International Journal of Scientific Research Updates

Journal homepage: https://orionjournals.com/ijsru/

ISSN: 2783-0160 (Online)

(RESEARCH ARTICLE)

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In vitro plantlet regeneration from cotyledon explants of *Capsicum annuum* L

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International Journal of Scientific Research Updates, 2022, 04(02), 195–200

Publication history: Received on 14 October 2022; revised on 27 November 2022; accepted on 30 November 2022

Article DOI: https://doi.org/10.53430/ijsru.2022.4.2.0181

Abstract

An efficient *In vitro* plant regeneration was established in *Capsicum annuum* L. from cotyledon explants. The best performance i.e., 80% of the explants responded for multiple shoot bud proliferation on MS medium containing 10 mg/l Zeatin. The maximum number of buds per explants was 50.28 ± 2.69 and the bud length of 0.5 ± 0.22 cm was obtained in this medium after 30 days. The average number of 80.32 ± 0.16 multiple shoot bud and the average shoot bud length of 1.0 ± 0.19 cm was attained on the same medium after 90 days. These shoot buds on transfer to MS + 10 mg/l Zeatin + 2.0 mg/l GA3 yielded 6.3 ± 0.35 number of multiple shoots with an average shoot length of 4.6 ± 0.62 cm after 30 days. Shoots rooted well (80% of induced shoots) on half strength MS medium supplemented with 0.5 mg/l IBA. The average number of roots per shoot was 8.2 ± 0.79 and the average root length of 4.5 ± 0.58 cm was recorded. About 80% of *In vitro* derived plantlets were survived under open field conditions.

Keywords: *In-vitro*; Capsicum; Cotyledon; Regeneration; Zeatin

1 Introduction

The genus Capsicum is an important vegetable and spice crop in Bangladesh and many other regions around the globe. The genus consists of about 25 wild ad 5 domesticated species [1]. Among the domesticated species, *Capsicum annum* is one of the most economically important species of the genus and includes both mild and pungent fruit types. It contains numerous chemicals including steam volatile oil, fatty oils, Capsaicinoids, carotenoids, vitamins, protein, fibre and mineral [2]. It serves as an indispensable spice in various cuisines in a great variety of food all over the world due to its nutritional value, flavor and aroma and glossy color. It also has medicinal uses to treat various disorders is attributed to the presence of a group of alkaloids called capsaicinoids [3]. Begin an important economic crop, the propagation suffers from many constraints due to cross pollinating behaviour, shoot viability span and low germination rate [4]. Moreover, plants are also highly susceptible to fungal and viral pathogens [5]. Thus, micropropagation system is an alternative and unique method to overcome those variabilities for conserving, *In vitro* genetic improvement, and mass propagation of this important plant. Several attempts have been made on *In vitro* plant regeneration from different explants tissues such as shoot tip [6], nodal segments [7-8] rooted hypocotyls [9], leaf, stem, hypocotyls, cotyledon root,

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shoot tip and embryo [10-11], induced somatic embryogenesis from shoot tip [12] and protoplast inducting from leaf of *In vitro* shoots [13]. But many of these investigations did not report satisfactory result in terms of enhanced number of shoots. It is reported that plant regeneration in chilli is severely limited due to the formation of ill define buds or shoot like structures either resisting elongation or producing rosettes of distorted leaves which generally do not produce normal shoots [14-16]. Therefore, the present investigation was undertaken to develop an efficient *In vitro* clonal propagation protocol for *capsicum annum* L. through cotyledon culture.

2 Material and methods

2.1 Explant collection & Surface Sterilization

Cultures were initiated from aseptic germinated seeds from *capsicum annum* L. which were collected from the local market. Seeds were washed with running tap water for 30 minutes to remove adherent particles. The seeds were then surface sterilized with an aqueous solution of 0.1% $HgCl_2$ with two drops of tween 20 for 10 minutes under aseptic condition and rinsed four times with sterile distilled water to wash out any traces of $HgCl_2$. The surface sterilized seeds were then placed on MS medium devoid of plant growth regulators. Seeds were started to germinate within seven days after inoculation and became about 5 to 6 cm in height within 3 weeks.

2.2 Culture media

Then cotyledon explants were excised aseptically from *In vitro* raised 21 days old seedlings and cultured on to media containing MS supplemented with BA and Zeatin alone and in combinations with BA+IAA and BA+NAA for multiple shoot proliferation. Different concentrations of GA_3 with 10 mg/l zeatin were used for shoot elongation. Subcultures were done 30 days interval for promoting strong and healthy multiple shoots. Healthy shoots were excised individually and transferred to half strength of MS media supplemented with different concentrations of IBA, IAA and NAA for root induction. The sucrose (table sugar) concentration was used 30 g/l and the pH of the media adjusted to 5.8 prior to autoclaving. Cultures were incubated at 24 ± 2 °C with 16 hours illumination of 50 µmol/cm^2/S provided by cool white fluorescent tubes. The experiment was conducted with 10 explants per media type and replicated thrice.

2.3 Data collection and analysis

Data were collected on different characters at day 30 and 90 for shoot bud proliferation and shoot elongation and at day 30 for rooting of shoots. Observation of cultures was carried out every alternate day. The experiments were arranged in a completely randomized design (CRD). A descriptive analysis was carried out using the recorded data. Each value represents Mean ± Standard errors.

3 Results and discussion

Cotyledon explants were excised into small pieces of 1.5cm long and inoculated on MS media supplemented with different concentrations of BA and zeatin alone and in combinations with BA+IAA and BA+NAA (Table 1) to investigate its effect of multiple shoot bud proliferation. Profuse shoot like structures or buds were formed in a clumps inoculation after 30 days on MS medium supplemented with 10 mg/l zeatin, in which 80% of explants were found to respond shoot bud proliferation. The average number (50.28 ± 2.69) of buds and the average bud length (0.50 ± 0.22 cm) were recorded in this medium of MS + 10 mg/l Zeatin inoculation after 30 days.

Some of the buds turns into shoots at inoculation after 90 days whilst subcultures were done 30 days interval at the same inoculation medium. The number of shoot bud was 80.32 ± 0.16 and the average length of shoot bud was 1.0 ± 0.19 cm were observed in this medium inoculation after 90 days. These shoot buds were then transferred into different concentrations of GA_3 with 10mg/l zeatin on MS supplemented media (table 2) for obtaining elongated shoots. The maximum average number of elongated shoots was 6.3 ± 0.35 and the average shoot length of 4.6 ± 0.62 cm were observed in this shoot elongation medium of MS + 10 mg/l Zeatin + 2.0 GA_3 inoculation after 30 days. On the other hand, very minimum percentage of explants response with poor shoot bud proliferation was observed on MS media containing BA singly (2.5 mg/l) and 2.5 mg/l BA + 0.1 mg/l NAA and also 2.5 mg/l BA + 1.0 mg/l IAA (Table 2). The rest of the media tested in this study did not response shoot bud proliferation any indicating imbalanced media compositions were involved. Among the media composition tested in this study, it was found that MS + 10 mg/l Zeatin performed the best for shoot bud proliferation which is in agreement with the study reported by Sanatombi and Sharma [1].

Table 1 Effect of plant growth regulators on MS supplemented media for multiple shoot bud induction from cotyledon explant of *capsicum annum* L

Plant growth regulators (mg/l)				**% Explants response at day 30**	**Number of shoot bud/explant**		**Shoot bud length (cm)**	
BA	Zeatin	IAA	NAA		Day 30	Day 90	Day 30	Day 90
2.5	-	-	-	20	20.46±0.72	35.80±1.39	0.25±0.09	0.50±0.19
3.5	-	-	-	-	-	-	-	-
4.5	-	-	-	-	-	-	-	-
2.5	-	-	0.1	20	10.22±0.43	17.52±0.19	0.27±0.04	0.33±0.13
5.0	-	-	0.1	-	-	-	-	-
2.5	-	1.0	-	40	25.33±0.29	36.49±1.54	0.29±0.07	0.50±0.17
5.0	-	1.0	-	-	-	-	-	-
-	6.0	-	-	-	-	-	-	-
-	8.0	-	-	10	21.27±0.66	32.64±1.22	0.25±0.03	0.29±0.11
-	10.0	-	-	80	50.28±2.74	80.32±1.37	0.50±0.02	1.0±0.16

Table 2 Effect of different concentrations of zeatin+GA3 on MS supplemented media for shoot elongation of *In vitro* proliferated shoot bud derived from cotyledon explants of *Capsicum annuun* L. inoculation after 30 days

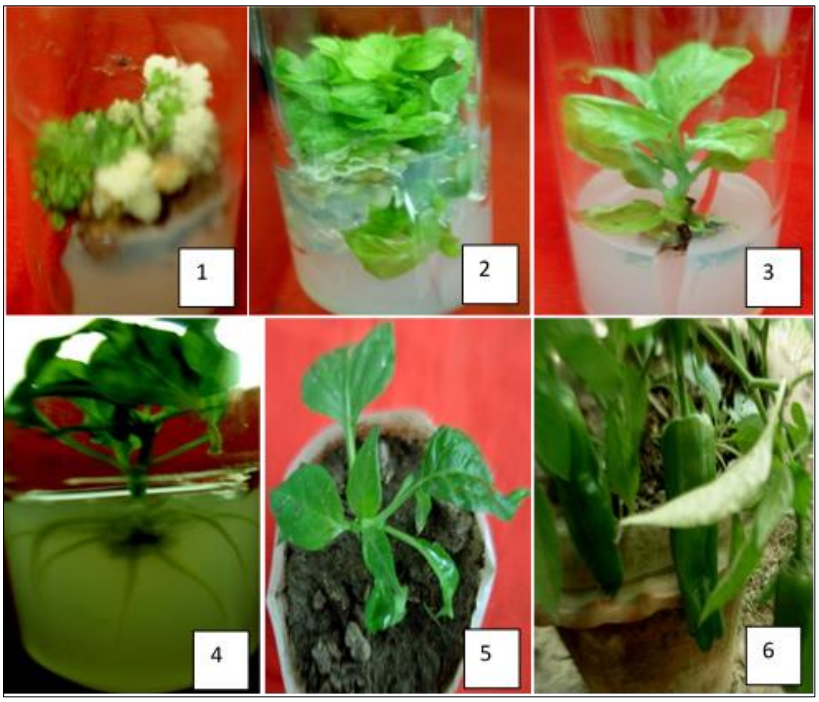
Plant growth regulators (mg/l)		**Number of elongated shoots (Mean ± SE)**	**Shoot length (cm) (Mean ± SE)**
Zeatin	**GA3**		
10.0	1.0	2.5 ± 0.72	2.8 ± 0.69
10.0	2.0	6.3 ± 0.35	4.6 ± 0.62
10.0	3.0	2.7 ± 0.26	1.9 ± 0.38
10.0	4.0	-	-

Earlier investigators Arroyo and Revilla [17], Franck *et al*. [14], Gunay and Rao,[18], Phillips and Hubstenberger,[19] and Szasz *et al*. [20] reported better results with BA in combinations with IAA for multiple shooting of Capsicum. On the other hand, Verma *et al*. [11]and Ahmad *et al*. [7] found better results for multiple shooting in Capsicum using TDZ. Our investigation was differed from them which might be due to the different genotypes with different type of explant tissue used. The effective ness of GA_3 for shoot elongation of Capsicum is evidence in this study. This study suggests that using GA_3 in the media could be overcome the difficulties of ill define bud formation, producing rosettes of distorted leaves and shoot like structures and also to produce normal shoots. The differential effect of various concentrations of TDZ, BA, Zeatin and silver nitrate on shoot bud induction of Capsicum has also been reported [21].

Presence of GA_3 in the MS medium have been reported to increase in length of shoot in Capsicum [22]. Differential response caused by different explants and genotypes and also media composition has been suggested by many authors. This is also in agreement with the present investigation. The rooting response was found differed according to concentrations of different anxins used (Table 3). A well develop shoots of 5-6 cm long were excised and transferred to MS media supplemented with different concentrations of IBA, IAA and NAA for root imitation.

Table 3 Effect of different types of auxins and their various concentrations on half strength of MS media for root induction of *In vitro* raised shoots of *Capsicum annuum* L. at inoculation after 30 days

Different type and concentrations of auxins			% Shoots responding root induction	Number of root/shoots (Mean ± SE)	Root length (cm) (Mean ± SE)
IBA	IAA	NAA			
0.5	-	-	80	8.2±0.79	4.6±0.58
1.0	-	-	40	5.6±0.23	2.9±0.49
1.5	-	-	10	2.3±0.44	2.5±0.45
2.0	-	-	10	1.6±0.39	1.4±0.22
-	0.5	-	-	-	-
-	1.0	-	-	-	-
-	1.5	-	-	-	-
-	2.0	-	-	-	-
-	-	0.5	50	3.7±0.36	1.7±0.56
-	-	1.0	20	1.9±0.34	1.2±0.33
-	-	1.5	-	-	-
-	-	2.0	-	-	-



Figures 1 *In vitro* shoot bud initiation and plant regeneration from cotyledon explants of *Capsicum annuum* L. 1). 1)Bud initiation on MS + 10.0 mg/l Zeatin after 1 month of culture 2). Multiple shoot formation on transferring buds to MS + 10.0 mg/l Zeatin + 2.0 mg/l GA_3 after 2 months of culture 3). Healthy shoot formation and shoot elongation in the same medium of MS + 10.0 mg/l Zeatin + 2.0 mg/l GA_3 after 2 months of culture 4). Root induction on half strength MS + 0.5 mg/l IBA after 1 month of culture 5). *In vitro* raised plant resumed new growth in the polybag. 6). Four months old *In vitro* raised plant at fruiting stage

Among the anxins used, IBA was found to be the best for root induction of Capsicum and 0.5 mg/l was recorded most optimum, in which 80% shoots rooted within 30 days after inoculation of rooting media. The average number of roots induced per shoot was 8.20 **±** 0.79 and the average root length of 4.5 **±** 0.58 cm were observed in this medium of MS + 0.5 mg/l IBA. Similar observations by using IBA in *Capsicum annuum* L. was reported [1,7-8, 10]. The effectiveness of IBA and IAA on the rooting of *In vitro* regenerated chilli plantlets has been reported [11,18,20,23-27]. The superiority of IBA for rooting over other auxins has also been reported [28-31]. Sanatombi and Sharma also found effective root induction in *capsicum annum* L. using NAA [1]. Differential effect of rooting response observed by different authors with the use of different auxins in their experiments which might be due to the effect of genotypes and explant tissue. Morphologically strong and healthy rooted plantlets of about 5 to 6.5 cm tall were taken out from the culture vessels and washed gently under running tap water to get rid of agar. These plantlets were then transferred to polybags and earthen pots containing soil and compost (2:1) for acclimatization and establishment in natural environment. The *In vitro* raised plantlets showed 80% survival during hardening and acclimatization period of 30 days. The transplanted plantlets were established well in earthen pots and in the field.

4 Conclusion

The present study developed a method for efficient regeneration of *Capsicum annuum* L. from cotyledon explants using Zeatin and GA_3 for multiple shooting and IBA for rooting which could be useful for large scale production of propagules and genetic improvement of this economically important crop.

Compliance with ethical standards

Acknowledgments

I would like to gratitude to all co-authors and other related personnel who helped in every part of research such as planning, methodologies, data collection, manuscript writing and reviewing.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest related to this article.

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