from the data that the hypocotyls and the shoot tip calli were embryogenic and responded with relatively better frequency to regeneration. The root callus was found to be non embryogenic and failed to regenerate. BAP 1mg/l + NAA 0.5 mg/l treatment combination was the best when added with either MS or B_5 basal media for induction of multiple shoots from calli.

The regenerated shoot lets responded to rooting with almost all the treatments, but the intensity of rooting was found relatively better with B_5 medium supplemented with NAA 1mg/l, NAA 2mg/l + 0.1 mg/l KIN. It was noticed that the roots developed in ICCV-2 cultivars were apparently healthy and longer.

In vitro produced plants in any crop system have to finally reach to the field where in vivo conditions are totally different with practically no control on any of the environmental parameters. This situation essentially necessitates acclimatization of plants obtained through *in vitro* cultivars.

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