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Research Article

IN VITRO CLONAL PROPAGATION OF NAGA KING CHILLI (*Capsicum chinense* Jacq.)

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Abstract: Shoot tip explants were used to standardize the protocol for plantlet regeneration of Naga King Chilli (*Capsicum chinense* Jacq.) at the Department of Horticulture, Nagaland University. Shoot tips (1-2 cm) were cultured on MS medium supplemented with either BAP or KIN @ 0-8 mg L^{-1} respectively for shoot proliferation, alone and in combination with 0.5 mg L^{-1} IAA. The medium containing 8 mg L^{-1} BAP + 0.5 mg L^{-1} IAA recorded the maximum response (83.33%) for shoot proliferation. After six weeks of culture, maximum shoots (3.39) and shoot length (1.3cm) was recorded in the same medium which was further taken up for further sub culturing upto third cycle. MS basal medium was used for further elongation of the regenerated shoot buds. MS medium supplemented with auxins *viz*. IBA and IAA @ 0 - 1.5 mg L^{-1} alone was used for root formation. 1mg L^{-1} IBA enriched media recorded highest number of functional roots (11.36 per explant) and the rooted plantlets were acclimatized in pre-sterilized moist cocopeat, sand and farmyard Manure @ 1:1:1 ratio and were maintained under shade condition.

Keywords: *Naga King Chilli, Shoot Tip, Shoot Regeneration, Subculture, Rooting*

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Introduction

Naga King Chilli (*Capsicum chinense* Jacq.) belongs to the family Solanaceae, native to North-Eastern India more particularly to Nagaland [1]. *Capsicum chinense* Jacq. has received the attention of scientific community throughout the world due to its unique aroma and high capsaicin content. Most of the chilli species and varieties cultivated in India contain around 1% capsaicin but Naga King chilli has around 2–4% capsaicin as reported by various researchers [2, 3]. Capsaicin is mainly used as a spice, as food additives and in pharmaceutical applications showing anti-cancer effect [4], antimicrobial and carminative, against obesity and cholesterol [5]. It is a self-pollinated species but occurrence of high cross pollination leads to the formation of variants within Naga King Chilli, leading to heterozygosity and true-to-type plants cannot be maintained. Even though other solanaceous members easily undergo morphogenesis, chilli was found to be highly recalcitrant due to the formation of ill-defined bud like structures and rarely of well-developed shoots. The conventional method of propagation using seeds is restricted by the short span of viability and low germination rate of chilli seeds. Chilli tissue culture is mostly confined to *Capsicum annum* L. and *Capsicum frutescens* L. and very merge information for C. *chinense* Jacq. Since, the plant lacks natural vegetative propagation, plant tissue culture technique provides an alternative method of propagating novel genotypes asexually. The amount of capsaicinoids in a chile pepper pod is dependent on the genetic makeup of the plant and the environment where it is grown [6, 7]. Growing environment, sowing time and crop geometry influenced the synthesis of capsaicin or capsaicinoids in Chilli pepper [8]. Based on the commercial importance of naga King Chilli, the Nagaland government obtained the Geographical Indication of Goods (Registration and Protection) Act in 2008, to provide some safety net to Naga farmers in the cultivation of the King Chilli [9]. The present research involving culture of shoot tip as explants of *Capsicum chinensis* was undertaken to study the effect of different levels of *cytokinin* (BAP & kinetin) alone and in combination with IAA and levels of auxins (IAA & IBA) in producing shoots and roots respectively.

Materials and Methods

The present study entitled "*In vitro* clonal propagation of Naga King Chilli (*Capsicum chinense* Jacq.)" was carried out at the Department of Horticulture, School of Agricultural Sciences and Rural Development, Nagaland University. The popular genotype *Capsicum chinense* Jacq. cv. Naga King Chilli was chosen for the study, because the cultivar is native to North-Eastern India for its unique aroma and high capsaicin content which is location specific and the plants of the same genotypes grown under different environmental condition vary from one another in various aspects which proves to be a boon to bring about improvement works. Healthy and ripe fruits were taken from the selected plants which were grown under proper care at Horticulture Instructional Farm, Nagaland University. Seeds extracted from mature ripe fruits were washed in running tap water and treated with Bavistin solution (0.1%) for 15 minutes followed by rinsing several times with distilled water. The seeds were surface sterilized by immersing in 70% ethyl alcohol for 1 minute under the laminar low cabinet with vigorous shaking, followed by 4% sodium hypochlorite for 5 minutes. The seeds were then rinsed with sterile distilled water to remove the traces of disinfectant liquid and blot dried. Seeds were then sown in petri dishes containing sterile filter paper soaked in distilled water and incubated in dark for 7-10 days at 25±2ºC. After germination, the seeds were inoculated on MS basal medium and allowed to grow. Six-weeks-old *in vitro* germinated seedlings were used as the source of explants. Shoot tips (1–2 cm) were excised from these seedlings and implanted in the culture medium. Murashige and Skoog (1962) medium which has relatively high concentration of nitrate, potassium and ammonium ions when compared to other nutrient media were used in the present investigation.

Shoot regeneration

Shoot tips (1–2 cm) high were cultured in modified MS medium supplemented with ±30gm sucrose in 9 different concentrations of cytokinins (BAP & KIN) @ 0, 2, 4, 6, 8 mg L^{-1} respectively alone and in combination with 0.5 mg L^{-1} IAA, for shoot proliferation.

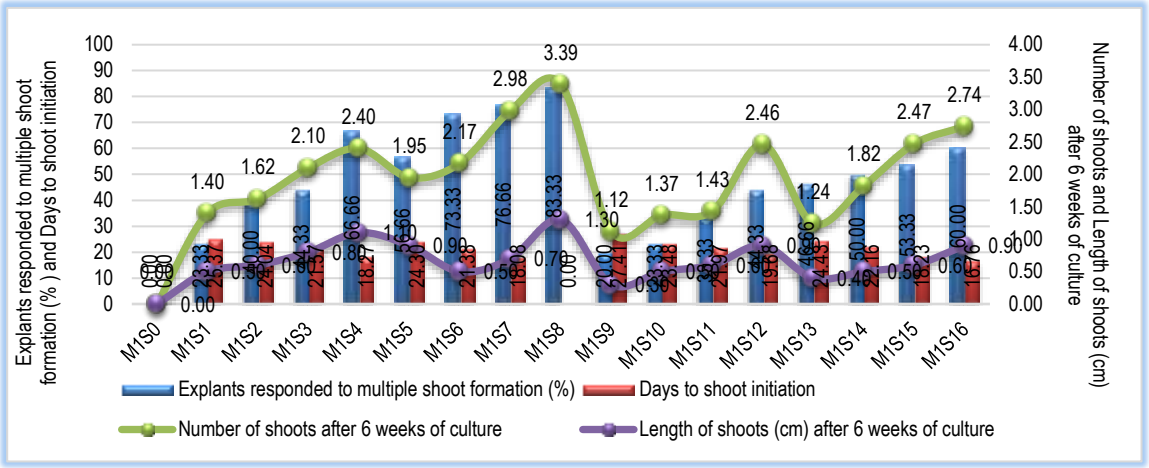

Fig-1 Response of different growth regulators to multiple bud induction of shoot tips of *Capsicum chinense* Jacq. cv. Naga King Chilli.

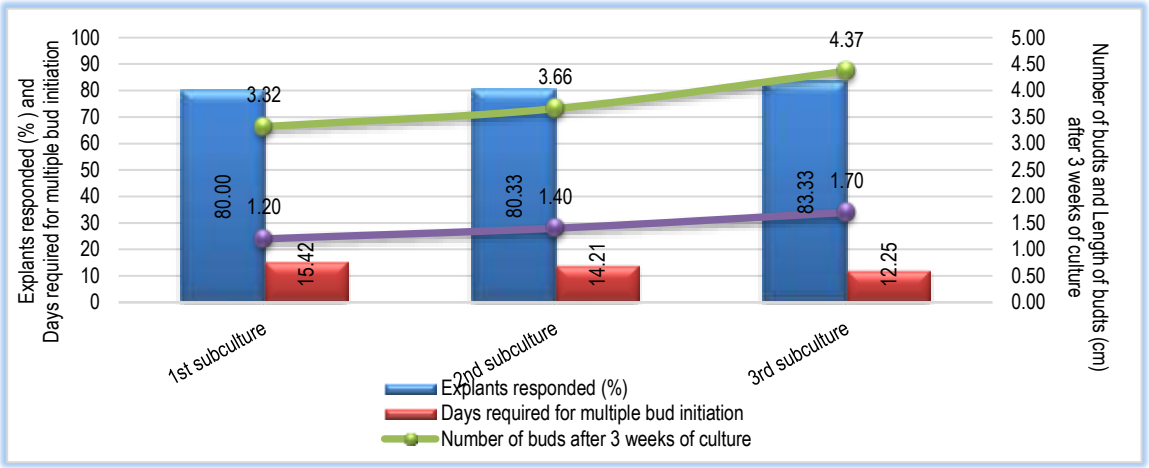

Fig-2 Subculture of Naga King Chili (*Capsicum chinense* Jacq.)

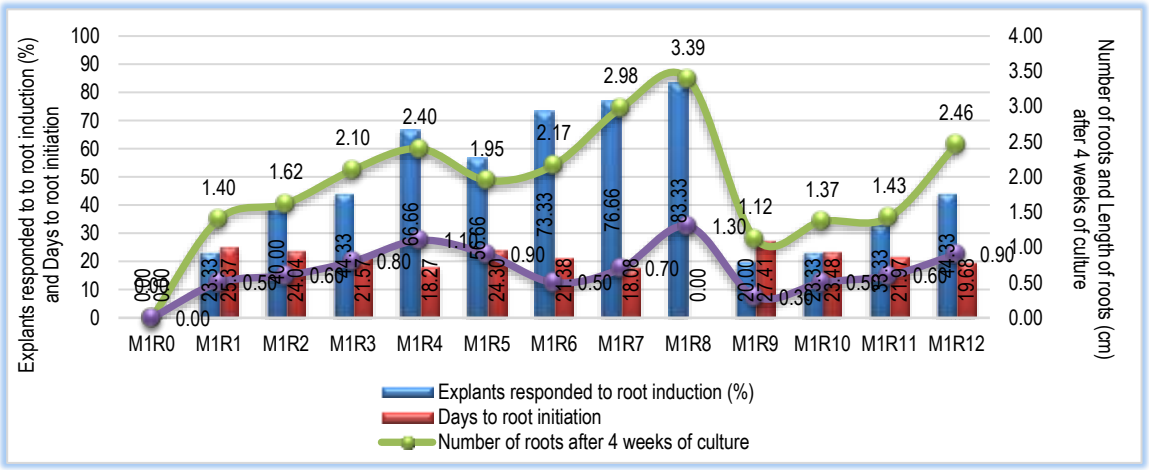

Fig-3 Response of different growth regulators on rooting of shoot tips of Naga King Chili (*Capsicum chinense* Jacq.)

After 4 weeks of multiple buds' initiation, Number of shoots responded in each medium, number of buds developed, average length of multiple buds was also recorded. After 4 weeks of culture, best recorded medium was used for further multiplication of propagules, for further regeneration of shoots and growth response at lower concentration of cytokinin in different sub culture treatments. They were subculture every 3 weeks interval in the best recorded modified MS medium for multiple buds' proliferation. The multiple buds were cut separately (containing 1-2 buds each) and transferred into separate bottles containing well-prepared medium for getting enough multiple buds for testing multiplication potentiality in each media. The regenerated shoot buds were placed on MS basal media for shoot elongation.

Root induction

Well elongated shoots were transferred to MS medium supplemented with various concentrations of auxins (IAA & IBA) @ 0, 0.2, 0.5, 0.8, 1.0, 1.2, 1.5 mg L^{-1} alone to optimize protocol for root formation. The rooted plantlets were hardened in pre-sterilized moist cocopeat, sand and farmyard manure @ 1:1:1 ratio and were maintained under shade condition.

Statistical analysis

The effects of treatments were tested by Analysis of Variance; differences among the treatment means were tested by Duncan's Multiple Range Test (DMRT), (Duncan, 1955). The levels of probability used for 'F' test was at 5%.

Results and Discussions

Response of different growth regulators to multiple bud induction

Plant growth regulators are the essential part of *in vitro* regeneration of crop plants in any artificial medium. Organ differentiation in plant is regulated by the interplay of auxins and cytokinins [10]. By changing the amounts and types of growth regulators in the medium, the cells can be stimulated to develop into shoots and/or roots or may even die, if the medium solidification is changed, the micro-propagation efficiency also altered [11]. Generally, cytokinin helps in shoot proliferation and auxin helps in rooting of proliferated shoots. The regeneration systems of *Capsicum* reported so far had shown the critical effect of cytokinin, cytokinin-cytokinin or cytokinin-auxin ratio in regeneration from various explants [12-14]. The nodal segment explants differed significantly in respect to shoot proliferation under various medium concentrations. After 6 weeks of inoculation, the morphogenic responses of the shoot tips (1–2 cm) were recorded (Fig: 1). Treatment difference for the number of shoots per explants was noticed in a time period of 15±1.41 to 27±1.41 days. Shoot bud induction was observed earliest (15.61 days) in M_1S_8 (8 BAP mg L^{-1} + 0.5 mg L^{-1} IAA) medium with maximum response (83.33%) for shoot formation and maximum days taken (27.41 days) in M_1S_9 (2 mg L^{-1} KIN) medium (Fig. 1). The results are in conformity with the findings of [15, 16, 17] in diverse cultivars of *Capsicum annum* L in the regeneration of plants from nodal explants. After 6 weeks of inoculation, it was recorded that the maximum number of shoots (3.39) was observed in M1S8 which was closely followed by M_1S_7 (6 BAP mg L^{-1} + 0.5 mg L^{-1} IAA) with the value of 2.98 and medium 8mg KIN+ 0.5 mg L^{-1} IAA with 3.25 and found to be at par. In the present study, the length of the multiple buds did not elongate satisfactorily but the media showed significant differences among them. Among the various media tested, the highest length of multiple buds was recorded in M^1S^8 (1.8 cm). This investigation was initiated to study the effect of cytokinins *viz*. BAP and KIN (2-8 mg L^{-1}) alone and in combination with 0.5mg L^{-1} IAA on shoot proliferation from shoot tips and nodal segments of C. *chinense Jacq*. cv. King Chili. The highest mean shoots were obtained on medium supplemented with high levels of BAP alone and in combination with IAA than in KIN. The results are in close conformity with [18] who reported that BA was found to be more effective than Kn for multiple shoot proliferation from apical shoot meristem explants of Capsicum. The results are constant with those of previous reports in which BAP was used successfully to regenerate shoots [12, 19, 20]. Reports show that low concentrations of BAP (8.8-22.2 µM) alone or in combination with 0.6-11.4 µM IAA was found to be effective for shoot bud induction from various explants in chilli tissue cultures [12, 21, 22]. However, in the present study, when BAP was used in combination with 0.5 mg L^{-1} IAA, the number of shoot buds increased with the increase in BAP concentration beyond mg L^{-1}. Similar results in *Capsicum chinense Jacq*. cv. Umorok was reported [23]. Similar conclusions that, BAP along with low concentrations of other auxins promoting organogenesis, have been drawn by others [3, 24].This might be attributed to the BAP uptake and metabolism which was subsequently converted to isopentyl adenine (iP) and isopentyl adenosine (ipR) inhibiting biosynthesis activity of cytokinin on cytokinin action of shoot development. The results partially agreed with those of [25] with respect to the media composition and better induced shoot bud number. They reported that the maximum number of shoot buds per nodal explants was in MS medium supplemented with BAP (5.0 mg L^{-1}) and IAA (0.5 mg L^{-1}) for all the three genotypes of Capsicum annum L tested. However, [26, 27] reported that TDZ alone or combined with IAA ranks as the best shoot bud inductor in nodal explants of the cultivar *Capsicum annum* L. cv. Pusa Jwala. Such results obtained may be due to auxins or cytokinins used alone or in combination, which is supposed to be the result of the promotion of biosynthesis or inhibition of degradative metabolism [28].

Sub culturing of Naga King Chili (*Capsicum chinense* Jacq.)

For further regeneration, the medium showing best performance for shoot bud induction was taken forward. In this study the medium M_1S_8 recorded the best response and therefore was taken forward for sub culturing studies upto the third cycle. The culture cycle was repeated at three weeks interval. Regeneration with 1-2 shoot tip per explants were subculture in M_1S_8 medium that remained quiescent for nearly a week. After about 2-3 weeks, explants showed signs of bud growth significantly. It was recorded that minimum days (12.25 days) required for bud initiation was in the 3rd subculture and the maximum (15.42 days) on the 1st cycle. Significant differences in the number of buds per explants were observed with significant increase at every cycle. The 1st subculture recorded 3.32 numbers of buds and the highest was proliferated during the third subculture (4.37). Maximum length (1.7cm) of the bud was also recorded on 3rd subculture. (Fig: 2). It was found that higher concentration of cytokinin and lower levels of auxin is found beneficial to influence shoot buds formation in the various stages of subculture. It was also observed that explants cultured and subculture to the same treatment consistently gave lowest production of buds. However, [29] reported that bud formation could be enhanced when the explants were subculture to medium containing same concentration of BAP but reduced concentration of IAA. They also reported that Cytokinin to auxin ratio, their concentration in culture and subculture media, duration of culture and subculture are crucial determinants in the induction of buds and shoot formation. However, they recommended sub culturing every two weeks in order to obtain higher percentage of bud formation and avoid browning of the explants. Prolonged culture in high concentration of BAP inhibited bud development and leaf expansion, hastened browning and caused abnormality of the explants. Explants without subculture exhibited callus formation and a slower rate of bud induction. The findings are in accordance with the results of [30, 25]. The regenerated shoots were separated and replanted for shoot elongation in the basal medium. It was noticed that the regenerated shoots underwent fair shoot elongation of (2.5-4) cm on transfer to growth regulator free MS basal medium. Only vigorous grown shoots after transfer to shoot elongation medium produced elongated shoots. It was observed that among the explants used, the nodal segments gave better elongation after transfer to shoot elongation medium. The results are in accordance with [31, 32].

Effect of growth regulators on rooting

Induction of roots was observed with varying degree of response in all the media tested. Days to root initiation were recorded the earliest (13.64 days) in medium M_1R_{10}, followed by M_1R_{11} (14.41 days). The medium devoid of growth regulator auxin showed the slowest growth (29.30days).The effect of IBA and IAA on the number of functional roots per explants by different combinations at 4 weeks after inoculation recorded in Fig: 3 showed significant variation. The medium with M_1R_{10} (1 mg L^{-1} IBA) recorded the highest response of 83.33% while the controlled medium showed least response to rooting (16.66). The highest number of functional roots were produced by 1mgl^{-1}IBA (11.36 per explant), which was statistically significant than the other treatments. The lowest number of root was produced by M_1R_0. Vigorous root length (3.88) of *in vitro* grown plantlet on MS media supplemented with 1mgl-1IBA. Length of the root was not increased with the increasing of auxins concentration and the range was 1.0-3.8 cm. The present observations are consistent with the earlier finding in which IBA was successfully employed for rooting in Capsicum [33, 34, 25, 24]. The effectiveness of IBA on rooting of *in vitro* regenerated C. *chinense* Jacq. plantlets were also reported [23].

Acclimatization

In the present study, shoots with several well ramified roots were transferred into 10 cm diameter polycups containing pre-sterilized moist cocopeat, sand and farmyard Manure @ 1:1:1 ratio and were maintained under shade condition. This investigation showed that survival of transferred plants improved significantly when plantlets were initially covered with clear polythene bags having a few holes in it and were frequently watered to maintain high humidity and prevent desiccation. After 10 days the humidity was gradually decreased by increasing the size of holes in the polythene bags to harden the plantlets. The successfully hardened plantlets were indicated by emergence of new apical leaves. After 6 weeks, survival percentage of 60% was recorded in polythene bag covers cups. This confirms the earlier observations made by [35, 24].

Conclusion

The experimental study revealed that the MS medium combinations of BAP and low levels of IAA could be successfully used for shoot proliferation and 1 mgL^{-1} IBA for root induction in respect to *in vitro* regeneration of Naga King Chilli.

Application of Research: This efficient and reliable plant regeneration system can be exploited for the production of healthy and disease free plants to enhance yield and productivity through genetic transformation and other cellular techniques.

Research Category: Micropropagation, Naga King Chilli.

Abbreviations

BAP : Benzyl Amino Purine
KIN : Kinetin
IAA : Indole Acetic Acid
IBA : Indole Butaric Acid
MS : Murashige and Skoog

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Study area/ Sample Collection: Horticulture Instructional Farm, Nagaland University.

Cultivar/ Variety/ Breed name: Naga King Chilli (*Capsicum chinense* Jacq.)

Conflict of interest: None declared.

Ethical approval: This article does not contain any studies with human participation or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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