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Low Cost in Vitro Multiplication of Capsicum Annuum

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Abstract*: *Due to high cost of plant tissue culture technology, Attempts have been made in the present study to explore the possibility of cost reduction in micropropagation of capsicum by input of low cost components. Media having cheaper input sugar instead of sucrose and tap water instead of distilled water was found to be the best for in vitro seed germination as well as multiplication of Capsicum annuum L. var. solan bharpur. The maximum percent germination was comparable where as maximum average shoot number and leaves shoot was better on cost effective medium than on standard MS medium. Thus by reducing the cost of in vitro raised plantlets, make the micropropagation of capsicum annuum cv. solan bharpur a viable proposition for commercialization of this variety.

Keywords: Capsicum, cost reduction, micropropagation, in vitro seed germination, sugar and tap water

I. INTRODUCTION

Bell pepper (Capsicum annuum L. var. solan bharpur.) popularly known as Shimla mirch. It belongs to family Solanaceae. It is an important vegetable crop grown worldwide for its delicate taste, pleasant flavor and colour. It is used as salad, cooked as vegetable, pickled or processed. Its fruits contain appreciable quantities of ascorbic acid, β-carotene and other carotenoid pigments such as lycopene and zeaxan thin which are beneficial for prevention of cancer and cardiovascular human diseases [1].Capsicum is an excellent source of vitamins A, B, Cand E and also rich in minerals like molybdenum, potassium, manganese, sulphur and thiamine[2]. β Carotenoids and vitamins C and A are powerful antioxidants that destroy free radicals[3],[4]**.**Seeds are commonly utilized for multiplication and production in the conventional plant breeding systems. In order to meet the increasing demands of crop and to improve the methods of propagation of capsicum, in vitro propagation methods provide a better way for the asexual multiplication of pepper plants as compared to seeds. The propagation through seeds is further restricted by short span of viability, low germination rate, high risk of infection by various diseases. Though the advantages of micropropagation are tremendous, cost is a limiting factor. To reduce the cost of plant tissue culture technology, Attempts have been made in the present investigation to explore the possibility of cost reduction in mass multiplication of capsicum by input of cheaper medium components.

II. MATERIALS AND METHODS

Source of material**:** The seeds of Capsicum annuum L. var. solan bharpur were collected from Department of Extension Activities, Dr. Y.S. Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh.

Surface Sterilization: Healthy, uniform size seeds were selected for in vitro germination studies. The dirt particles if any, adhered to seeds were removed by cleansol (3-4 drops) followed by fungicide treatment bavistin 5% for 10 minutes. Seeds were thoroughly washed with distilled water 2-3 times after treatment. The fungicide treated seeds were further treated with 0.1% mercuric chloride for 2-3 minutes, followed by washing with autoclaved distilled water under laminar air flow conditions. The seeds were inoculated singly on MS standard [5]and MS modified media and incubated at culture room conditions .This protocol is followed throughout for surface sterilization of Capsicum seeds. All the media compositions shown in Tables I & II were supplemented with 3 mg/1 thiamine-HCl, 0.1 g/1meso-inositol, 3% sucrose; pH was adjusted to 5.8 before autoclaving at 121 °C and 15 psi for 15 min. Cultures were maintained at 25±2°C under 16 h photoperiod. The data is analysed by using [6].

III.RESULTS AND DISCUSSION

A. Effect of different Growth Regulators NAA & BA on seed germination of Capsicum Annuum

Different Growth Regulators (NAA & BA) combinations were used in MS standard media and *Capsicum annuum* seeds were inoculated onto them. out of them, best growth was observed after 14 days of inoculation on the M_2 medium (NAA 0.5mg/l, BA1mg/l) which gave percentage germination of 86% with maximum average shoot length 1.76±0.207cm.MS medium having NAA0.5mg/l and BA 3mg /l was found to be the least effective. Percent germination of 33.3% with minimum average shoot

length of 0.45±0.35cm was observed on it. Higher concentration of cytokinin is found to be inhibitory .that may be due to presence of endogeneous level of cytokinin in seeds. Low concentrations (0.08, 0.22 or 0.35 µM) of all cytokinins were more effective as compared to their higher levels (2.20 or 3.50 µM) for improved growth of seedlings of teak[7].The nodal segments from well grown seedling are used for further multiplication of capsicum annuum L var. solan bharpur. M_2 medium is further modified by using cheaper inputs i.e. Tap Water instead of distilled water, Sugar instead of sucrose and Isabgol instead of agar and is used for mass multiplication of shoots of capsicum.

B. Effect of different low cost Media on Seed Germination of Capsicum Annuum L

Different combinations of low cost components were used along with MS basal media and *Capsicum annuum* cv. Solan bharpur seeds were inoculated onto them in which initial growth was observed after 14 days of inoculation, out of which best growth was observed on the media $M_{2tw+sugar}$ which gave percentage germination of 85% which was at par with the standard MS medium(M_2) used for in vitro seed germination. However maximum average shoot length observed was 3.97±0.42cm on $M_{2tw+sugar}$ .which was almost two times than that of standard MS medium(M_2) i.e.1.76 ±0.27cm (Table I). This may be probably due to efficient translocation and assimilation of cheaper components as compared to the standard components by the explants .[8] in Centella asiatica used MS medium containing tap water and table sugar as cost effective components of culture medium to bring down the cost of mass multiplication. Similar findings were also reported in banana[9] ginger[10], anthurium[11],strawberry [12]. Minimum growth was observed on medium M_{2all} containing Tap Water, Sugar and Isabgol having percentage germination of 40% having minimum average shoot length of 0.8±0.42cm after 14 days of inoculation on medium. on contrary Culture medium containing all the cost effective components was found to be the best for *in vitro* establishment of cultures of lilium yielding 6.00 bulblets per explants[13].

Table-1: To Study Effect of Different Low Cost Media Combinations On*In Vitro* Seed

C. Germination of Capsicum Annuum

S. no	Label	Components	Percent germination(%)	Shoot length
1	Standard M_2	Tap water +Sucrose + Agar	86.00	1.76±0.207cm
2	$M_{2tw+sugar}$	Tap water +Sugar+ Agar	85.00	3.97±0.42cm
3	M_{2tw}	Tap water +Sucrose +Agar	66.60	0.21±0.21cm
4	M_{2sugar}	Distilled water + Sugar +Agar	66.60	1.31±0.59cm
5	$M_{2isabgol}$	Distilled water + Sucrose +Isabgol	50.00	3.25±0.35cm
6	M_{2all}	Tap water + Sugar+ Isabgol	40.00	0.8±0.42cm

For the above data $p<0.01$, so the data is statistically significant. The in vitro regenerated capsicum shoots were further multiplied on four modified MS medium by using cheaper inputs i.e. $M_{2tw+sugar}$, $M_{2isabgol}$ M_{2tw}& M_{2all} and Standard MS medium. Out of these four cost effective media, best multiplication of shoots was observed on the medium $M_{2tw+sugar}$ after 7 days of inoculation. The maximum average shoot no. of 5.2±0.1 with average no of 3.0±0 leaves/shoot followed by M_{2tw} which gave avaerage multiplication rate of 4.5±0.5 with same number of leaves as in case $M_{2tw+sugar}$. we have found significant differences with respect to the induction of shoots per explant on media prepared either with tap water and commercial sugar and Standard medium prepared with distilled water and tissue culture-grade sucrose.). Our results are consistent with the results of Kaur et al. 2005[11].They reported maximum in vitro multiplication of shoots of strawberry on MS medium supplemented with kn 0.5 mg/l, BAP 1.0 mg/l and GA3 2.0 mg/l and table sugar in place of sucrose[14] in banana and [15] in Brassica campestris and [9]in ginger in vitro multiplication used tap water and sugar as cheaper inputs in MS media and found encouraging results .The minimum rate of multiplication of shoots was observed on $M_{2isabgol}$ having average shoot no.

Table-II: To Study Effect of Different Low Cost Media Combinations on Multiplication of

D. Capsicum Annuum

SN o.	Media &labeling	No.of Shoots	No. of leaves/shoot	Callus
1	M2	4.02±0.32	2.27±0.5	-
2	$M_{2tw+sugar}$	5.2±0.1	3.0±0	-
3	$M_{2isabgol}$	1.35±0.21	2.5±0.70	+
4	M_{2all}	4.1±1.41	3.0±0	+
5	M_{2tw}	4.5±0.5	3.0±0	-

of 1.35±0.21 with average no of 2.5 ±0 leaves/shoot . The rate of multiplication of shoots on standard MS M2 was 4.02±0.32shoots/explant which is found to be comparable to M2 all medium i.e 4.1±1.41shoots/explant. Isubgol as alternative gelling agent for in vitro culture of Curcuma longa L. non-significant differences were observed between media solidified with isabgol and agar during multiplication[16].The use of chemicals such as carbon sources, gelling agents, inorganic and organic supplements, and growth regulators in culture media, make this technique expensive. Sucrose is usually used as a source of carbon and it constitutes the most expensive component of the culture media. so by replacing it with cheap sugar can substantially decrease the cost of micropropagation and If tap water is free from heavy metals and contaminants, it can be substituted for distilled water. Isabgol having a distinct cost advantage over agar, none is likely to be used as routinely as agar because of some inherent drawbacks i.e. problem in adjustment of pH and dispensing of the medium to culture vessels [17].Micropropagation of vegetables has become a routine procedure but high production cost is limiting the commercial use of tissue culture technology so from The above studies, it may be concluded that table sugar instead of sucrose and tap water instead of distilled water can be effectively used at different stages of micropropagation of capsicum annuum cv solan bharpur. The use of low cost components could reduce the cost of in vitro raised plantlets and thus making micropropagation of capsicum annuum cv solan bharpur a viable proposition for commercialization of this variety.

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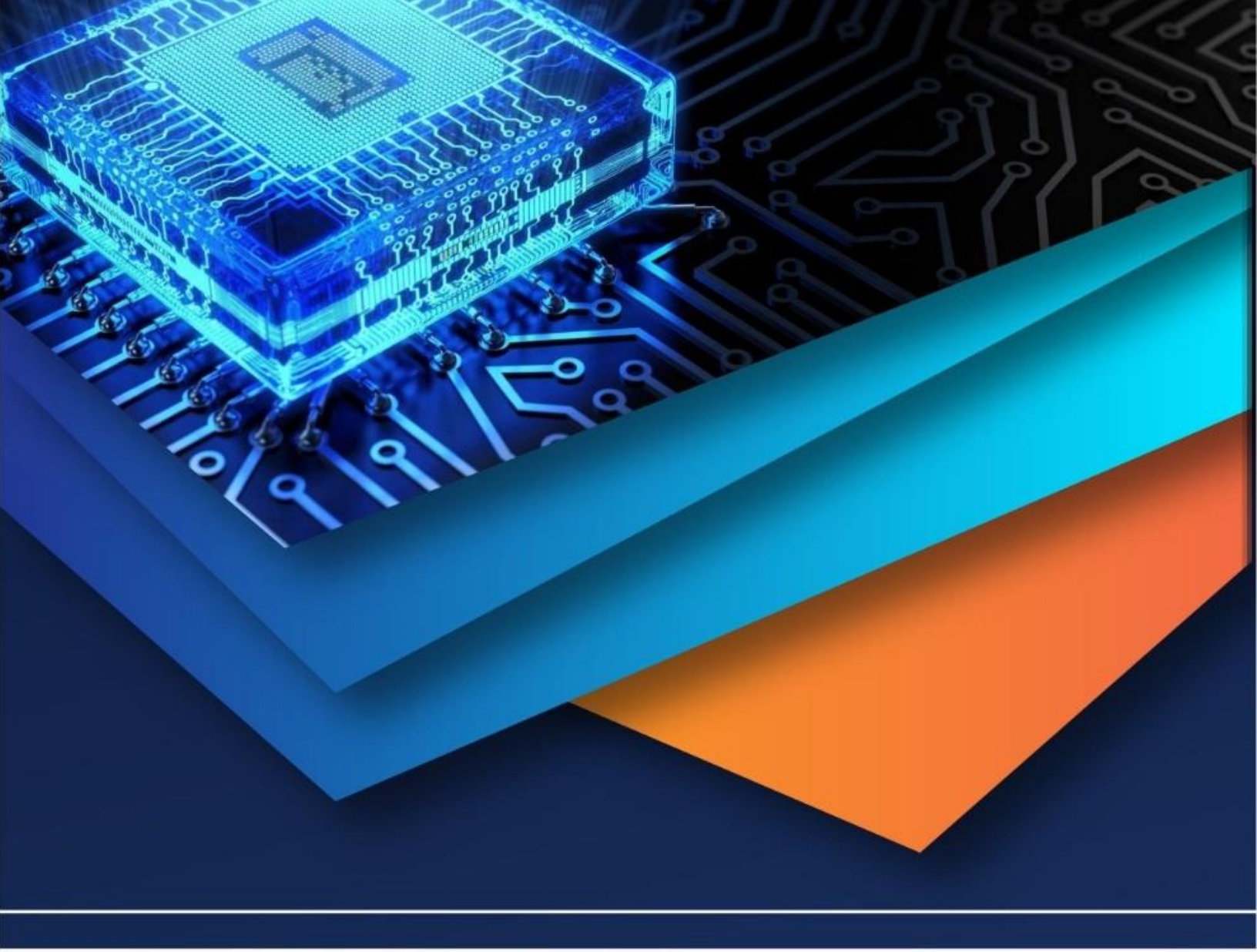

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