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ADVENTITIOUS SHOOT BUD INDUCTION IN CHILI PEPPER (C*APSICUM ANNUUM* L. CV. X-235)

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ABSTRACT
Adventitious shoot buds were produced *in vitro* by using cotyledon explants of 14-day-old *Capsicum annuum* L. cv. X-235. The effect of plant growth regulators i.e., BAP and IAA at various concentrations on cotyledon explants for adventitious shoot bud differentiation was observed. Among the media tested MS medium supplemented with 5mg/l BAP and1mg/l IAA gave maximum shoots (19.2) per explant. Effective *in vitro* shoot elongation and rooting was achieved on 0.5mg/l GA_3 and 0.5mg/l IBA, respectively. The rooted plants were hardened and transplanted in to the soil and the plants showed 80-90% survival during transplantation.

KEY WORDS: Adventitious shoot, BAP, cotyledon, IAA, PGR.

INTRODUCTION:

Chili pepper (*Capsicum annuum* L.) is one of the most important vegetable cum spice crop around the world. The chili fruit powder is the most important savory ingredient (capsaicin) in the Indian traditional dishes. However, it is highly susceptible to many pathogens including viruses, bacteria, fungi and insect-pests (Morrison *et al*., 1986). Spraying of fungicides and pesticides can control the diseases to some extent, while effective resistance against several destructive pathogens is still not possible. Approaches are being made to produce genetically engineered disease resistance pepper varieties, but they have been delayed due to its recalcitrant nature (Ochoa-Alejo and Ramirez-Malagon, 2001; Steinitz *et al.*, 1999). In chili peppers, based on the *in vitro* responsiveness to plant regeneration via different explants i.e., hypocotyl, node, leaf, shoot tip, immature embryos and anthers have been reported earlier, but the success rate is very meager to meet genetic engineering protocols (Ahmad *et al*., 2006; Aniel Kumar and Subba Tata, 2010; Binzel *et al*., 1996; Koleva-Gudeva *et al.*, 2007; Sanatombi and Sharma, 2006). However, most of the previous *in vitro* work on chili peppers suggest that cotyledons remain the better choice of explant for adventitious shoot proliferation (Arroyo and Revilla, 1991; Ashrafuzzaman *et al*., 2009; Berljak, 1999; Dabauza and Pena, 2001; Golegaonkar and Kantharajah, 2006; Gunay and Rao, 1978; Hyde and Phillips, 1996; Qin *et al.*, 2005; Rodeva *et al.*, 2006; Sobhakumari and Lalithakumari, 2005; Sripichitt *et al.*, 1987; Subhash and Prolaram, 1987; Valadez-Bustos *et al.*, 2009). As reported in most published papers on cotyledon explants of chili peppers (*Capsicum* L.) BAP and IAA have the ability to induce various frequencies of shoots *in vitro* (Husain *et al.*, 1999; Mezghani *et al.*, 2007; Mok and Norzulaani, 2007; Sanatombi and Sharma, 2008). Therefore the present study was undertaken to induce adventitious shoot buds by optimizing the levels of BAP and IAA on cotyledon explants of *Capsicum annuum* L. cv. X-235.

MATERIALS AND METHODS

Explant preparation

Seeds of *Capsicum annuum* L. cv. X-235 genotype were obtained from Sutton and Seeds, Calcutta, India. The seeds were surface sterilized with 0.1% $HgCl_2$ and repeatedly washed in sterile distilled water. The seeds were then inoculated in glass containers with 50ml of half-strength MS medium (Murashige and Skoog, 1962) for germination. The cotyledon explants were about 2-3cm in length, derived from 14 days old seedlings grown *in vitro* transferring on to shoot multiplication media.

Micropropagation

The basal nutrient medium containing MS salts and vitamins was used with IAA(indole -3- acetic acid) and BAP(6-benzyl aminopurine). In the first experiment, the effects of BAP were examined individually at the concentrations of 1.0-9.0mg/l and in the second experiment, IAA at the concentrations of 1.0 and 2.0mg/l was combined with BAP(1.0-9.0mg/l) and subculture at every two weeks to the same medium. The number of shoot buds were recorded after five weeks of culture, then the shoot buds were elongated on MS medium with various levels of GA_3 (0.25-0.75mg/l) after one week of culture. To test their rooting capacity, the *in vitro* elongated shoots were excised and transferred on MS medium fortified with IAA or IBA with 0.25-1.0mg/l concentrations, respectively. The rooting i.e., frequency of rooting (%), number of roots and root length(cm) per shoot were noted after two weeks of culture.

In vitro conditions

All media were supplemented with 3% sucrose and 0.8% agar, the pH of the media was adjusted to 5.8 with IN NaOH or IN HCl prior to autoclaving. The cultures were maintained at 25 ± 2^0C air temperatures in a culture room with a 16 hour photoperiod under an illumination of 20 m mol $m^{-2}s^{-1}$ photosynthetic photon flux density, provided by cool-white fluorescent light. All the experiments were repeated thrice; each treatment consisted of twenty replicates.

Acclimatization

Plants with roots were transferred during two weeks, after washing of the agar with distilled water, to pots with a mixture of soilrite (1:1). Potted plantlets were covered with transparent polythene membrane to ensure high humidity and watered every three day with half-strength MS salts solution for two weeks, in order to acclimatize plants to field conditions. After two weeks the acclimatized plants were transferred to pots containing normal garden soil and maintained in greenhouse under natural day length conditions.

Statistical analysis

Experiments were set up in Randomized Block Design (RBD) and each experiment was replicated thrice. Observations recorded on the percentage of response, number of shoots per explant, number of roots per shoot and root length. Mean and
Standard errors were carried out for each treatment.

RESULTS AND DISCUSSION

Table 1 shows the morphogenetic response of *Capsicum annuum* L. cv. X-235 cotyledon explants under the influence of BAP and IAA plant growth regulators.

TABLE 1. Effects of BAP and IAA on cotyledon explants

S. No.	Plant growth regulator(mg/l)	Shooting (%)	Shoot No. /explant
1	MS	-	-
2	MS+BAP(1.0)	69.0±0.08	2.4±0.08
3	MS+BAP(2.0)	70.4±0.22	3.1±0.12
4	MS+BAP(3.0)	70.0±0.13	5.3±0.06
5	MS+BAP(4.0)	74.0±0.10	8.2±0.22
6	MS+BAP(5.0)	76.0±0.20	11.0±0.04
7	MS+BAP(6.0)	75.0±0.16	8.4±0.18
8	MS+BAP(7.0)	74.6±0.11	6.0±0.24
9	MS+BAP(8.0)	74.0±0.04	4.8±0.15
10	MS+BAP(9.0)	63.0±0.18	2.2±0.15
11	MS+BAP(1.0) + IAA(1.0)	85.0±0.18	3.9±0.20
12	MS+BAP(2.0) + IAA(1.0)	89.4±0.24	5.8±0.16
13	MS+BAP(3.0) + IAA(1.0)	90.0±0.11	9.4±0.09
14	MS+BAP(4.0) + IAA(1.0)	90.4±0.10	13.0±0.12
15	MS+BAP(5.0) + IAA(1.0)	95.0±0.22	19.2±0.29
16	MS+BAP(6.0) + IAA(1.0)	88.0±0.26	10.2±0.26
17	MS+BAP(7.0) + IAA(1.0)	88.0±0.20	8.6±0.12
18	MS+BAP(8.0) + IAA(1.0)	76.5±0.18	5.5±0.18
19	MS+BAP(9.0) + IAA(1.0)	70.8±0.26	3.2±0.09
20	MS+BAP(1.0) + IAA(2.0)	70.0±0.14	3.0±0.08
21	MS+BAP(2.0) + IAA(2.0)	75.0±0.22	4.3±0.05
22	MS+BAP(3.0) + IAA(2.0)	83.2±0.14	6.8±0.12
23	MS+BAP(4.0) + IAA(2.0)	84.8±0.28	8.0±0.16
24	MS+BAP(5.0) + IAA(2.0)	86.4±0.24	14.2±0.22
25	MS+BAP(6.0) + IAA(2.0)	86.0±0.20	9.5±0.18
26	MS+BAP(7.0) + IAA(2.0)	76.4±0.18	7.0±0.14
27	MS+BAP(8.0) + IAA(2.0)	74.0±0.22	4.2±0.08
28	MS+BAP(9.0) + IAA(2.0)	64.2±0.14	2.8±0.20

Results are mean of twenty replicates Mean±SE.

TABLE 2. Effect of Gibberellic acid (GA_3) on *in vitro* shoot elongation.

S. No.	Plant growth regulator(mg/l)	Shoot length(cm)
1	MS	2.4±0.12
2	MS+GA_3(0.25)	4.8±0.08
3	MS+GA_3(0.50)	6.6±0.10
4	MS+GA_3(0.75)	5.4±0.15

Results are mean of twenty replicates Mean±SE.

TABLE 3. Effects of IAA and IBA on rooting of *in vitro* regenerated shoots

S. no.	Plant growth regulator(mg/l)	Rooting (%)	Root no. /shoot	Root length(cm)
1	MS	-	-	-
2	MS+IAA(0.25)	71.4±0.22	5.8±0.24	6.5±0.08
3	MS+IAA(0.50)	76.4±0.13	10.4±0.18	11.0±0.12
4	MS+IAA(0.75)	72.0±0.18	8.2±0.14	9.2±0.22
5	MS+IAA(1.0)	69.6±0.25	6.9±0.22	7.6±0.06
6	MS+IBA(0.25)	79.0±0.22	8.2±0.20	10.5±0.09
7	MS+IBA(0.50)	84.0±0.09	12.4±0.10	15.4±0.18
8	MS+IBA(0.75)	80.2±0.18	10.6±0.08	12.0±0.16
9	MS+IBA(1.0)	80.0±0.19	8.8±0.23	9.00±0.11

Results are mean of twenty replicates Mean±SE.

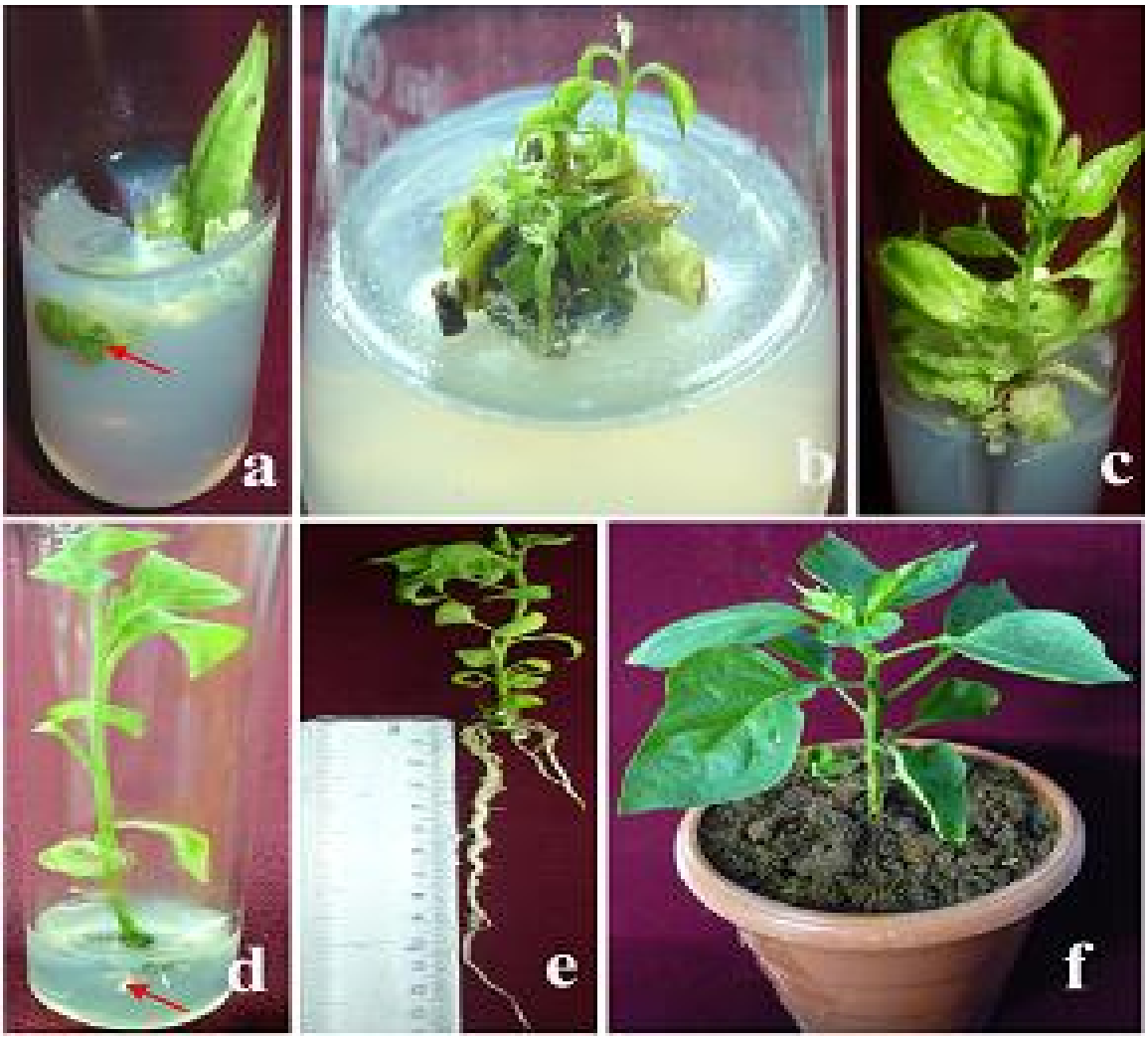

FIGURE 1. Direct adventitious shoot bud induction in cotyledon explants of *Capsicum annuum* L. cv. X-235

Legends:
1a): Initiation of shoot bud primordial
1b): Adventitious shoot buds proliferation
1c): Elongated shoot
1d): Plantlet showing root initiations
1e): Plantlet showing maximum root length
1f): Acclimatized plant.

The cultured explants showed direct organogenesis on MS media containing various levels of 1-9mg/l BAP alone or in combination with 1-2mg/l IAA; however, the explants cultured on MS medium without growth regulators failed to induce shoot proliferation. A progressive frequency of shoot bud proliferation was observed at lower concentrations of BAP, alone or combined with IAA (1.0mg/l), while decreased at higher concentrations of

BAP(6-9mg/l), alone or supplemented with IAA(2mg/l). The results obtained were strongly supported by earlier reports on chili peppers (*Capsicum* L.) (Arroyo and Revilla, 1991; Gunay and Rao, 1978; Mezghani *et al*., 2007; Mok and Norzulaani, 2007; Rodeva *et al*., 2006; Sanatombi and Sharma, 2008; Sripichitt *et al*., 1987; Valadez-Bustos *et al*., 2009) who stated that lower concentrations of BAP and IAA enhanced the maximum *in vitro* response. This may be due to the synergetic effect of IAA in combination with BAP on the enhancement of shoot multiplication (Sudha and Seeni, 1994). In the present investigation the shooting frequency and mean number of shoots per explant ranged from 63 to 95 and 2.2 to 19.2 respectively. It was found that MS medium supplemented with 5.0mg/l BAP and 1.0mg/l IAA promoted adventitious shoot buds with maximum number of shoots i.e., (19.2±0.29) which was significantly superior to other combinations (Fig. 1A and 1B). These results were agreed with according to the data reported by (Qin *et al*., 2005 and Ashrafuzzaman *et al*., 2009). The shoots 1.0-1.5cm in length were excised from the shoot cluster and transferred individually to cultures tubes containing MS medium with various levels of 0.25-0.75mg/l GA_3 for shoot elongation (Table 2). Elongation of shoots was higher i.e., (6.6cm) on MS medium containing 0.5mg/l GA_3 (Fig. 1C). This may due to the action of GA_3 on cell elongation (Qin *et al*., 2005). The elongated shoots were excised and implanted on MS medium fortified with different levels of IAA and IBA alone with 0.25-1.0mg/l concentrations respectively (Table 3). The optimum rooting frequency (%) (84.0±0.09), root number (12.4±0.10) and root length (15.4±0.18cm) per shoot was obtained on MS medium supplemented with IBA (0.5mg/l) (Fig. 1D and 1E). Similar results were also obtained by (Aniel Kumar and Subba Tata 2010). In the present investigation it was proved that the PGRs i.e., BAP and IAA could make the explants more responsive to induce adventitious shoot bud proliferation. The regenerated plants showed 80-90% survival during hardening and acclimatization, and there were no observable differences between the parent plant and *in vitro* raised plants. The transplanted plantlets established well in pots and in the field (Fig. 1F).

CONCLUSION

In conclusion of the present study underlines the importance of IAA and BAP concentrations for the induction of adventitious shoot regeneration from cotyledon explants of *Capsicum annuum* L cv. X-235, which could be recommended to future gene transformation protocols via agro infection.

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