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Influence of Plant Growth Regulators on Seed Germination and Regeneration of Shoots and Roots in Chili (*Capsicum annuum* L.)

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A B S T R A C T

Capsicum annuum L. is an important vegetable crop of India and grown widely in open field as well as polyhouses. Low seed germination and higher price of seeds is major problem associate with the cultivation of this crop. To overcome these problems, plant tissue culture offers technology to generate a nursery in minimum duration with several other benefits. For this purpose, a study was designed to evaluate the effect of benzyl amino purine (BAP) on seed germination and multiplication shoot induction from shoot apex and nodal segment. Explants were obtained from in vitro grown seedlings. The germination of seeds was early on MS medium supplemented with BAP with compare to MS media without plant growth regulators. The shoot apex explants found superior over nodal segments on the basis of number of shoots regenerated per explants. MS medium with 0.50 mg/l IBA was fount optimum for root induction in regenerated shoots. The results of present study shows a way to overcome the problem with germination rate with chili seeds and also can be used to regenerate multiple number plants within a short span.

Introduction

Chili (*Capsicum annuum* L.) is an economically important spice crop cultivated throughout the world. Chili is reported to be a native of South America and it is widely distributed in all tropical and subtropical countries including India. Chili is grown in both tropical and subtropical climate and grown throughout the year at one or the other parts of the country for vegetable purpose. World chili production is primarily concentrated in South Asian countries to an extent of about 55% of total world production. India is the largest producer contributing for about 38 % followed by neighbors China with 7%. Pakistan and Bangladesh are also contributing for about 5% each. It is also gaining importance as an ornamental plant due to its attractive, colorful and long -lasting fruit which stand erect on the plant. The chili fruit powder is the most important savory ingredient in the Indian traditional dishes. It is also used for preparation of chutneys, masala, sauces and pickles. It is rich source of vitamin C. There is demand for export of raw chilies

and chili powder. Extract of green chilies can be used as bio–insecticide. The regeneration from tissue culture serves the following main purpose: micro propagation of elite plants, fixation of hybridization vigor, preservation and application of variants, and genetic transformation (Dabauza and Pena, 2001). Dicotyledonous species differ widely in their organogenesis potential and amenability to genetic transformation. Genetic engineering of dicotyledonous plants has been limited due to difficulties associated with efficient in vitro plant regeneration (Pozueta-Romero *et al.*, 2001).

The propagation through seeds is restricted by low germination rate and short span of viability of seeds (Santombi and Sharma, 2006). The price of seeds is very high, so the establishment of plant regeneration methods of these cultivars reduces the dependence of nursery plant production and seed price. Moreover, chili is highly susceptible to fungal and viral pathogens and these cause considerable damage to the crop. Tissue culture followed by gene transfer could be an easy, efficient and economic means for obtaining large number of disease-free. In vitro plant regeneration of chili has been achieved *via* protoplast, hypocotyls, cotyledons, young leaves and direct somatic embryogenesis (Kumar *et al.*, 2011; Grozeva *et al.*, 2012) and different medium combination (Sanatombi and Sharma, 2008). But many of these investigations did not report satisfactory result in terms of enhanced number of shoots because plant regeneration in chili is severely limited due to the formation of ill-defined bud or shoot like structure which either resist elongation or produce rosette of distorted leaves that do not produced normal shoot (Alejo and Ramirez-Malagon, 2001). So the present study was carried out to investigate the effect of plant growth regulators on seed germination and regeneration of shoots and roots in chili.

Materials and Methods

Seed sterilization

Seeds of *C. annuum* cv G-235 were obtained from local market for present investigation. For sterilization, seeds were taken in 50 ml conical flask and treated with Bavistin™ (1% w/v) for 5 min. seeds were washed with distilled water and further treated with four to five drops of Tween-20 for 15 min with intermittent shaking. Seeds were rinsed with distilled water till foam disappears. Further sterilization was carried out under aseptic conditions in laminar air flow. Seeds were dipped in ethanol (70% v/v) for 2 min and washed 2-3 times with sterilized distilled water. Again seeds were treated with different concentration of NaOCl i.e. 0.5, 1.0, 1.5, 2.0% v/v for 1 and 2 min. These seeds were washed with sterilized distilled water for 5-6 times. Sterilized seeds were kept on a blotting paper to remove excess water and used for experiment.

Culture media and seed germination

MS basal medium (Murashige and Skoog, 1962) was used in entire experiments with 3% w/v sucrose and 08% w/v agar for solidification. pH of medium was adjusted to 5.8±0.05 and medium was sterilized using autoclave for 20 min. MS basal medium with different concentration of (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) of Benzyl amino purine (BAP) was used to evaluate the effect of cytokinin on seed germination. Sterilized seeds were inoculated on medium with the help of sterilized forceps (Fig. 1A) and bottles were sealed with Parafilm^{M}.

Cultures were incubated at 25 ± 2^{o} C with photoperiod of 16/8h light/dark cycle at 3000 lux light intensity with cool white fluorescent light with chamber relative humidity 60-65 % in the tissue culture chamber.

Explant preparation and multiple shoot induction

Shoot apex and nodal segment from *in vitro* grown seedlings were used as explants (Fig. 1B and 1C). 30 days old seedlings were used to excise explants. For multiple shoot induction, MS basal medium with different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) of BAP. MS medium without any growth regulator was used as control. Cultures were incubated at similar condition as above for four weeks. Days to initiate multiple shoot induction were observed for all treatments. After completion of four weeks, number of explants responded, number of shoot regenerated and length of shoots (cm) were recorded.

Rhizogenesis in regenerated shoots and hardening

Regenerated shoots were separated from each other and transferred in root induction medium. Root induction medium consist of MS medium with different concentration (0.25, 0.50, 0.75, 1.0 mg/l) of indole butyric acid (IBA). Cultures were again incubated at similar conditions for 15 days.

Rooted plantlets were transferred in mixture of soil and sand (1:1) in polybags and covered with transparent polythene bag to maintain humidity. Polythene bag was removed gradually after plants get hardened in regular interval.

Statistical analysis

Each experiment was repeated three times and mean values and standard deviation were calculated. All data obtained were subjected to the single factor analysis of variance (ANOVA) using Microsoft excel. The critical difference (C.D.) values were calculated at p=0.05 level to find out the significant difference between the means of different treatments.

Results and Discussion

Establishment of aseptic cultures

Results are presented in Table 1 for the seeds treated for varying period of time at different concentrations with sodium hypochlorite in order to establish minimum contaminant free cultures. The optimum percentages (12.4 %) of contaminated explants with 6.7% non-viable seeds were observed when treated with 1.5 % v/v sodium hypochlorite for duration of two minutes. When seeds were exposed to 2.0 % v/v sodium hypochlorite for 1 and 2 minutes, 5.7% and 4.2% contaminated cultures were obtained respectively but this concentration inhibited the seed viability. In this regards, 1.5% v/v of sodium chlorite was used in further experiments. Sodium hypochlorite at 1.5% v/v was also used for sterilization of seeds of *Capsicum annuum* by Santos *et al.,* (2017).

Effect of BAP on seed germination

It has been observed that the seeds arise early when MS medium supplemented with BAP with compare to MS media without plant growth regulators. The minimum time taken for the seed germination was 6 days with 98% seed germination on MS medium fortified with 1.5mg/l BAP (Fig. 1D) followed by 6 days with 95% seed germination on MS medium containing 2 mg/l BAP.

Seeds took 14 days to germinate with 98% seed germination on MS medium without plant growth regulator (Table 2).

Higher concentration of BAP (<2 mg/l) reduced seed germination. Khan *et al.,* (2009) showed that pretreatment of seeds with acetylsalicylic acid and salicylic acid in

pepper (*Capscum annuum* L.), resulted in greater uniformity of germination and establishment of seedlings under high salinity.

Multiple shoot induction from shoot apex

Results obtained for multiple shoot induction from shoot apex are presented in Table 3. Time taken to initiate multiple shoot induction was decreased as concentration of BAP was increased up to 2 mg/l. Maximum response (93.33%) of explants was obtained on MS medium with 2 mg/l BAP followed by 86.66% response on MS medium with 2.5 mg/l BAP. Maximum number of multiple shoots (4.10 shoots/explant) with 7 cm length was obtained on MS medium fortified with 2 mg/l BAP (Fig. 1E) followed by 3.25 shoots per explants with 7.33 cm length on MS medium containing 2.5 mg/l BAP. Shoot apex inoculated on MS medium without growth regulator showed delayed shoot induction with minimum shoots per explants. Shoot tips posses meristematic cells which are highly dividing cells and have potential to produce multiple shoots when growth modulated using cytokinins. BAP was found effective for producing multiple shoots from shoot tips explants in *Capsicum annuum* (Sobhakumari and Lalithakumari, 2003; Rao *et al.*, 2006).

Table.1 Effect of different concentration of sodium hypochlorite and periods on incidence of contamination in chili

Conc. of NaOCl (v/v)	Time (min)	Contamination (%)	Non-germinated seeds (%)
0.5	1	46.3	0.8
	2	41.2	1.2
1.0	1	33.7	3.7
	2	28.7	4.1
1.5	1	24.8	5.8
	2	12.4	6.7
2.0	1	5. 7	10.4
	2	4.2	14.6

Table.2 Effect of BAP on seed germination of Chili

BAP (mg/l)	Explants responded (%)	Days of germination
0.0	98%	14
0.5	98%	11
1.0	98%	8
1.5	98%	6
2.0	95%	6
2.5	90%	7

Table.3 Effect of BAP on *in vitro* regeneration from shoot apex explants Chili

BAP (mg/l)	Responsive Explants (%)	Average shoots per explants	Day of shoot initiation	Length of shoot after 4 week (cm)
0.0	32.3	1.02	11.20	3.22
0.5	40.4	1.80	9.00	3.66
1.0	60.8	2.30	8.40	4.13
1.5	80.3	3.05	8.10	6.06
2.0	93.33	4.10	7.00	7.40
2.5	86.66	3.25	7.33	6.40

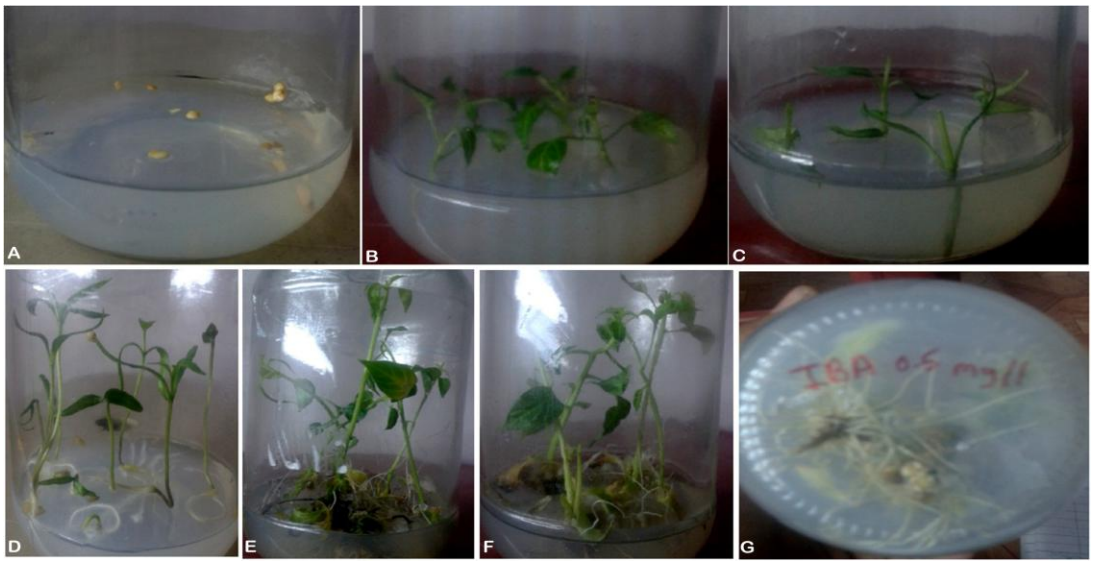
Table.4 Effect of BAP on in vitro regeneration from nodal segment explants of Chili

BAP (mg/l)	Responsive Explants (%)	Average shoots per explant	Day of shoot initiation	Length of shoot after 4 week (cm)
0.0	32.1	1.12	11.45	2.85
0.5	40.00	2.12	8.73	4.00
1.0	73.33	3.50	7.53	5.13
1.5	86.66	3.95	7.33	6.73
2.0	66.66	3.20	8.03	6.30
2.5	53.33	2.33	9.26	4.26

Table.5 Effect of different concentration of IBA on root growth

IBA (mg/l)	Days required induction	Rooted shoots (%)	No. of roots per shoot
0.25	9.0	75	7.9
0.50	8.0	85	11. 2
0.75	9.0	80	10.1
1.0	9.5	75	8.6

Fig.1- *In vitro* regeneration of *Capsicum annuum* with shoot apex and nodal segment (A) Seed inoculation on medium. (B) Inoculation of shoot apex and (C) nodal segment (D) effect of BAP (1.5mg/l) on seed germination (E) effect of BAP on multiple shoot induction from shoot apex and (F) nodal segment (G) root induction in regenerated shoots



Multiple shoot induction from nodal explants

Results obtained for multiple shoot induction induced from nodal explants are presented in Table 4. Minimum time (7.33 days) to initiate multiple shoot induction was observed in MS medium fortified with 1.5 mg/l BAP followed by 7.53 days on MS medium with 1 mg/l BAP. Maximum response (86.66%) of explants was obtained on MS medium with 1.5 mg/l BAP (Fig. 1F) followed by 73.33% response on MS medium with 1mg/l BAP. Highest number of multiple shoots (3.95 shoots/explant) with 6.73 cm length was obtained on MS medium fortified with 1.5 mg/l BAP followed by 3.50 shoots per explants with 5.13 cm length on MS medium containing 1 mg/l BAP. Nodal explants inoculated on MS medium without growth regulator displayed slow initiation with minimum number of shoots per explants. Nodal segment are important explants for direct regeneration of *Capsicum* species with a huge potential of multiple shoot induction. Several reports are their confirming utilization of nodal segment as explants (Ahmad *et al.,* 2006; Kehie *et al.,* 2012; Robinson and Maheswari, 2013)

Root induction and hardening of plantlets

The elongated shoots were excised and implanted on MS medium fortified with different levels of IBA. The highest root induction (85%) with 11.2 roots per shoot were obtained on MS medium with 0.50 mg/l IBA (Fig. 1G) followed by 80% root induction with 10.1 roots per shoot on MS medium containing 0.75 mg/l IBA (Fig. 1). Minimum days required for root induction (8 days) was showed on MS medium supplemented with 0.5 mg/l IBA. Similar results were also obtained by (Kumar *et al.,* 2012). Regenerated plantlets with well-developed roots were hardened and showed normal growth without phenotypic variation. IBA is extensively used auxin for root induction in several plants including Capsicum *annuum* (Ahmad *et al.,* 2006; Rao *et al.,* 2006) (Table 5).

A study was conducted with shoot apex and nodal segment of chili as explants for regeneration on MS medium supplemented with different concentrations cytokinins. In this study, the effects of different concentrations of

BAP on seed germination and multiple shoot induction was evaluated. Inclusions of BAP have positive impact on seed germination as these seeds require minimum days with improved germination rate. The shoot apex explants found better then nodal segments on the basis of number of shoots regenerated per explants. MS medium with 0.50 mg/l IBA was fount optimum for root induction in regenerated shoots. The results of present study shows a way to overcome the problem with germination rate with chili seeds and also can be used to regenerate multiple number plants within a short span.

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