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In vitro plant regeneration in Capsicum chinense Jacq. (Naga Chili)

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ABSTRACT

An *in vitro* plantlet regeneration protocol was developed for *Capsicum chinense* Jacq. Naga Chili, one of the world's hottest chili cultivars and an important horticultural crop of Northeast India. *In vitro* propagation plays a major role in conservation of genetically pure plants, crop improvement and production of disease free planting materials. The effect of different compositions of plant growth regulators on multiple shoot development and callus induction was investigated. Multiple shoot was induced by culturing explants in MS medium supplemented with Benzyl adenine (BA) in combination with Indole-3-acetic acid (IAA). Maximum numbers of shoot buds were induced in MS medium containing 5 mgl^{-1} BA and 0.5 mgl^{-1} IAA. Successful induction of callus from stem segments of *in vitro* raised plants were achieved in MS medium in combination with 3 mgl^{-1}Benzyl adenine (BA) and 1 mgl^{-1} 1-Naphthaleneacetic acid (NAA).Shoot elongation and rooting were achieved in MS basal medium. This is the first successful report of plant regeneration from calluses in *Capsicum chinense* Jacq. Naga Chili. This protocol can be used as a cost effective method for the production of disease free planting materials and for genetic improvement and conservation of the crop.

1. INTRODUCTION

Naga Chili, belongs to the family Solanaceae is mainly cultivated in the Indian states of Assam and Nagaland. It is a very pungent chili, measuring 1,001304 Scoville Heat Units (SHU). It is locally called as Bhootjolokia or Naga jolokia in Assamese, and is native to North-Eastern India [1].

In vitro plant regeneration is essential for the rapid multiplication of disease free planting materials and is an imperative for the application of biotechnology tools to plant breeding and genetic improvement. It is also important for the conservation of genetically pure planting materials. Micropropagation is advantageous over traditional propagation as it can be used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It also leads to simultaneous accomplishment of rapid large-scale propagation of new genotypes [2]. The conventional method of chili plant propagation using seeds is restricted by the short span of viability and low germination rate of seeds. Chili plants are also highly susceptible to fungal and viral pathogens [3]. Lack of natural vegetative propagation in Chili is the limiting factor in conserving genetic purity and micropropagation can be a remedy for it.

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Even though there are several reports available on micropropagation of *Capsicum annum* [4-6] and *Capsicum frutescens* [7, 8], research on micropropagation of *Capsicum chinense* is still in infant stage. Only few reports are available on the *in vitro* regeneration in *Capsicum chinense* [9-11] and this is the first report on successful plant regeneration from callus in *Capsicum chinense* Jacq.

2. MATERIALS AND METHODS

2.1 Source of explants

Fresh and healthy ripe fruits were collected from Dimapur area of Nagaland state with the help of collaborating institute, Nagaland University.

2.2 Preparation of explants

Seeds were taken out using forceps, washed with tap water, then treated with 0.1% copper oxychloride for 10min and rinsed three times with distilled water. This was followed by surface sterilization with systemic fungicide, 0.1 % Bavistin, under aseptic conditions for 5 min, followed by several washes with sterile distilled water. The surface-sterilized seeds were inoculated in petri plates containing sterile filter paper soaked in sterile distilled water and incubated in the culture room for 7-10 days at 25±2 °C. After germination, the seeds were transferred to culture tubes containing MS basal medium [12] and allowed to grow for one week. These plants were used for multiple shoot induction

2.3 Culture medium and condition

MS medium was augmented with plant growth regulators *viz*., Indole-3-acetic acid (IAA), ∝-naphthalene acetic acid (NAA) and Benzyl adenine (BA) at different concentrations were used in this study. Sucrose was used as the carbon source at the rate of 30 gl^{-1} and 0.8% agar used in all experiments. The pH of the medium was adjusted to 5.8 and sterilized by autoclaving at 121°C for 20 min at 15lbs pressure. The cultures were incubated at 25±1°C and were given a photoperiod of 16 h with a light intensity of 3000 lux.

2.4 Multiple shoot induction

Plants grown in MS basal media were inoculated in multiple shoot induction medium consisting of MS basal medium supplemented with 8 different combinations of Benzyl adenine (2-7 mgl^{-1})and Indole-3-acetic acid (0-0.5 mgl^{-1}). The numbers of shoots were counted after four weeks of inoculation. The multiplied shoot obtained were excised and transferred in culture flasks containing MS basal media for shoot elongation and rooting.

2.5 Callus induction and plant regeneration

Stem segments of *in vitro* raised plants were used as explants. Explants were inoculated in callus induction medium consisting of MS basal medium supplemented with 6 different combinations of Benzyl adenine (1-3 mgl^{-1})and 1-Naphthaleneacetic acid (0-1 mgl^{-1}). Developed calluses were inoculated in multiple shoot induction media, MS medium supplemented with 5 mgl^{-1} BA with 0.5 mgl^{-1} IAA, for plant regeneration.

3. RESULTS AND DISCUSSION

In the present study, *in vitro* plantlet regeneration from shoot explants and callus induction in MS medium supplemented with various concentrations of auxins and cytokines are reported for *Capsicum chinense* Jacq. Naga Chili. Successful initiation of *in vitro* cultures is a crucial factor in the application of biotechnological tools.

Fig. 1: Seeds showing germination.

3.1 Micropropagation

There are several factors affecting the successful initiation cultures. *In vitro* regeneration of *Capsicum* species is reported to be difficult [13]. Successful germination of seeds achieved *in vitro*. Chili plants are more likely to be contaminated by fungus [3]. In the present study, only a few percentages (6.15 %) of cultures were found contaminated. Seed germination started after 7-9 days of inoculation and 90% of germination was obtained within 18-20 days {Fig. 1}.

3.2 Multiple shoot induction

An efficient protocol for the induction of multiple shoots *in vitro* has developed with an average of 5.2±0.16 shoots per culture. There are reports available on multiple shoot induction in other *Capsicum* species using BAP and IAA, where the maximum number of shoot proliferation achieved was 4.2±0.44 per explant [14]. Multiple shoot induction occurred in high concentration of BA, either alone or with a low concentration of IAA. Out of 8 different combinations tested (Table 1), media with a composition of MS basal + 5 mgl^{-1} BA + 0.5 mgl^{-1} IAA found to be more suitable for multiple shoot regeneration in Naga Chili {Fig 2}. In this study, an average of 5.2±0.16 shoots per explant achieved which could successfully transferred to induce roots and shoot elongation. Multiple shoot formation was observed after 14 days of inoculation. These shoots were excised and subcultured in MS basal media for shoot elongation and rooting. Similar results were achieved in *Capsicum chinense* where MS basal medium supplemented with Thidiazuron (TDZ) and 6-benzylaminopurine (BAP) as supplements [15, 16].

Fig. 2: Multiple shoot formation.

Table 1: Mean number of shoots per different combinations of plant growth regulators.

Growth regulators (mgl^{-1})			Mean number of shoots per explants (mean±SE)
Media	BA	IAA	
A	2	-	2.1 ± 0.17^{e}
B	2	0.5	2.4 ± 0.16^{de}
C	3	-	2.3 ± 0.15^{de}
D	3	0.5	2.8 ± 0.15^{d}
E	5	-	4.6 ± 0.16^{b}
F	5	0.5	5.4 ± 0.16^{a}
G	7	-	3.8 ± 0.13^{c}
H	7	0.5	4.2 ± 0.17^{bc}

3.3 Callus induction and plant regeneration

Successful induction of callus from stem segments were achieved *in vitro* and plantlets were regenerated successfully. Stem segments of *in vitro* germinated plants were used as explants. Explants were cultured on MS medium supplemented with

different combinations of BA and NAA and successful callus induction was achieved in a combination of MS basal + 3 mgl^{-1} BA + 1 mgl^{-1} NAA.91.01 % cultures showed callus development {Fig. 3}.Callus formation was observed after 2 weeks of culture initiation. The other media compositions had a very less or null influence in callus induction. Plant regeneration from callus was obtained in MS medium supplemented with 5 mgl^{-1} BA and 0.5 mgl^{-1} IAA {Fig. 4}.Previous report of induction of callus from placental tissues of *Capsicum chinense* were achieved in MS medium supplemented with 2mg/l 2,4-D and 0.5mg/l Kin [17]. But there is no report available on successful plant regeneration from callus in Capsicum *chinense*.

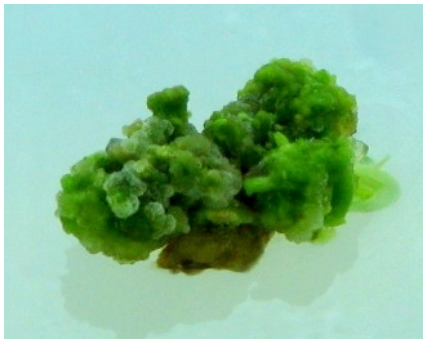

Fig. 3: Callus formation.

Fig. 4: Plant regeneration from callus.

Fig. 5: Shoot elongation with wide leaves.

3.4 Shoot elongation and rooting

Shoot elongation and rooting were achieved in MS basal medium though there is report available on the role of auxins in root development of *Capsicum chinense* [11]. Long shoots with wide leaves and long root formation was observed after 16-18 days in MS basal media (Fig 5 & 6).

Fig. 6: Root formation.

4. CONCLUSION

The present study demonstrates a simple and promising protocol for *in vitro* plantlet regeneration of *C. chinense* from shoot explants and callus derived from stem segments of *in vitro* raised plants. The use of BA in combination with IAA or NAA favored plant regeneration and callus induction respectively. This protocol can be applied for the conservation and multiplication of genetically pure and disease free genotypes and also for transgenic experiments in this species.

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