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Effect of different explant and hormones on *in vitro* callus induction and regeneration of Pepper (C*apsicum annuum* L.)

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Chilli (*Capsicum annuum* L.) is an important horticultural crop widely used as fresh vegetable. Developments in plant cell, tissue and organ culture as well as on plant genetic transformation lagged far behind from the other members of the same family such as tobacco, tomato and potato which are frequently used as model systems because of their facility to regenerate organs. Callus was initiated on Murashige and Skoog (MS) medium containing different combinations of growth regulators. Callus derived from cotyledons was white and friable and showed excellent growth. Different media were tried to initiate callus in two varieties Sujamukhi and Bally. With the increase in auxin (NAA) concentration in the media, callusing response increased particularly at 0.1 and 0.5mg/L concentration of Kn and BAP. MS medium containing MS+2.0mg/LNAAor NAA+0.5mg/L Kn+5.0mg/L 2,4-D was the best for callus initiation. For shoot induction 0.5mg/L IAA+ 1.0mg/L BAP showed good response.

Key words: *Capsicum annuum*, Callus, Growth regulators, Vitamins

INTRODUCTION

Capsicum annuum L. commonly known as red chilli, chilli pepper, hot red pepper, tobasco, paprika, etc is an important horticultural crop used as fresh vegetable. Capsaicin (8-methyl-N-vanilly-6-noneamide), a pungent compound found only in *Capsicum*. In addition, the fruit contains coloring pigments, resin, protein, cellulose etc. *Capsicum* is the richest source of vit C which may be present up to 340mg/100g in some varieties (Purseglove et al 1981). Chilli production is badly affected by various pests diseases like phytopthora root rot, Verticillium wilt, Rhizoctonia root rot and Fusarium wilt (Leonian 1922, Skaggs et al 2000). Plant cell culture offers a promising approach for large scale production of disease free seedlings. The basic steps in tissue culture is to standardize the culture media, hormones and explants combinations to obtain first , undifferentiated mass of cells called "callus" and finally regeneration of complete plants from the induced calli.

Capsicum is a Solanaceous crop but tissue and organ culture as well as on plant genetic transformation lagged far behind from the other members of the same family such as tobacco, tomato and potato which are frequently used as model systems because of their amenability to generate organs.

Not many reports available on tissue culture studies on Indian chillies. In the present study, the effect of different hormones on in vitro callus induction and regeneration of Pepper (*Capsicum annuum* L.) in two popular Indian chilli varieties *viz.* Surjamukhi and Bally will be reported.

MATERIALS AND METHODS

Two popular *Capsicum annum* L. varieties, *viz.* Surjamukhi and Bally were used in this investigation. The seeds were washed thoroughly with 1% SDS for 10 min followed by thorough washing with water for 12 min. The seeds were surface sterilized with 0.1% mercuric chloride for 5 to 8 minutes, rinsed 3 to 4 times with autoclaved distilled water under aseptic conditions. Seeds were inoculated aseptically on MS basal media containing 0.8% agar but devoid of sucrose for germination. Within three to four weeks the well-developed seedlings were formed. Hypocotyls and cotyledons were exercised and utilized for callus induction studies.

Two different media *viz.* MS (Murashige and

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Skoog, 1962) and B5 (Gamborg *et al.*, 1968) supplemented with either alone or in combination of 2,4-dichlorophenoxyacetic acid (2,4-D), indole 3 acetic acid (IAA), naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP) and kinetin (Kn) were used for callus induction and regeneration studies. In the media 2% sucrose was used as carbon source. pH of the media was adjusted to 5.8. The media were sterilized at 1.06 kg/cm2 and 121 C for 20 minutes. To get good callus, all cultures were incubated at 25±2 C in dark for a period of four weeks.

Final weight and volume of callus were taken 40 days after inoculation. Callus weight was recorded as the difference between the weight of the flasks before inoculation and after 40 days of inoculation. Volume was calculated by measuring the length, width and height of the calli before sub culturing. Friable white and soft textured callus, formed at the cut ends of the explants, was maintained over the same fresh callusing medium, as was used for initiation, by regular subculturing at a 3 week interval.

The subcultured calli were incubated in shooting media at 27 C for four weeks, under alternating cycles of light and dark (16h/8h respectively). After formation of enough shoots calli were incubated in the rooting media at 27 C under dark for four weeks. Response of cultures in different rooting media was measured four weeks after subculturing. Response of cultures were investigated by measuring the numbers and length of regenerated roots and shoots. Experiment was carried out in complete randomized block design (CRBD) with three replications. In each replication data was taken an average of ten observations.

RESULTS AND DISCUSSION

Callus induction:

Callusing percentage in different media is presented in Table 1 and Fig.1. Out of sixty-three combinations of media twenty combinations (Table 1) were showing callus. Data suggested that hypocotyls explants exhibited

Table 1: Callus induction 30 days after inoculation

Media composition Hormone conc. in mg/L	Callusing Percentage (%)			
	Surjamukhi		Bally	
	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon
MS+0.5 NAA+0.5Kn	10	-	-	-
MS+1.0 NAA+0.01Kn	10	10	-	-
MS+1.0 NAA+0.1Kn	10	-	10	-
MS+2.5 NAA+0.1Kn	30	10	10	-
MS+2.5 NAA+0.5KNn	20	-	10	-
MS+2.5 NAA+1.0Kn	10	-	-	-
MS+5.0 NAA+0.01 Kn	10	-	-	-
MS+5.0 NAA+0.1 Kn	70	50	60	20
MS+5.0 NAA+0.5Kn	80	20	70	20
MS+5.0 NAA+1.0 Kn	30	-	10	-
MS+7.5 NAA+0.1 Kn	40	-	-	10
MS+7.5 NAA+0.5 Kn	30	20	20	10
MS+7.5 NAA+1.0 Kn	10	-	-	-
MS+1.0 NAA+0.1 BAP	30	10	30	20
MS+1.0 NAA+0.5 BAP	20	-	10	10
MS+5.0 NAA+0.1 BAP	90	60	90	50
MS+5.0 NAA+0.5 BAP	80	50	80	20
MS+2.0IAA+0.5Kn+5.02,4-D	80	50	80	50
MS+2.0NAA+0.5Kn+5.02,4-D	100	60	90	60
B5+2.0NAA+0.5Kn+5.02,4-D	80	50	80	50

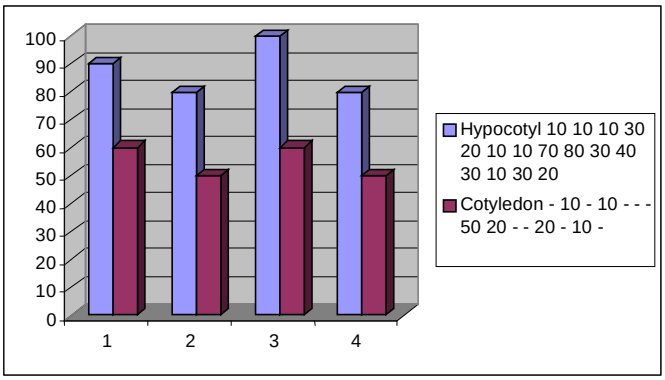

Fig: Compative callusing response in Surjamukhi using hypocotyls and cotyledon as an explant

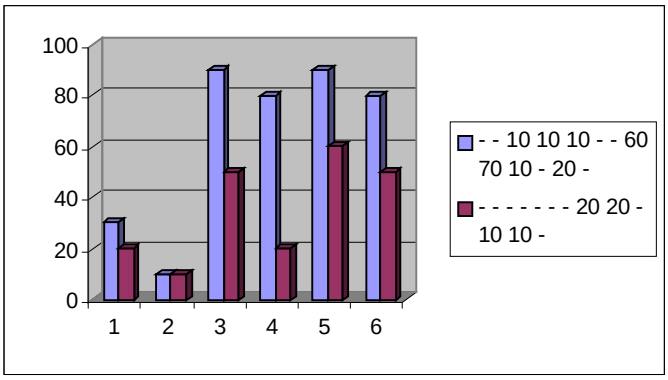

Fig: Compative callusing response in Bally using hypocotyls and cotyledon as an explant

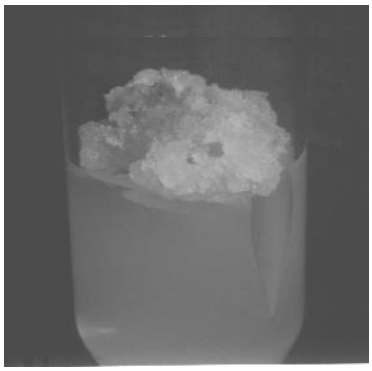

Plate 1

Plate 2

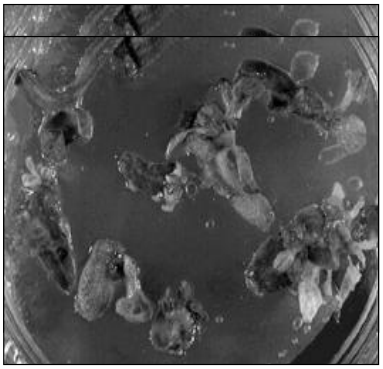

Plate3

Plate 1 : Callus induction in Surjamukhi variety in MS medium containing 2.0mg/L NAA+0.5mg/L Kn+5.0mg/L 2,4-D
Plate 2 : Callus induction in Bally variety in MS medium containing 2.0mg/L NAA+0.5mg/L Kn+5.0mg/L 2,4-D
Plate 3 : Shoot induction in Surjamukhi variety

significantly better callusing response than the other parts of the fruits. In case of hypocotyls callusing response was 76.5% while that for cotyledon 36%. This contradicts the observation of Gupta *et al*. (1990) and Shen *et al*. (1994) who reported better response in cotyledonary explants. With the increase in auxin (NAA) concentration in the media, callusing response increased particularly at 0.1 and 0.5mg/L concentration of Kn and BAP. But response diminished when NAA concentration was increased above 5.0mg/L. Similar response was observed with Kinetin and BAP when hormone concentration was below 0.1mg/L and above 0.5mg/L. Best response was recorded with MS+2.0mg/L NAA+0.5mg/L Kn+5.0mg/L 2,4-D (Plate1 and 2). No callusing was recorded in any medium containing basal medium B5 except in one case (Table1), where media composition was 2.0mg/LNAA+0.5mg/LKn+5.0mg/L2,4-D. This contradicts the report of Gupta *et al*. (1990). In all cases substantial callus was formed after

Table 2 : Average response of two varieties in shooting midium

Media composition	Shoot number	Shoot length
MS + 0.1 IAA + 0.5 BAP	0.8	5.6 ± 0.7
MS + 0.O1 IAA + 1.0 BAP	1.2	2.7 ± 1.3
MS + 0.05 IAA + 1.0 BAP	2.4	2.9 ± 0.7
MS + 0.1 IAA + 1.0 BAP	3.2	2.1 ± 0.5
MS + 0.5 IAA + 1.0 BAP	5.6	1.7 ± 1.1

Table 3 : Average response of two varieties in rooting medium

Media composition	Root number	Root length
MS + 2.0 IAA	4.6	2.760±0.580
MS + 2.0 NAA	6.4	3.88 ± 0.670
MS + 5.0 NAA + 0.1Kn	15.6	2.680 ± 0.551
MS + 5.0 NAA + 0.5Kn	10.4	4.88 ± 1.12
MS + 5.0 NAA + 0.5BAP	7.2	7.4 ± 1.34

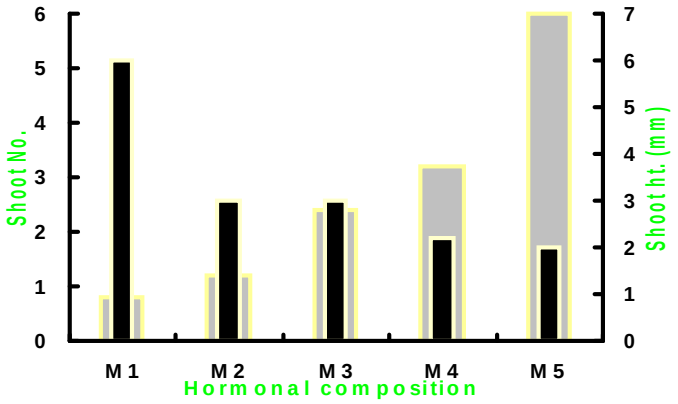

Fig 2: Response of cultures in different shooting media

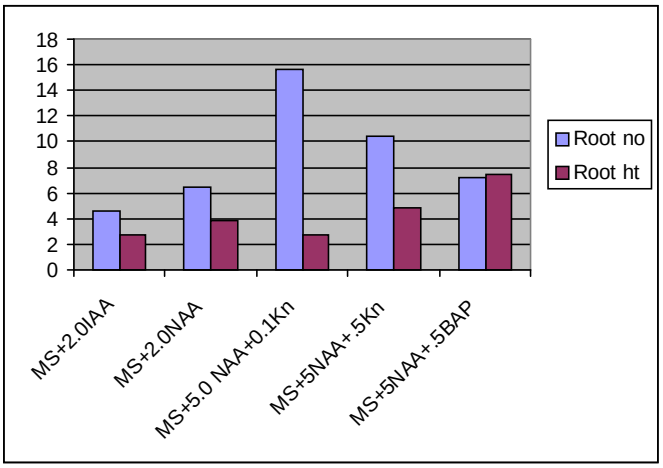

Fig 3: Response of cultures in different rooting media

30days. The callus mass was friable for Surjamukhi but for Bally the callus was solid. All of them were yellowish white in colour.

Induction of Shoots

Data recorded in shooting media 30 days after subculture. Maximum shooting was recorded in MS media supplemented with 0.5mg/L IAA+ 1.0mg/L BAP (Table2 and Fig 2). Highest length of shoots were recorded in media of MS+ 0.1mg/L IAA+ 0.5mg/L BAP (Plate 3). Induction of shooting number was negatively correlated with shoot length.

Induction of Roots

After shoot formation calli were transferred into rooting media. Maximum multiple rooting was recorded in MS+ 5.0mg/L NAA+0.1mg/L Kn (Table3 and Fig 3). Number of roots and length of the roots were negatively correlated.

Callus initiation involves three major criteria like-selection of explants, medium and culture condition. For commercial purposes it is recommended to culture white, well grown, friable callus. The best medium for callus formation in two varieties was MS medium supplemented with MS+2.0NAA+0.5Kn+5.02,4-D. MS media supplemented with 0.5mg/L IAA+ 1.0mg/L BAP proved to be efficient for shoot induction and MS+ 5.0mg/L NAA+0.1mg/L Kn showed good response for root induction.

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