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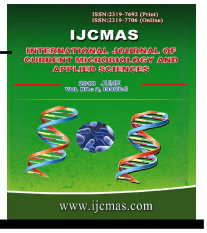

Original Research Article

In vitro multiple shoot induction from nodal explants of *Capsicum annum* L. of kandhari variety

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A B S T R A C T

An effective micropropagation system was developed through an enriched culture system for *Capsicum annum* L. of Kandhari variety, a traditional medicinal plant and nutritionally important spice crop. Proliferation of shoots were achieved on MS medium supplemented with 15 g l^{-1} sucrose, 8 g l^{-1} agar and fortified with benzyladenine (BAP)in the rage of 0.1 -1mg/l. A higher number of shoots were observed on an enriched MS medium supplemented with 0.5mg/l BAP. Then the shoots were subjected for root induction with the concentration of 1mg/ml. For the multiple shoot induction different combination of harmones were used. The well developed microshoots were transferred to rooting medium supplemented with IAA(1.0mg/l) for efficient rooting. The well developed shoots were transferred for hardening and the efficiency was 75% survival. This system can be successfully applied for mass propagation of Kandhari variety of *capsicum annum*.

Keywords

Multiplication; BAP; MS medium; rooting; accli-matization.

Introduction

Chillies are the dried ripe fruits of *Capsicum annum* L. Comes under the family of *Solanaceae* which has about 90 genera and 2000 species (Clark, 1997). This family includes tobacco a commercial and cash crop and important vegetables like tomato, potato, brinjal etc. Chillies are widely cultivated mainly in tropic and subtropic countries like India, China, Africa and Japan. The centre of origin of chilli is said to be Mexico, Guatemala and Bulgaria. Chilli is most famous for its pleasant aromatic flavour, pungency and high colouring material. Among the spices consumed per head in india, dried chillies both as a condiment or culinary supplement and as a vegetable.

Chilli (kandhari variety) is an important crop in South India and is grown for its pungent fruits, which are used both green and ripe (the latter in the dried form) to impart miniature pungency to the food (Lee *et al*., 2004) . The miniature fruits are widely used for its specific pungency and its significant medicinal value. It is also used medicinally, and in chutnies and pickles. The pungency is due to the higher accumulation of active principle `capsicin' contained in the skin and the septa of the

fruit. Tissue culture is an important technique for the rapid multiplications and for the crop improvement of medicinal and aromatic and horticulturally important crops.

The members of *Capsicum annuum* have been commonly used to various cultures to treat a wide range of afflictions, including bronchitis, arthritis, diabetes, fatigue, and sore throats. They have also been used to relieve the symptoms of migraines, colds, psoriasis, and kidney disorders

In vitro propagation

Plant cell and tissue culture has become a major tool in the study of an increasing number of fundamental and applied programs in plant science(Rolando et al., 2010) .Tissue culture techniques are being used globally for the exits conservation of plants (Kale, 2005) . In the present study aims the conservation and rapid multiplication of miniature chilli variety for its utilization.

Materials and Methods

The plants for this study Kandhari variety of Chilli were collected from Chilli cultivated region of Erode district of Tamilnadu. A clonal stock were maintained in the experimental garden of K.S.Rangasamy College of Technology, Trichengode. The *in vitro* seed germination was achieved on MS basal media. Previously the media was sterilized at 121ºC with 15 psi for 15 minutes. The cotton bed method was used with water and MS media for *in vitro* regeneration of of plantlets from the collected seeds of Kandhari variety. All the materials used in culture work must be free from microbes. This is achieved by one of the following approaches flame sterilization, wiping with 70% ethanol and other surface sterilants.

The Shoot tip and nodal region of in vitro derived plantlets were collected for the induction of multiple shooting. The surface sterilised seeds were transferred in Murashige and Skoog (MS) medium supplemented with 0.8% agar, 3% Sucrose and 1% myoinositol. The pH of the medium was adjusted to 5.7 and the maximum growth of plantlets was observed and it was sequentially subcultured in the media containing varying concentrations of BAP, IAA and NAA. Then subculturing process was carried out with the shoot tip and the nodal part of the plants. Then it was optimized with the maximum shootlets formed from the subcultured excised shoot tips. The maximum shoot length and internodes were determined.

Result and Discussion

Seeds which shown for *in vitro* regeneration were responded successfully. At the 15^{th} day seeds were germinated and by the 20^{th} day the maximum rate of growth were achieved. The plants which are grown in *in vitro* were utilized for *In vitro* cultivation studies. (Fari *et al*., 1990).The high frequency of germination was recorded on MS basal media, similar results was achieved in *in vitro* regeneration of chilli by Zhao *et al*., (2003).

In *capsicum* both apical and all nodal segment were used as primary explants, nodal explant is the most commonly used explant for producing micro shoots (Gunay *et al*., 1978). In present study high frequency of multiple shoot induction was achieved on auxillary bud explants. Media supplemented with 0.5mg/l BAP and 1.5mg/l of KIN shows high frequency

Table.1 Effect of BAP on multiplication of shoots per shoot tip cultured on MS media

Harmone BAP(mg/l)	Total no of explants	No.of explants responded	Percentage of responding cultures	M±SD
0.1	50	24	48%	2.16±1.158
0.2	50	35	70%	2.40±1.130
0.3	50	41	82%	3.31±2.198
0.4	50	38	76%	2.26±1.114
0.5	50	48	96%	4.48±3.292
0.6	50	34	68%	2.98±2.341
0.7	50	29	58%	1.46±1.198
0.8	50	34	68%	2.83±2.106
0.9	50	40	80%	3.09±2.347
1.0	50	32	64%	2.67±2.159

Figure.1 Effect of different concentration of BAP on multiplication of shoots

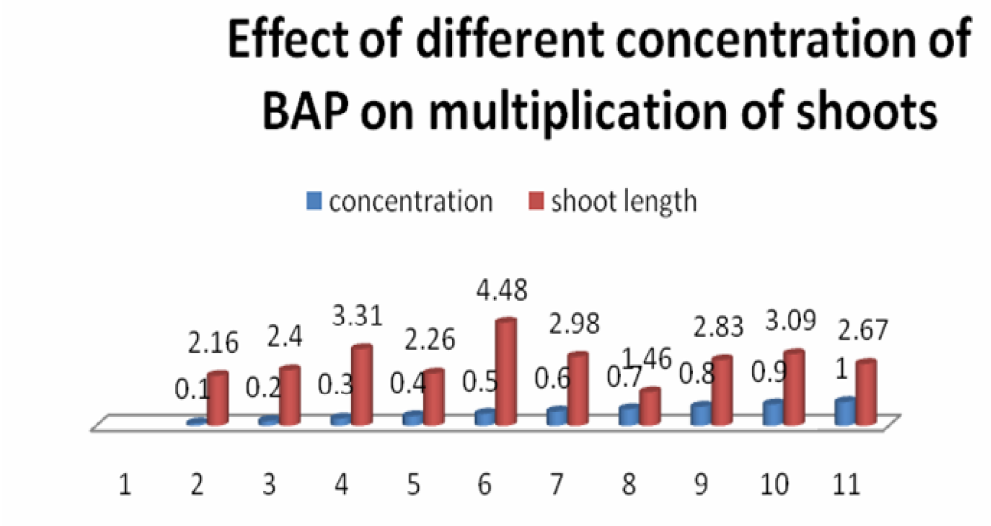


Table.2 Effect of BAP and NAA on multiplication of shoots per shoot tip as explants

Concentrtion of harmone (mg/l)		Total no of explants	No.of explants responded	Percentage of responding cultures	M±SD
BAP	NAA				
0.5	0.5	50	32	64%	3.27±2.742
0.5	1.0	50	38	76%	3.16±2.483
0.5	1.5	50	44	88%	4.45±3.946
0.5	2.0	50	23	46%	2.54±2.06
0.5	2.5	50	18	36%	1.78±1.53

Table.3 Effect of BAP and KIN on multiplication of shoots per shoot tip as explants

Harmone (mg/l)		Total no of explants	No.of explants responded	Percentage of cultures responding	M±SD
BAP	KIN				
0.5	0.5	50	37	74%	3.48±2.651
0.5	1.0	50	24	48%	3.14±2.63
0.5	1.5	50	42	84%	4.66±3.254
0.5	2.0	50	20	40%	2.87±2.16
0.5	2.5	50	15	30%	2.38±1.59

Figure.2 Effect of BAP and KIN on multiplication of shoots per shoot tip as explant

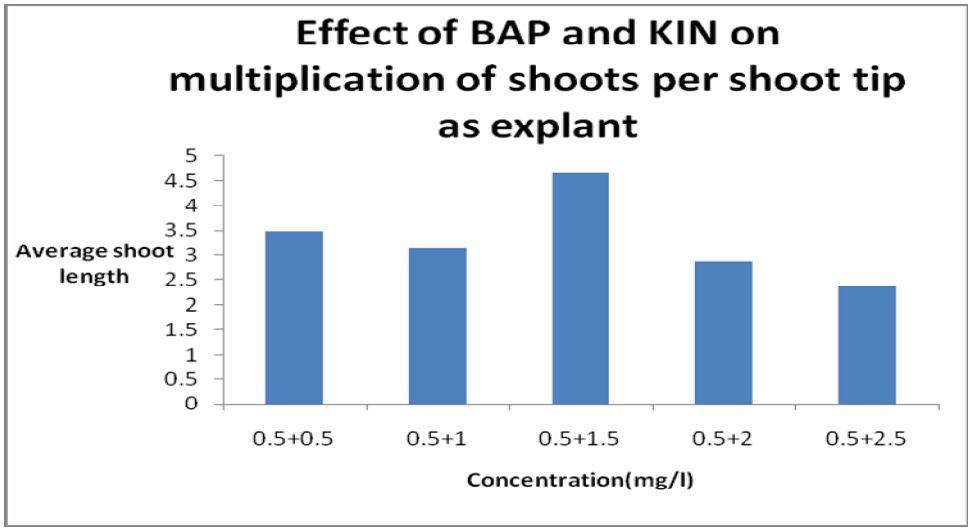


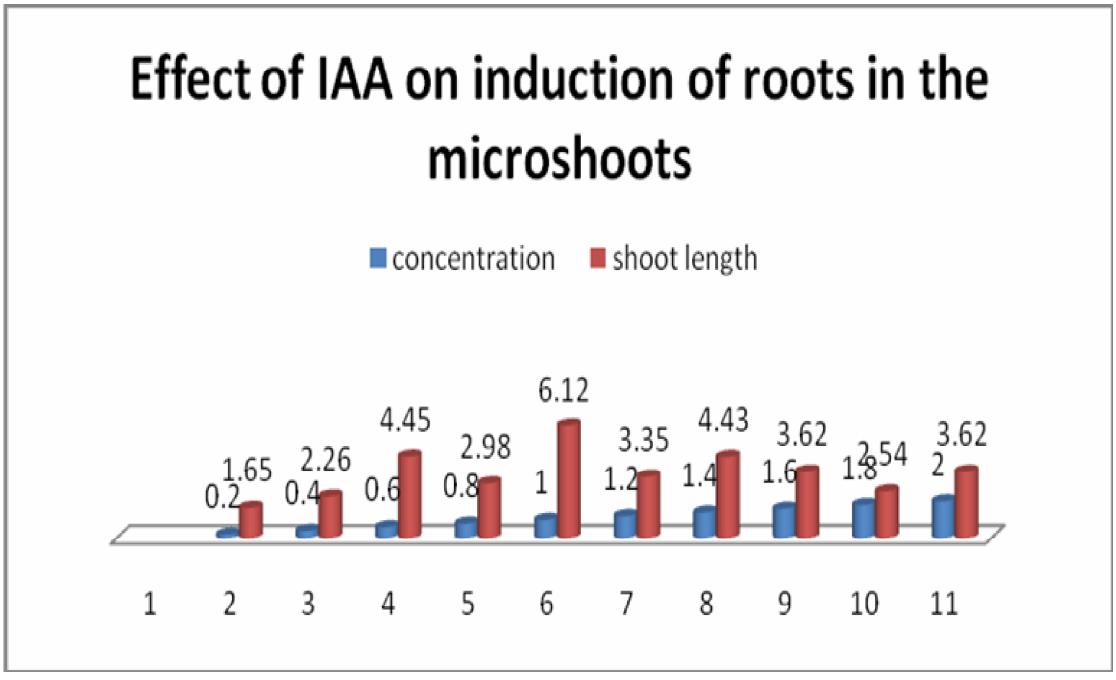
Table.4 Effect of BAP and IBA on multiplication of shoot per shoot tip as explants

Concentration of harmone (mg/l)		Total no of explants	No.of explants responded	Percentage of responding cultures	M±SD
BAP	IBA				
0.5	0.5	50	25	50%	3.83±2.492
0.5	1.0	50	18	36%	3.36±2.571
0.5	1.5	50	44	88%	4.57±3.635
0.5	2.0	50	27	54%	3.68±2.051
0.5	2.5	50	37	74%	3.24±2.856

Table.5 Effect of IAA on root induction per shoot tip cultured on MS medium

Harmone IAA(mg/l)	Total no of explants	No.of explants responded	Percentage of responding cultures	M±SD
0.2	50	18	36%	1.65±0.986
0.4	50	23	46%	2.26±2.187
0.6	50	36	72%	4.45±3.685
0.8	50	22	44%	2.98±2.602
1.0	50	45	90%	6.12±5.062
1.2	50	21	42%	3.35±2.517
1.4	50	29	58%	4.43±3.253
1.6	50	18	36%	3.62±2.163
1.8	50	14	28%	2.54±1.967
2.0	50	27	54%	3.62±2.385

Figure.3 Effect of IAA on induction of roots in the microshoots



(84%) of multiple shoot induction (Table-3). These results were in accordance with (Liu *et al.*,1990).

Acclimatization

The well rooted developed plantlets were transferred to the potting mixture in the ratio of sand:soil:farmyard manure on 1:1:1ratio.The 70% of germination percentage was recorded.

In the present study an efficient and reproducible protocol for standardization for the multiplication of kandhari variety of chilli were cultivated and formulated comparatively efficient and reproducible direct shoot induction system has been worked out utilizing nodal and apical bud explants. Root induction was performed to increase the efficiency of kandhari variety. The study revealed that apical buds and nodal segments per explants were achieved in *Capsicum annum* L. on MS medium supplemented with BAP (0.5mg/l) and IAA(1.0 mg/l).The *in vitro* grown microshoots were successfully developed and it was transferred to potting mixture in the ratio of sand: soil: farmyard manure

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