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In vitro micropropagation of *Capsicum Chinense* Jacq. (Naga King Chili)

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ABSTRACT

An in vitro micropropagation protocol was developed for Capsicum chinense Jacq. popularly known as habenaro pepper which is one of the world's hottest chilli and it is a traditional food item of the Naga people. Nagaland is believed to be the original home for the chilli pepper. The Guinness book of World record has awarded this chilli as hottest one as it contains 1,041,427 Scoville Heat units (SHU). In vitro propagation plays an important role in conservation, preservation of genetically pure plants, crop improvement, and production of disease free and disease resistant plants. The effect of Plant growth regulators were utilised in different concentration with combination for the regeneration of multiple shoot proliferation and callus induction. The study revealed that MS medium supplemented with BAP alone or in combination with IAA, Adenine Sulphate for multiple shoot development. Maximum numbers of shoot buds were produced in MS medium containing 19.98 µM BAP with 5.71 µM IAA and 13.31 µM BAP with 13.56 µM Ad.S. Successful induction of Callus from leaf and internodal part of in vitro raised plants were achieved in MS medium in combination of 6.66 µM BAP with 9.05 µM 2.4.D and 6.66 µM BAP with 8.06 µM NAA respectively. The rooting was achieved on MS medium containing 7.36 µM IBA, and the rooted plantlets were transplanted to the sterile soil to acclimatize the green house condition for resumed growth.

Keywords: *In vitro* micropropagation, Habenaro pepper- *Capsicum Chinense*, Multiple shoot proliferation, Callus induction.

INTRODUCTION

Capsicum chinense Jacq. is a semi-perennial herb belongs to the family Solanaceae, native of Northeast of India and Cultivated in West Bengal and Nagaland. Fruits of *Capsicum chinense* Jacq. has been confirmed as the World hottest chilli from the Guinness book of World records (2006). It is locally called as Bhut jolokia, Naga jolokia, Bih jolokia [1, 2].

The application of Modern Biotechnology to enhance the productivity of habenaro pepper requires an efficient *in vitro* protocol. In order to improve propagation of the commercial cultivars of this species and to meet the increasing demand for the crops, more reliable propagation approaches were need for the mass multiplication. The habenaro pepper plant propagation using seeds is restricted as it has a short span of viability with low germination rate and also it is highly susceptible to fungal and viral pathogens [3].

The natural vegetative propagation is limited in chilli, so conservation of genetic purity is very important through micropropagation. It has several medicinal properties like anti-inflammatory, analgestic, carminative, rubefacient and also possess powerful antioxidant, anti-mutagenic, anti-tumoural, hypoglycaemic, antifungal and antimicrobial activities have also been seen [4]. It is used as a counter irritant for the treatment of rheumatism, lumbago and neuralgia. It has been popularly used in both Ayurveda and Homoepathy system of medicine.

In vitro propagation through shoot tip and axillary shoot culturing is an easy and less economic way for obtaining large number of disease-free, consistently uniform and true-to- type plants within a short span of time. However, many attempts had been made by several workers which shows very mere satisfactory results with respect to the

increase in shoot number because the regeneration capacity is very poor in this species. Therefore, it is very essential to develop an efficient protocol for *in vitro* micropropagation of haberano pepper for the subsequent multiplication of the plantlets [5]. Chilli pungency is most commonly measured in Scoville heat units (SHU) by High Performance liquid chromatography (HPLC) methods which usually determine both the amount of capsaicin and pungency in a chilli sample [6].

The effect of plant growth regulators were supplemented with MS medium with different concentration and combination with Adenine sulphate is tried and successful induction of multiple shoots is seen in *Capsicum chinense* Jacq.

MATERIALS AND METHODS

Collection of plant materials

The fresh and healthy riped fruits were collected from the farm and local market of Assam and Gwahati and grown in the earthen pots in the shade house of Department of Botany, Pachaiyappa's college, Chennai-30, Tamilnadu, India.

Preparation of explants

Seeds were separated from fruits by using forceps and were sown in various pots and were kept in the shade house of our college to maintain humidity because it is a sun loving plant [7]. The seeds were disinfected with 70% ethanol for 60 minutes followed by three to four rinses with sterile distilled water. This was followed by Surface sterilization with lebolene for 5 minutes and then with systematic fungicide 0.1% bavistin under aseptic condition for 5 minutes, and were washed with sterile distilled water for several times. Then, the seeds were inoculated in sterilized petriplates containing sterile filter paper soaked in doubled distilled water and incubated in the culture room for 7-10 days at 25±2°C. After germination, the seeds with plantlets were transferred to culture tubes containing MS basal medium and allowed to grow for a week [8]. These Plantlets were used for further process. Shoot tip, axillary buds, leaves, nodal and internodal parts of *Capsicum chinense* were used as the explants for further study.

Media preparation and culture conditions

MS medium containing plant growth regulators such as, BAP, IAA, Ad.S, NAA, 2.4.D, at different concentration were used in this study. Sucrose was used as a carbon source 3 % (W/V) sucrose, 0.4 %(W/V) Phytagel a gelling agent and media is adjusted to pH 5.8 to 6.0**.** It is sterilized by autoclaving at 121°C for 10 min at 15 psi pressure. The cultures were incubated at 24±2°C and a photoperiod of 16 h with light intensity of 3000 Lux and 8 hours dark [9].

Multiple shoot bud induction

Shoot tips, nodal parts and axillary buds were used as explants. The explants were inoculated in the MS medium containing 3%(W/V) sucrose, 0.4%(W/V) Phytagel and supplemented with BAP alone, or in combination with 5.71 µM IAA and Adenine Sulphate for Shoot induction [7]. The numbers of shoot buds developing on the shoot tip explants were recorded after four weeks of culture.

In vitro Callus induction and plant regeneration

Internodal segments of *in vitro* grown shoots were used as explants. Leaves and Callus were induced on MS medium supplemented with different concentration and combination of BAP, NAA and 2,4-D. Developed Calli were inoculated in multiple shoot induction media for Plant regeneration [10].

Experimental design and Statistical analysis

The experiment was carried out in a completely randomized factorial design. Data's were statistically analyzed using the SPSS software and One way analysis of Variance (ANOVA) indicated significant treatment effects.

RESULTS AND DISCUSSION

Sprouting of seeds was observed after 7th day of inoculation and 95% of sterile seedlings were obtained. *In vitro* plantlet regeneration from shoot explants and callus induction in MS medium supplemented with various concentrations of auxins and cytokinins are reported for *Capsicum chinense* Jacq. (Naga King Chilli). *In vitro* regeneration of Capsicum species is reported to be difficult [6,11] from nodal and shoot tips explants loosely arranged pale green friable calli were obtained after 30 days of inoculation.

Multiple shoot bud induction

An effective protocol was developed for multiple shoot induction under *in vitro* condition and an average of 3-4 shoots per culture were obtained. [18,19]. The maximum number of shoot induction were observed on MS medium

supplemented with BAP+IAA, BAP+Ad.S (19.98 µM +5.71 µM) and (13.31 µM +13.56 µM) were shows the number of multiple shoots [12,13,14]. Multiple shoot induction developed in higher concentration of BAP, either alone or with low concentration of IAA and also with Adenine Sulphate. Out of different combination tested **(Table-1)** media with composition of MS basal medium +19.98 µM BAP with 5.71 µM IAA and 13.31 µM BAP+13.56 µM Ad.S. Adenine sulphate is found to be the most suitable for multiple shoot proliferation in *Capsicum chinense*. In the present study shows that 19.98+5.71(µM) BAP +IAA and 13.31+13.56 (µM) BAP+Ad.S shows to the 3.40±0.69 and 3.30 ±0.67 shoots per explants achieved which could be a successfully transferred to induce shoot elongation and rooting. Multiple shoot formation was observed after 14 days of culture. These shoots were excised and subcultured on MS medium along with plant growth regulator for shoot elongation and rooting. The similar results were achieved in *Capsicum chinense* Jacq. where MS medium supplemented with TDZ and Zeatin [11, 15].

Table-1. Effect of different concentrations of BAP, IAA and Ad.S on multiple shoot bud induction from explants of *Capsicum chinenese* Jacq. after four weeks of culture

Medium (µM)	Percentage of shoot initiation	Number of shoots (mean±SD)	Length of shoot(cm) (mean ±SD)	Number of leaves (mean ±SD)
BAP+IAA				
13.31+5.71	58.31±0.91 ^{a}	1.40±0.51 ^{a}	2.26±0.72 ^{b}	3.90±1.10^{a}
15.53+5.71	64.09±0.8 ^{c}	1.50±0.52 ^{a}	2.40±0.56 ^{bc}	7.00±0.94 ^{bc}
17.74+5.71	78.19±0.32 ^{e}	2.50±0.70 ^{b}	2.08±0.58 ^{c}	7.90±1.10 ^{cd}
19.98+5.71	85.57±0.14 ^{f}	3.40±0.69 ^{c}	3.50±0.52 ^{d}	9.80±1.47 ^{e}
BAP+Ad.S				
13.31+5.42	60.14±0.18 ^{b}	1.60±0.51 ^{a}	1.45±0.28 ^{a}	4.10±0.56 ^{a}
13.31+8.13	63.95±0.79 ^{c}	2.40±0.51 ^{b}	2.40±0.51 ^{bc}	6.20±1.22 ^{b}
13.31+10.85	71.53±0.59 ^{d}	2.60±0.51 ^{b}	2.85±0.47 ^{c}	7.00±0.94 ^{bc}
13.31+13.56	84.53±0.85 ^{f}	3.30±0.67 ^{c}	3.55±0.49 ^{d}	8.20±1.93 ^{d}

Means followed by the same letters are not significantly different at p=0.01

Table-2. Effect of different concentrations of BAP and IBA on root induction of *Capsicum chinense* Jacq after six weeks of culture

Plant growth regulator(BAP) (µM)	Plant growth regulator(IBA) (µM)	Percentage of root initiation (mean±SD)	Number of roots per shoot (mean±SD)	Length of roots(cm) (mean ±SD)
8.87	-	30.00±8.16 ^{a}	2.40±0.69 ^{a}	2.55±0.68 ^{a}
11.09	-	39.53±6.83 ^{b}	3.00±0.94 ^{ab}	3.31±0.41 ^{b}
13.31	-	45.58±9.55 ^{b}	3.30±1.05 ^{b}	3.42±0.20 ^{b}
8.87	3.68	39.00±7.37 ^{b}	2.80±0.91 ^{ab}	3.49±0.27 ^{b}
8.87	4.90	42.84±5.64 ^{b}	4.10±0.31 c	4.42±0.24 ^{c}
8.87	7.36	56.72±8.96 ^{c}	4.60±0.69 ^{c}	4.54±0.22 ^{c}

Means followed by the same letters are not significantly different at p=0.01

Table-3. Response of callus to combination of hormones (µM)

Combination of hormones(µM)			Proliferation	Percentage of response	Colour and Texture
BAP	2,4-D	NAA			
8.87	-	-	Moderate	60	White friable
11.09	-	-	Moderate	67	Soft, white friable
13.31	-	-	Moderate	70	Soft, white friable
4.44	4.52	-	High	80	Soft, white friable
4.44	6.78	-	High	83	Soft, white friable
6.66	9.05	-	High	87	Soft, white friable
4.44	-	5.37	High	83	Pale green
4.44	-	6.71	High	84	Pale green hard and off-white
6.66	-	8.06	High	87	Pale green hard and off-white

Callus induction

Callus induction was observed in culture tubes with different concentration and combination of growth hormones **(Table-3)**. The explants such as node, internode and leaves segments which show a better response in callus induction, particularly the leaves which showed 90% capacity for the formation of callus. Mostly leaf produced calli are white friable callus and internode produced calli are compact pale green and creamish coloured callus. Mostly calli are friable granulose callus initially white, they become creamy white after second subculture. As the result of rapid growth and density of the cellular suspension, sub culture was carried out at every 14 days. The spongy and compact calli showing different tone of green, although creamy white and brown tones where observed. The spongy and compact calli grew quickly and turned brown colour. Explants were cultured on MS medium supplemented with different combination of BAP, NAA and 2,4-D and successful callus induction were obtained [10]. MS basal medium + 6.66 µM BAP+8.06 µM NAA and 6.66 µM BAP+ 905 µM 2,4-D were showed successful callus development. 90 % of cultures showed callus development (fig 2). Callus formation was observed after 2 weeks of

culture. Plant regeneration from callus was obtained in MS medium supplemented with 19.98+5.71(µM) BAP + IAA [16].

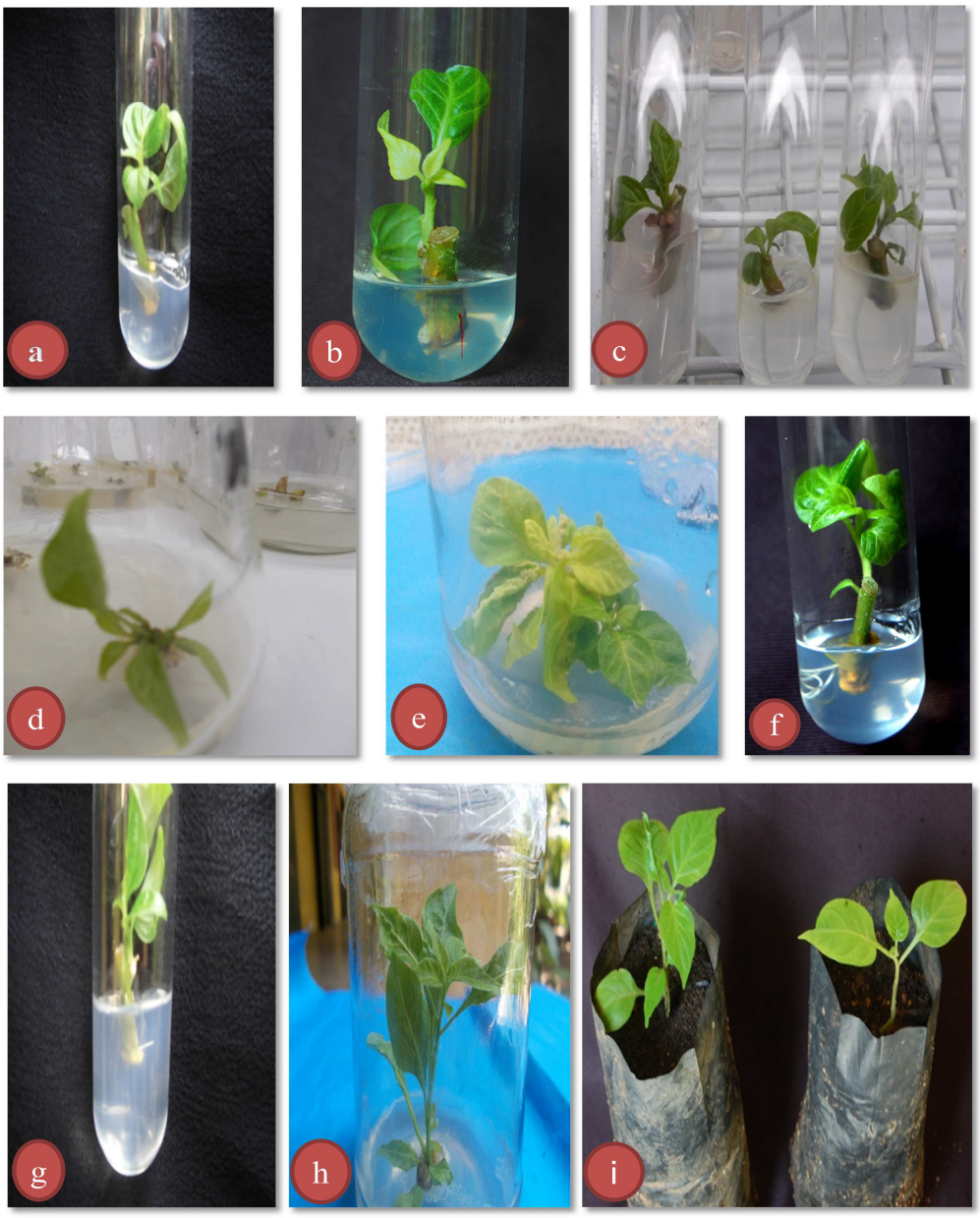

Figure: 1. Effect of 6-benzylamino purine, Indole 3-acetic acid and Adenine sulphate on shoot induction of *Capsicum chinense* Jacq.cv. Naga king chilli on MS medium. (a-c) Shoot proliferation from the nodal segment explants on MS medium supplemented with BAP+IAA and BAP+Ad.S after 16 days of culture; (d-e) formation of multiple shoot buds; (f-g) formation of roots from regenerated shoots cultured on MS medium supplemented with various concentration of IBA; (h) Complete plantlets of *Capsicum chinense* Jacq. Developed *In vitro*; (i) secondary hardening: transferred to a polythene bags containing hardening mixture

Shoot elongation and Rooting

The proliferated shoot buds showed rooting and elongation in media containing IAA and IBA. Shoot formed roots on media containing 8.87µM BAP and 7.36 µM IBA which showed very long and thick roots helps for food conduction [17]. Number of roots also increased by adding the correct concentration and combination of growth hormones. **Table-2** Shows the results of root induction and percentage of root proliferation. These rooted plantlets were decapitated again and used for further induction of axillary shoots. Rapid multiplication of the plantlets was achieved within a short span of time [18,19].

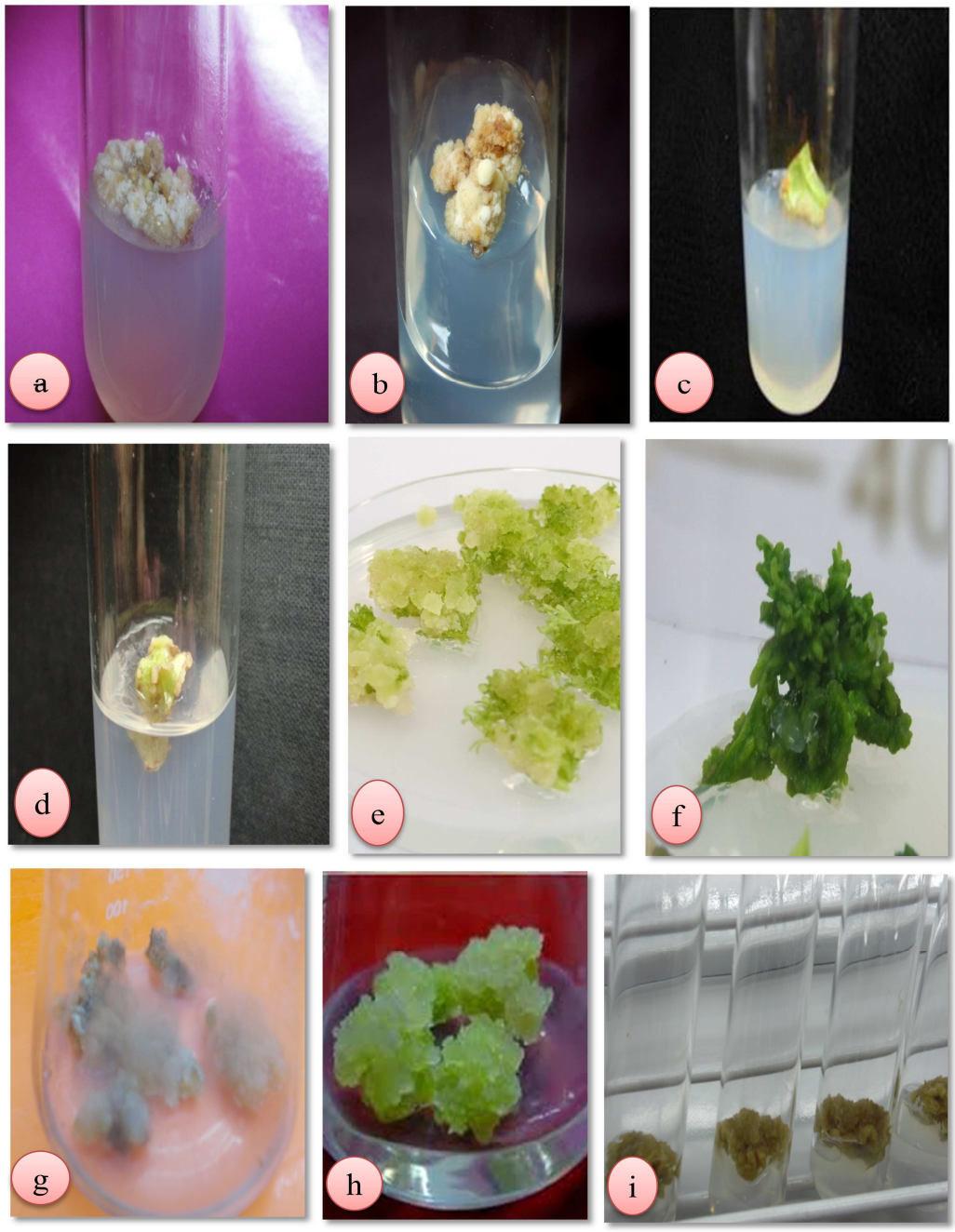

Fig. 2. Callus induction from leaves, nodal and intermodal segment explants on MS medium supplemented with BAP alone, BAP+2.4-D and BAP+NAA.(a-b) white friable callus; (c-d) callus induction from leaves; (e-f) green callus; (g-h) callus mass propagation; (i) pale brown callus

CONCLUSION

The experimental study reveals that a simple and standard protocol for *in vitro* micropropagation of *Capsicum chinense* from nodal segment and shoot tips which helps to produce true-to-type and disease free plants. The use of BAP, BAP+IAA, BAP+Ad.S, BAP+NAA, and BAP+2,4-D favour the development of plant and callus induction respectively [10]. The similar results were also obtained by Christoper & Rajan, Sanatombi K & Sharma GJ, reported the formation of shoot buds and elongated shoots from the explants. This protocol may be applied for conservation and large scale propagation of individual genotype of this habenaro pepper species [11,19, 20].

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