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# *In vitro* regeneration of *Capsicum chinense* Jacq.

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**Abstract**

An efficient *in vitro* regeneration protocol was developed for *Capsicum chinense* Jacq. cv. 'Chiengpi' using shoot-tip and axillary shoot explants. Shoot-tip explants proliferated shoot buds in Murashige and Skoog (MS) medium supplemented with 5 or 10 mg/l 6-benzylaminopurine (BAP). The regenerated shoot buds showed rooting on medium containing 0.5 mg/l IAA. Axillary shoots were induced in the rooted plantlets by decapitating them and multiple shoots were further induced from the axillary shoot explants in medium containing BAP alone or in combination with IAA. Maximum shoot bud proliferation from the axillary shoot explants occurred in medium supplemented with 5 or 10 mg/l BAP. The proliferated shoot buds also showed rooting and elongation on medium containing 0.5 mg/l IAA. Rooted plantlets were successfully established in the soil.

**Keywords:** axillary shoot culture, *Capsicum chinense*, decapitation, shoot-tip culture.

## Introduction

Chillies are the fruits of the genus *Capsicum* belonging to the Nightshade family, Solanaceae. The genus *Capsicum* consists of about 25 wild and 5 domesticated species. The five domesticated species are *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum chinense* Jacq., *Capsicum baccatum* L., and *Capsicum pubescens* R & P.(1). Chillies have been forming a part of the human diet since the beginning of civilization in the western hemisphere from about 7500 B.C. (2). Chillies contain numerous chemicals including steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre and mineral elements (3) and are used in a wide assortment of foods, drugs, and cosmetics. Besides the above-mentioned uses, chillies also have medicinal uses. The medicinal use of chillies has a long history, dating back to the Maya and Aztec tribes of ancient America who used them to treat asthma, coughs and sore throats (4). The medicinal value of *Capsicum* is well-recognized today and it is included in the American Illustrated Medical Dictionary, the Merck Manual and Materia Medica, where it is referred to as a rubefacient, local stimulant and diaphoretic (5). Recently, several studies have also demonstrated anti-cancer or anti-mutagenic effect of chilli extracts (6-8).

*Capsicum chinense* Jacq. cv. 'Chiengpi' is an indigenous chilli cultivar cultivated in different parts of Manipur. The fruits of the cultivar are pungent with a characteristic aroma and flavour and both fresh and dried fruits of

this cultivar are used as hot spice. Although the plants of this cultivar are perennial and persist for 2-3 years, the production decreases successively from the first year and requires new planting material every year for optimum production. The production potential of this cultivar does not often show the achievable targets due to various reasons, and to meet the increasing demand for the crops, faster propagation techniques for mass multiplication has become necessary. Since the plants also lack natural vegetative propagation tissue culture methods provide a novel way for the asexual multiplication of these chilli pepper plants.

In *Capsicum*, several procedures are available for inducing *in vitro* plant regeneration using different explants (9-27). However, several of these reports suggest a strong influence of genotype on the regeneration process (13,15,16,19). Moreover, *Capsicum* tissue culture is mostly confined to the more common species, *Capsicum annuum* L. and there has been no report for the *in vitro* plant regeneration of *Capsicum chinense* Jacq. Therefore, the present study was undertaken to develop efficient *in vitro* plant regeneration protocol for the economically important chilli cultivar.

## Materials and Methods

Seeds extracted from fresh and healthy ripe fruits collected from local cultivation fields were used for initiation of *in vitro* cultures. The seeds were first washed with tap water and treated with 0.1% *Dhanustin* (carbendazim 50% w/w) for 10-15 min followed by washing with distilled water. The seeds were then surface sterilized under aseptic conditions with 0.1% $HgCl_2$ solution for 5 min followed by several washes with sterile distilled water. The surface-sterilized seeds were inoculated in 250 ml flasks containing sterile filter papers soaked in sterile distilled water and incubated in the dark for 7-10 days at 25±2 °C. After germination, the seeds were transferred to culture tubes containing Murashige and Skoog (MS) (28) basal medium. Shoot-tip explants (1-1.5 cm long shoot apices) were derived from four week-old *in vitro* germinated seedlings and inoculated on shoot bud multiplication medium consisting of MS basal medium supplemented with different concentrations of cytokinins, 6-benzylamino-purine (BAP) or kinetin (Kin) alone or combinations of BAP with indole-3-acetic acid (IAA). The number of shoot buds was counted after four weeks. The elongated shoot buds (about 2 cm long) proliferating from the shoot-tip explants were excised and cultured in 250 ml flasks containing 70 ml of MS medium supplemented with concentrations of 0.5 or 1 mg/l of IAA, indole-3-butyric acid (IBA) or α-naphthalene acetic acid (NAA) for rooting of the shoot buds. The percentage of rooting, number of roots (including the main roots and laterals), shoot length and the length of the roots were recorded after six-weeks of culture.

For induction of enhanced axillary shoot development, the axillary shoots were induced on four week-old rooted plantlets. These plantlets having 5-9 leaves were decapitated for inducing axillary shoot development by cutting the tips with a sterile blade. Axillary shoots developing in the axils of leaves of the decapitated plantlets were used for further multiple shoot bud induction by culturing on medium containing BAP alone or in combination with IAA and the number of shoot buds were counted after six weeks. The shoot buds elongating from axillary shoot-tip explants were excised and cultured on rooting medium consisting of MS medium supplemented with 0.5 mg/l IAA or IBA. All cultures were maintained in a growth chamber at a temperature of 25±2 °C and 16-h photoperiod provided by white fluorescent tubes (30 μmol $m^{-2}s^{-1}$).

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The rooted plantlets were gently removed from the flasks and the roots were washed in tap water to remove traces of agar. The plantlets were then transplanted in perforated paper cups containing sand: soil (1:1) and kept covered with clear polythene bags having a few holes on it for the initial 10 days. The plantlets were kept in a 50% shaded net-house and watered daily with tap water to maintain high humidity. After 10 days, humidity was gradually decreased by increasing the size of holes in the polythene bags and the polythene bags were finally removed. Four week-old hardened plants were then transplanted to bigger earthen pots or to the field.

All the experiments were repeated thrice and each treatment for shoot bud induction and rooting of the shoot buds consisted of ten replicates. Data on multiple bud induction and rooting were analyzed by Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test.

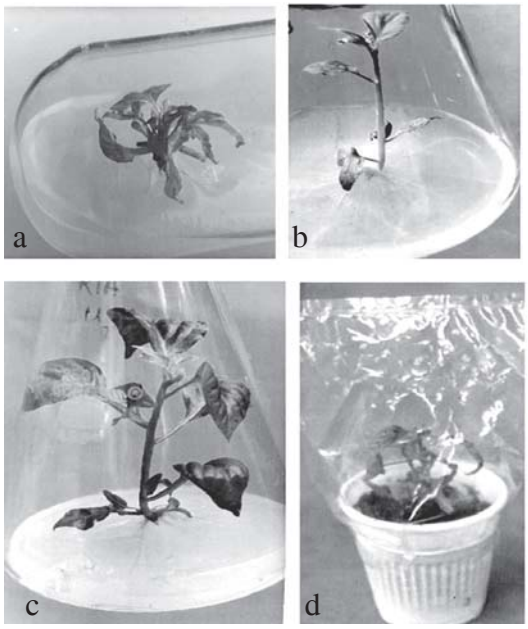


**Fig. 1**. *In vitro* plant regeneration of *Capsicum chinense* Jacq. cv. 'Chiengpi': a) shoot bud multiplication from shoot-tip explant; b) rooting of shoot buds; c) axillary shoot bud induction by decapitation of rooted plantlet; d) hardening of plantlet in plastic pots.

## Results

After 2-3 weeks of culture on the shoot bud multiplication medium, about 2-8 multiple shoot buds developed from the shoot-tip explants derived from *in vitro* germinated seedlings. The effect of growth regulators in shoot bud multiplication from shoot-tip explants is shown in Table 1. Maximum proliferation of shoot buds occurred on medium containing 5 or 10 mg/l BAP (Fig. 1a). The frequency of shoot buds obtained on MS medium containing Kin alone was low (1 to 3) with explants showing little or no growth followed by browning and therefore were not used for further experiments. When the regenerated shoot buds (about 1 cm long) were separated and transferred to MS medium supplemented with IAA, IBA or NAA, rhizogenesis occurred followed by elongation of the shoots (Fig. 1b). IAA and IBA were found superior to NAA with respect to the induction of roots and 42-100% rooting efficiency was recorded for the cultivar (Table- 2). On NAA containing medium, the roots produced were thick and short with fine root hairs while on medium containing IAA or IBA, the roots were thin and long with branches and root hairs. Best rooting occurred on medium containing 0.5 mg/l IAA or IBA and maximum shoot elongation occurred on medium supplemented with 0.5 mg/l IAA or 1 mg/l IBA (Table- 2).

In the next set of experiments, the effect of decapitation on enhanced axillary branching in the rooted plantlets and the effect of growth regulators on the multiplication of shoot buds from the axillary shoot-tip explants were studied. The decapitated plantlets showed the development of axillary shoots/branches within

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**Table 1.** Effect of growth regulators on multiple shoot bud multiplication from shoot-tip explants of *Capsicum chinense* Jacq. cv. 'Chiengpi' after four-weeks of culture

| Growth regulators (mg/l) BAP | IAA | Mean number of shoots per explant (mean ± S.E.) 'Chiengpi' |
|---|---|---|
| 2 | - | 1.2 ± 0.13$^{c}$ |
| 2 | 1 | 1.3 ± 0.21$^{c}$ |
| 2 | 2 | 1.1 ± 0.10$^{c}$ |
| 5 | - | 3.3 ± 0.5$^{4b}$ |
| 5 | 1 | 1.4 ± 0.22$^{c}$ |
| 5 | 5 | 1.2 ± 0.13$^{c}$ |
| 10 | - | 4.5 ± 0.43$^{a}$ |
| 10 | 1 | 2.6 ±0.45$^{b}$ |
| 10 | 5 | 1.3 ± 0.21$^{c}$ |

Means followed by the same letters are not significantly different at $P$ <0.01

**Table 2.** Effect of auxins on rooting and elongation of *in vitro* induced shoot buds from shoot-tip explants of *Capsicum chinense* Jacq. cv. 'Chiengpi' after six-weeks of culture

| Auxins (mg/l) | | | Rooting | Shoot length (cm) | No. of roots | Root length (cm) |
|---|---|---|---|---|---|---|
| IAA | IBA | NAA | (%) | (mean ± S.E.) | (mean ± S.E.) | (mean ± S.E.) |
| 0.5 | - | - | 100 | 2.5$^{a}$ ± 0.28 | 21.2$^{b}$ ± 1.29 | 6.3$^{a}$ ± 0.31 |
| 1 | - | - | 50 | 1.4$^{b}$ ± 0.25 | 5.7$^{cd}$ ± 1.93 | 2.1$^{b}$ ± 0.72 |
| - | 0.5 | - | 100 | 1.2$^{b}$ ± 0.16 | 28.4$^{a}$ ± 1.17 | 5.3$^{a}$ ± 0.49 |
| - | 1 | - | 60 | 2.7$^{a}$ ± 0.46 | 7.7$^{cd}$ ± 2.16 | 2.2$^{b}$ ± 0.63 |
| - | - | 0.5 | 100 | 1.3$^{b}$ ± 0.17 | 10.8$^{c}$ ± 0.81 | 1.0$^{bc}$ ± 0.14 |
| - | - | 1 | 40 | 0.6$^{b}$ ± 0.08 | 2.4$^{d}$ ± 1.06 | 0.2$^{c}$ ± 0.07 |

Means followed by the same letters are not significantly different at $P$ <0.01

**Table 3.** Effect of growth regulators on multiple shoot bud multiplication from axillary shoot explants of *Capsicum chinense* Jacq. cv. 'Chiengpi' after six-weeks of culture

| Growth regulators (mg/l) BAP | IAA | Number of shoot buds/explant (mean ± S.E.) |
|---|---|---|
| 2 | - | 1.1$^{d}$ ± 0.10 |
| 2 | 1 | 1.2$^{d}$ ± 0.13 |
| 5 | - | 3.1$^{b}$ ± 0.18 |
| 5 | 1 | 1.7$^{cd}$ ± 0.21 |
| 10 | - | 3.9$^{a}$ ± 0.31 |
| 10 | 1 | 2.1$^{c}$ ± 0.23 |

Means followed by the same letters are not significantly different at $P$ <0.01

two weeks of culture. About 3-6 young shoots per plantlet were formed within two weeks of culture (Fig. 1c). On culturing the axillary shoots with a few leaf primordia in bud induction media, these proliferated to produce multiple shoot buds. The maximum proliferation of shoot buds from the axillary shoot-tip explants occurred on medium containing 5 or 10 mg/l BAP (Table-3). The proliferated shoot buds also showed rooting and elongation on medium containing 0.5 mg/l IAA. The regenerated plants showed 80-90% survival during hardening (Fig. 1d) and acclimatization and there were no morphological variations between the parent plants and *in vitro* raised plants. The transplanted plantlets were established well in pots and later in the field.

**Discussion**

The effectiveness of different combinations of BAP and IAA in inducing shoot buds from different explants of *Capsicum* was reported in earlier studies (9,10,12,14,16,19,20). Therefore, *in vitro* culture response of shoot-tip and axillary shoot explants of the cultivar cultured on MS medium supplemented with various concentrations of cytokinins (BAP or Kin) and combinations of BAP with IAA have been investigated. Proliferation of multiple shoot buds from shoot-tip explants of *Capsicum* were reported in limited cases (15,24-26) and since *in vitro* clonal propagation via meristem culture is one of the ways for producing large number of disease-free plantlets, protocol for *in vitro* clonal propagation of the cultivar was developed using shoot-tip explants. The maximum proliferation of shoot buds occurred on medium containing 5 or 10 mg/l BAP. Similar effectiveness of MS medium containing BAP alone in inducing multiple shoot buds in chilli tissue culture was reported earlier (12,15,17,18). MS medium containing Kin alone was found to be the least effective between the two cytokinins (BAP and Kin) tested. Such ineffectiveness of Kin in shoot bud induction from chilli tissue culture has also been reported earlier (9,10,12). The shoot buds derived from the shoot-tip explants rooted efficiently on MS medium containing 0.5 or 1 mg/l IAA or IBA. Similar effectiveness of IBA and IAA on rooting of *in vitro* regenerated chilli plantlets was reported by several workers (9,12,15-17,22,23,26). Studies also reported higher effectiveness of NAA in inducing rhizogenesis of the regenerated shoots in *Capsicum* (21,29,30). However, in the present study, NAA was found to be less effective for root induction.

Earlier, we reported axillary shoot proliferation (up to 5) from *in vitro* raised chilli seedlings by decapitation (27). The response of axillary shoo-tip explants to bud multiplication media containing different concentrations and combinations of growth regulators were identical to the shoot-tip explants. Since the shoot buds derived from the shoot-tip explants rooted efficiently on MS medium containing 0.5 or 1 mg/l IAA or IBA, the buds derived from the axillary shoot-tip explants were also rooted on medium containing 0.5 or 1 mg/l IAA or IBA.

Thus, by inducing multiple shoots from the shoot-tip explant of a seedling followed by *in vitro* induction of axillary shoots from the regenerated plantlets and further induction of multiple shoot buds from the axillary shoot explants, it is possible to produce large number of plantlets from a single seedling. This technique, therefore, presents an efficient system of *in vitro* clonal propagation for conserving and mass multiplication of the chilli cultivar.

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