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In vitro clonal propogation of *Capsicum annum* L. var. (*Arka lohit* and *Arka suphal*) for fruit borer disease resistance

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ABSTRACT

An efficient protocol of *in vitro* clonal propagation has been developed from epicotyls, hypocotyls, cotyledons, leaf disc of *Capsicum annum. L.var. (Arkalohit and Arka suphal).*Different types of explants and developmental stages sub cultured in different media combinations were studied. Explants cultured on Murashige and Skooge (MS) medium supplemented with silver nitrate ($AgNO_3$), Benzyl Amino Purine (BAP) and 2,4-Dichlorophenoxy acetic acid (2,4-D) combination. Shoot regeneration recorded best with 4mg/l $AgNO_3$ and 5mg/l BAP and 2mg/l 2, 4-D combination. The best rooting media was recorded as MS media supplemented with 1.0mg/l NAA and 5.0mg/l IBA.

Key words: Capsicum , Clonal propogation , Explants , Epicotyls, Hypocotyls, Cotyledons, Leafdisc , BAP, IAA, Silver Nitrate.

INTRODUCTION

Chilli(*Capsicum annum)* is an important cash crop, cultivated as a vegetable and used around the whole world as an indispensable ingredient of the cuisine belonging to the family Solanaceae. It grows in tropical, subtropical as well as temperature regions. It is rich in vitamins A, C, and E also it is used as a coloring agent and in other innovative ways of industrial application. The alkaloid capsaicin is an important ingredient of the pharmaceutical industry. The demand for chilli boosts the production of high yielding and disease resistant varieties in India and the world market. To meet the increasing demand for these crops *In vitro* tissue culture followed by gene transfer would be an efficient and economic method to obtain large number of disease free plants within short span of time. Although excellent progress has been made in obtaining transgenic variety plant from solanaceae family, chilli has lagged behind due to its recalcitrant nature[1].

The genus capsicum is recalcitrant in its regeneration potential which becomes difficult to apply genetic transformation techniques. Therefore in the present study, an attempt has been made to develop efficient regeneration protocol by using epicotyls, hypocotyls, leaf disc and shoot tips using different growth hormones for the fruit borer disease resistance[2].

MATERIAL AND METHODS

Culture media and conditions

Explants epicotyls, hypocotyls, cotyledons, leaf disc were transferred to shoot inducing media containing MS salts, [3] B5 vitamins[4] and 3% Sucrose supplemented with different growth regulators. Explants were inoculated on tissue culture bottles with different combination of growth regulators. The explants were transferred and sub cultured to fresh medium every three weeks and regenerated shoots were transferred to rooting media supplemented with NAA and IBA.

Surface sterilization of explants

Certified Seeds of *Capsicum annum.L.var.Arka lohit and Arka suphal* were obtained from Indian institute of horticulture (IIHR, ICAR Constituent Laboratory) Bangalore. Seeds are washed and soaked in tap water for 45 minutes and are allowed to germinate in an autoclaved manure of coco pith for 15 days. The explants hypocotyls, epicotyls and cotyledons of Chilli were obtained from the plant after 15 days and are surface sterilized using the following procedure[5].

Washed the explants with tap water 3-4 times.

↓

Washed the explants with double distilled water twice.

↓

Washed the explants with Tween 20 and Sodium hypochlorite for 4 minutes.

↓

Washed the explants with autoclaved double distilled water 4 times.

↓

Washed the explants with 70%alcohol for exactly 30 seconds

↓

Immediately washed the explants with $HgCl_2$ for exactly 2and a half minutes.

↓

Washed the explants with double distilled water 4 times to remove traces of ethanol And $HgCl_2$.

Shoot bud induction

Explants from 10-12 days were excised and surface sterilized with Tween 20 and Sodium Hypochlorite leaves, cotyledons were removed from hypocotyls and epicotyls , nodal culture , leaf disc is done and inoculated in regeneration media which is a combination of basic MS media supplemented with growth hormones in different combinations.[6] Out of the 20 different combinations of Auxin, Cytokinen used in this study for the regeneration, the best regeneration was achieved in the following 3 combinations. Again out of this 3 different combinations the first one was recorded the maximum regeneration percentage (Table 1,2).

- Silver nitrate ($AgNO_3$) 5.0mg/l Benzyl Amino Purine (BAP) 5.0mg/l and 2,4 Dichloro phenoxy acetic acid (2,4-D) 2.0mg/l were added to the basic MS media .hypocotyls, epicotyls , leaf disc preparation from young leaves, matured leaves and cotyledons were inoculated and best results of elongated shooting were obtained in 5 weeks in all explants(Figure 1,2,3,4).
- a-Napathalene Acetic Acid (NAA) 0.1mg/l and Benzyl Amino Purine 5.0mg/l (BAP) were added to the basic MS media. Hypocotyls, epicotyls, leaf disc preparation from young leaves, matured leaves and cotyledons were inoculated and we got a rosette of shoots from hypocotyls and epicotyls which did not elongate in 5 weeks.
- Indole-3-Acetic Acid 0.2mg/l (IAA) and

Table 1: Responding Regeneration Medium for *Capsicum annum (ARKA LOHIT*). 6 Combination of Medium Responded well, and A8 Media Responded Maximum Growth

Medium Number	Combination	ARKA LOHIT	8 Days Obser-vation	15 Days Observ-vation	25 Days Obser-vation	35 Days Obser-vation	45 Days Obser-vation
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Hypocotyl	0.4 cm	1.2 cm	2.7 cm	3.2 cm	3.8 cm
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Epicotyl	0.4 cm	2.1 cm	2.7 cm	4.2 cm	5.2 cm
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Cotyledon	0.4cm	0.2mm callus	0.3mmcal lus	0.4cm	1.0cm
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Leaf Disc	0.4cm	0.1mm	0.2mm	0.3mm	0.5cm
A7	0.1mg/l NAA + 5.0mg/l BAP	Hypocotyl	0.4 cm	0.9 cm	0.9 cm	0.0 cm	0.0 cm
A7	0.1 mg/l NAA + 5.0mg/l BAP	Epicotyl	0.4 cm	1.0 cm	1.0 cm	1.0 cm	1.0 cm
A7	0.1 mg/l NAA + 5.0mg/l BAP	Cotyledon	0.4 cm	0.0mm	0.1mm	0.1mm	0.0mm
A7	0.1 mg/l NAA + 5.0mg/l BAP	Leaf Disc	0.4 cm	0.0mm	0.1mm	0.1mm	0.0mm
A6	0.2 mg/l IAA + 5.0 mg/l BAP	Hypocotyl	0.5 cm	0.7 cm	0.7 cm	0.7 cm	0.7 cm
A6	0.2 mg/l IAA + 5.0 mg/lBAP	Epicotyl	0.6 cm	0.6 cm	0.6cm	0.6 cm	0.6 cm
A6	0.2 mg/l IAA + 5.0 mg/lBAP	Cotyledon	0.4 cm	0.1mm	0.2mm	0.4cm	0.6cm
A6	0.2 mg/l IAA + 5.0 mg/lBAP	Leaf Disc	0.4 cm	0.1mm	0.2mm	0.4cm	0.6cm
D6	0.2 mg/l IAA + 4.5 mg/l BAP	Hypocotyl	0.2 cm	0.2cm	0.0 cm	0.0 cm	0.0 cm
B6	0.2 mg/l IAA + 3.0mg/l BAP	Epicotyl	0.4 cm	0.4 cm	0.0 cm	0.0 cm	0.0 cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Hypocotyl	0.4 cm	0.4 cm	0.4 cm	0.5 cm	0.5 cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Epicotyl	0.4 cm	0.5 cm	0.5 cm	0.5 cm	0.5 cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Cotyledon	0.4 cm	0.0 cm	0.0cm	0.0cm	0.0cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Leaf Disc	0.4 cm	0.0 cm	0.0 cm	0.0cm	0.0cm

Table 2: Responding Regeneration Medium for Capsicum annum (ARKA SUPHAL). 6 Combination of Medium Responded well and A8 Media Responded Maximum Growth

Medium Number	Combination	ARKA SUPHAL	8 Days Observation	15 Days Observation	25 Days Observation	35 Days Observation	45 Days Observation
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Hypocotyl	0.5 cm	1.4 cm	2.9 cm	3.2 cm	3.8 cm
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Epicotyl	0.5 cm	2.5 cm	2.9 cm	4.3 cm	5.5 cm
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Cotyledon	0.6cm	0.15m mcallus	0.35mmc allus	0.6cm	1.4cm
A8	3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D	Leaf Disc	0.6cm	0.5mm	0.7mm	0.5mm	0.7cm
A7	0.1mg/l NAA + 5.0mg/l BAP	Hypocotyl	0.6 cm	1.0 cm	1.0 cm	0.0 cm	0.0 cm
A7	0.1 mg/l NAA + 5.0mg/l BAP	Epicotyl	0.6 cm	1.3 cm	1.5 cm	1.4 cm	1.4 cm
A7	0.1 mg/l NAA + 5.0mg/l BAP	Cotyledon	0.48 cm	0.0mm	0.14mm	0.12mm	0.0mm
A7	0.1 mg/l NAA + 5.0mg/l BAP	Leaf Disc	0.44 cm	0.0mm	0.19mm	0.1mm	0.0mm
A6	0.2 mg/l IAA + 5.0 mg/l BAP	Hypocotyl	0.7 cm	0.6 cm	0.8 cm	0.9 cm	0.9 cm
A6	0.2 mg/l IAA + 5.0 mg/lBAP	Epicotyl	0.9 cm	0.8 cm	0.7cm	0.8 cm	0.9 cm
A6	0.2 mg/l IAA + 5.0 mg/lBAP	Cotyledon	0.7 cm	0.5mm	0.6mm	0.5cm	0.8cm
A6	0.2 mg/l IAA + 5.0 mg/lBAP	Leaf Disc	0.6 cm	0.7mm	0.5mm	0.6cm	0.7cm
D6	0.2 mg/l IAA + 4.5 mg/l BAP	Hypocotyl	0.4 cm	0.3cm	0.0 cm	0.0 cm	0.0 cm
B6	0.2 mg/l IAA + 3.0mg/l BAP	Epicotyl	0.5 cm	0.6 cm	0.0 cm	0.0 cm	0.0 cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Hypocotyl	0.5 cm	0.5 cm	0.6 cm	0.5 cm	0.5 cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Epicotyl	0.6 cm	0.6 cm	0.7 cm	0.7 cm	0.8 cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Cotyledon	0.6 cm	0.0 cm	0.0cm	0.0cm	0.0cm
A1	0.1mg/l IAA + 5.0mg/l BAP	Leaf Disc	0.6 cm	0.0 cm	0.0 cm	0.0cm	0.0cm

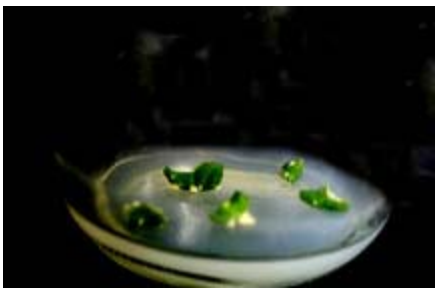
a) 15 day's old regeneration

b) 25 day's old regeneration

c) 35 day's old regeneration

d) 40 day's old regeneration

Fig. 1 : Cotyledon Explants regeneration media 3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D



a) 15 day's old regeneration

b) 25 day's old regeneration

c) 35 day's old regeneration

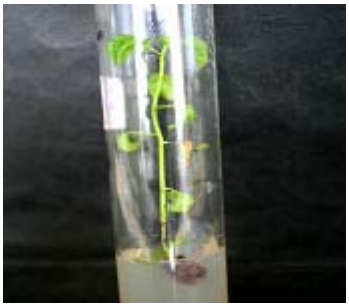
d) 40 day's old regeneration

Fig. 2: Chilli Leaf Disc as explants on regeneration medium 3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D

a) 15 day's old regeneration

b) 25 day's old regeneration

c) 35 day's old regeneration



d) 40 day's old regeneration

Fig. 3: Chilli Epiocotyl as explants on regeneration medium 3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D

a) 15 day's old regeneration

b) 25 day's old regeneration

c) 35 day's old regeneration

d) 40 day's old regeneration

Fig. 3: Chilli Hypocotyl as explants on regeneration medium 3.0mg/l$AgNO_3$ +5.0mg/l BAP +2.0mg/l 2,4D

Benzyl Amino Purine 5.0mg/l (BAP) were added to the basic MS media. Hypocotyls, epicotyls, leaf disc preparation from young leaves, mature leaves and cotyledons were inoculated and cotyledons and young leaf disc emerges to callus and from that shooting started in about 9 weeks

Standardization of Rooting Media for *Capsicum Species* Regeneration

Induced Shoots were placed in MS media supplemented with different combinations of NAA and IBA. Out of 5 different combination of rooting media the following two combinations were identified as suitable regeneration media of roots in *Capsicum Species*.[7]

- a-Napthalene Acetic acid 0.2mg/l (NAA) and Indole ButyricAacid 0.1 mg/l (IBA) were added to the basic MS media and better results were obtained in about 4weeks.
- a-Napthalene acetic acid 2.0mg/l (NAA) and Indole Butyric Acid 5.0mg/l (IBA) were added to the basic MS media and visible roots started appearing on 2 week and more roots were seen by the end of 4th week.

RESULTS AND DISCUSSION

Regeneration of shoots can be obtained in 2 Different ways direct regeneration of shoots, shoot regeneration via callus. Callus from explants is an important step for successful plant regeneration. Cotyledenary and leaf disc explants induced callus prior to shoot regeneration, While hypocotyls and epicotyls developed shoots directly. Explants were cultured on MS Medium supplemented with different combinations of hormones and other growth regulators. After 4 weeks from the formation of callus, calli are transferred to shoot regeneration media, after 2-3 weeks shoots emerged from callus. Shoots elongate within 3 weeks. In direct regeneration hypocotyls and epicotyls inoculated started responding in 8 days. Regeneration medium for all the 4 explants were tested on different combination of hormones. regeneration of chilli resulted best with MS medium supplemented with 5.0mg/l BAP+3.0mg/l $AgNO_3$ +2.0mg/l 2,4-D.[8] This result comply with the report that silver nitrate promotes shoot development and plant regeneration of chili pepper.[9, 10] From the different medium combinations tested with chilli regeneration of the following 6 media respondent very well. Out of the 6 media the A8 media recorded good growth, Biomass, Calli characters.

CONCLUSION

Based on the result of our present study we have concluded that the MS medium supplemented with 3.0 mg/ l $AgNO_3$ + 5.0 mg / l BAP+2.0 mg / l 2,4-D and the chilli cotyledon explants through indirect somatic embryogenesis is an ideal one for the fruit borer disease resistance in Chilli crops. chilli epicotyl explants through direct somatic embryogenesis is an ideal one for the fruit borer disease resistance in Chilli crops.

Note

In table 1 and 2 we have given the measurement for cotyledon and leaf disc in millimeter which represents the diameter of the calli.

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