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Effects of Phytohormones BA (6-benzylaminopurine) and IBA (Indole-3-butyric acid) on Shoot and Root Multiplication in *Allium hookeri* Thw. Enum.

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Abstract

An efficient system for *in vitro* regeneration of plants is a prerequisite for plant improvement, using advanced biotechnological techniques. In present study, effect of phytohormones (BA and IBA) was studied on the explants survival, shoot and root regeneration in daughter bulbs of *Allium hookeri*. The phytohormones BA (6-benzylaminopurine) and IBA (Indole-3-butyric acid) stimulated the process and effective in plant survival, shoot and root initiation. Percentage of explants survival was found higher (71-92%) with the application of phytohormones. The phytohormone BA was also found positive to shoot induction and growth as well as comparatively early (3-5 days) shoots initiation. MS medium supplemented with 1.5 mg/l act as an optimum concentration for shoot initiation (3-5 days) and maximum shoot length (8.00cm). Application of IBA (0.5mg/l) with BA (1.5 mg/l) promoted the root initiation and growth while no root initiation was observed in MS and MS supplemented with phytohormone BA.

Introduction

Advanced biotechnological techniques in plant breeding programs for developing new varieties demand an efficient and reliable system for *in vitro* regeneration of plants. Applying different conventional tools and techniques has regenerated many important plants. *Allium hookeri* Thw. Enum. is an important medicinal bulbous herbaceous plant of family Alliaceae. *Allium* is the largest genus of family Alliaceae that comprising 450 species, distributed mainly in temperate and alpine regions (Lonzotti, 2006). In Asia the species is mostly distributed, in India, China, Bhutan, Sri Lanka and Myanmar. The species is commonly found in North and Eastern Himalayan region of India and known

as Hooker Chive. The leaves and bulbs contain a good quantity of aromatic compounds and used as flavoring agent in different foods and vegetables as well as appetizers in and outside the India. The different species of *Allium* are well known worldwide for their therapeutic applications (Tapsell, 2006) and plants have been reported for high medicinal values as antioxidant, anti-cancer and anti-inflammation properties (Bae and Bae, 2012; Kim *et al*., 2012; Won *et al*., 2013). The species also contains good amount of protein, sugar, fibre, ascorbic acid, phytosterols, phenols (Ayam, 2011), and somewhere chewed as such to recall the lost taste of mouth.

Efficient procedures are already available for *Allium* species and cultivars of major commercial importance, such as onion, garlic and garden leek (Tubic *et al*., 2011). Although, *in vitro* propagation and culturing of some species of *Allium* have been studied with the application of phytohormones, 2, 4 D, BA and kinetin (Wawrosch *et al*., 2001; Stelmaszczuk and Kozak, 2013; Monemi *et al*., 2014) but the knowledge of proper conventional techniques for the *in vitro* propagation for *A*. *hookeri,* is still meager.

In the field cultivation, the propagation rate of present species is very slow (Stelmaszczuk and Kozak, 2013). To overcome the problem, *in vitro* plant culturing may be one of the prospective and potential way to propagate the species successfully. The optimization of media constituents is critical for *in-vitro* plant regeneration as it can ensure a better survival of explants. The establishment of *in-vitro* propagation protocol for *A. hookeri* is not only important for its mass production but also to conserve the species. Therefore, in the present investigation, an attempt was made to optimize the media and to test the effects of phytohormones on the propagation of *A. hookeri.*

Materials and Methods

Plant Materials

The plants were collected, on permission from NBPGR, Bhowali (Accessions Number IC353540). The daughter bulbs were isolated by detaching them from mother plant and used in the study. The daughter bulbs were placed on basal MS media with different PGR content, dispensed in sterile Petri-dishes.

Surface sterilization of used bulbs was carried out by dipping them into 2% (v/v) Molyclean (8-10 minutes), 1% Bavistin solution (10-15 minutes) and 0.1% $HgCl_2$ (2-3 minutes)

Basal Medium and Culture Conditions

The basal medium contained Murashige and Skoog (MS) mineral solution (Murashige and Skoog, 1962) and 30 g/l sucrose, 100 mg/l myo-inositol, 0.1 mg/l thiamine, 0.5 mg/l pyridoxine and 0.5 mg/l nicotinic, was used for study. The medium ingredients were boiled and sterilized by autoclaving at 121 ºC for 18 min.; pH was adjusted to 5.7 before sterilization by using pH-meter. BA (6-Benzylaminopurine) and IBA (Indole-3-butyric acid) were added to sterilized media cooled to approximately 35-

40 ºC. BA and IBA stock solution was prepared by dissolving them into NaOH and then diluted with the fix volume of double distilled water and stored at -20 ºC. The cultures were maintained under cool white fluorescent tubes with a photon flux density of 40 µmol m^{-2} s^{-1} and a 16 h day length at 25 ± 2ºC.

Induction of Adventitious Roots and Shoot

For the induction of shoot and root, the bulbs were placed on MS PGR free medium or media supplemented with 6-benzylaminopurine (BA) or Indole-3-butyric acid (IBA). After 3 weeks of culture, the clumps containing apical shoot with adventitious buds were transferred to media of the same composition for an additional 3 weeks. Thereafter, all shoots acquired were separated and planted to Erlenmeyer flasks with solid MS medium supplemented with 1.5 mg/l BA+0.5 mg/l IBA, for multiplication.

Recordings and Analysis of Data

All cultures were placed in a completely randomized design. For adventitious shoot induction, the experiment was performed in three replicates with six samples (Petri-dishes) and three subsamples (plants per Petri-dish) (n=27). The regenerating of adventitious shoots per bulb were recorded after 21 days of initiation in culture. The growth parameters; survival of explants, days of shoot initiation and length, days for root initiation and length were recorded, on their visualization.

For multiplication, adventitious shoots were used for treated bulbs, three replicates with three samples (Erlenmeyer flasks), each with three subsamples (plants per flask). The multiplication of adventitious shoots and growth parameters were evaluated after 8 weeks of culture (Tubic *et al*., 2011).

Results

The inoculated test bulbs of *A. hookeri* were found positive to respond the shoot initiation on MS medium, shoot induction were started within 6 to 8 days of incubation while MS supplemented with phytohormones was responsible for early shoot initiation (Table 1 and Figure 1). The applied concentrations of BA showed their effect in shoot initiation. The lower concentration (0.5 and 1 mg/l) of BA did not show any significant effects while higher concentration (1.5 BA and 1.5 BA + 0.5 IBA) were found effective for an early shoot initiation (Table 1; Figure 1). The combination of BA+IBA found much effective in shoot initiation, root initiation, root length as well as in root multiplication (Table 1). The treatments of BA improved the shoot initiation and lengths compared to MS medium. Root formation was not observed in MS medium and MS medium supplemented only with BA while root induction was observed in medium supplemented with phytohormones, BA 1.5 mg/l in combination with IBA 0.5 mg/l. The modified media (BA 1.5 mg/l) and (BA 1.5 mg/l + IBA 0.5 mg/l) were found suitable for explants survival (87.50%), shoot and root initiation compared with control (58.33%).

Both the used media, MS and modified MS (with BA) were not found positive for root multiplication. For shoot length, the best responses (7.41 cm and 8.00 cm) were recorded in medium modified with (BAP 1.5 mg/l +IBA 0.5 mg/l) and (BA 1.5 mg/l) that were better than others treatments and control (4.42cm). For root initiation, medium modified with BA 1.5 mg/l +IBA 0.5 mg/l, was found positive. Root length was measured after 120 days of incubation (1.03cm). On clubbing all these parameters, the modified medium with BA 1.5 mg/l was found as a best medium for shoot multiplication while medium modified with BA 1.5 mg/l +IBA 0.5 mg/l was observed as a better treatment for shoot formation with roots for *in-vitro* propagation of *A. hookeri* from the daughter bulbs.

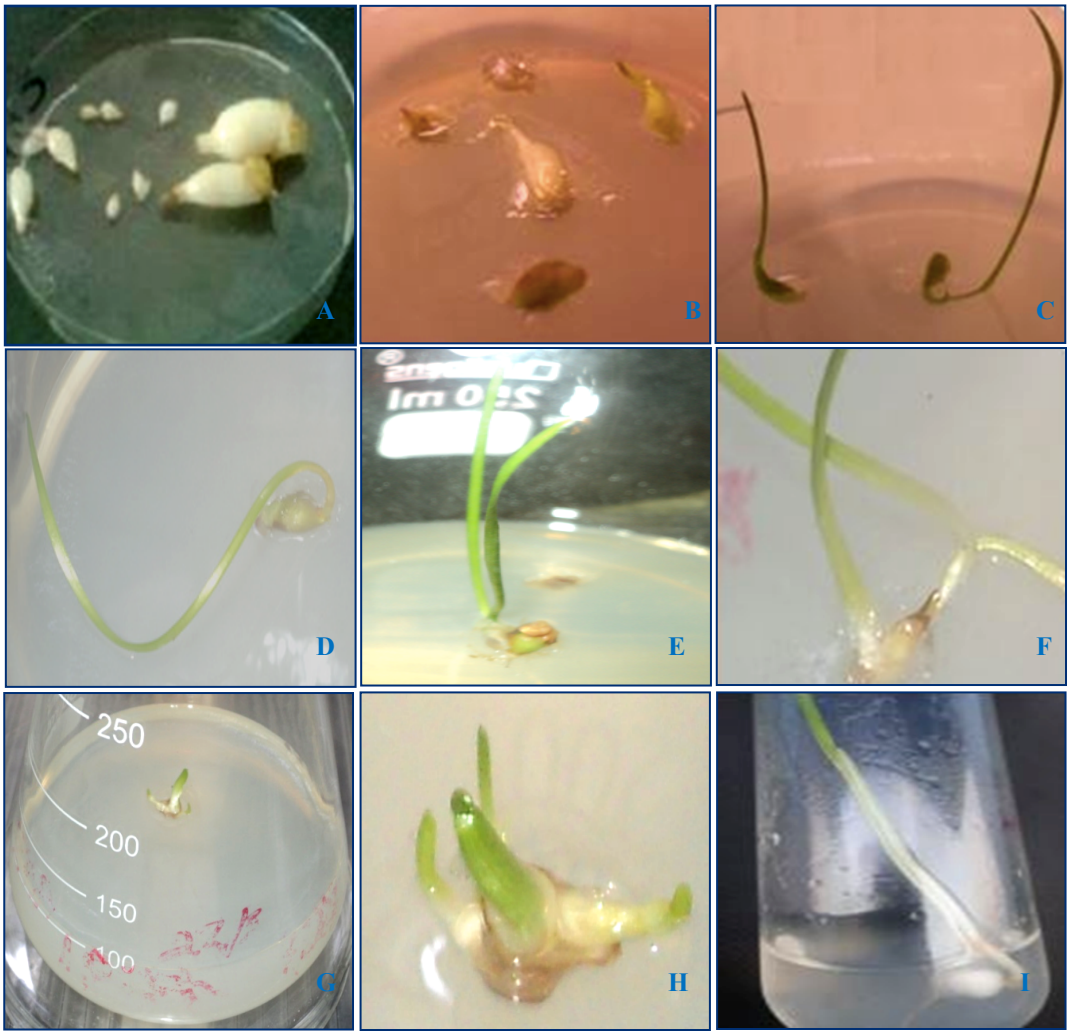

Figure 1 A- Explants (Daughter bulbs), B- Shoot initiation, C&D-Shoot elongation (medium supplemented with BA 0.5 mg/l), E&F- Shoot multiplication (medium supplemented with BA 1.0 mg/l), G & H- Shoot multiplication (medium supplemented with BA 1.5 mg/l), I- Root induction (medium supplemented with BA 1.5 mg/ l + IBA 0.5 mg/l)

Figure 2 A – Acclimatized plants growing under the glasshouse B – Growing plants, used for propagation.

Table 1. Effect of BAP and IBA on the morphogenic response of daughter bulbs of *Allium hookeri* Thw. Enum. reared on MS medium Means ± SE, n = 27.

BA mg/l	IBA mg/l	Bulbs response (%)	Shoot initiation (Days)	Shoot length (cm)	Root initiation (days)	Root length (cm)
0.0	0.0	58.33 (± 3.40)	6-8	4.42 (± 0.24)	-	-
0.5	0.0	70.83 (± 9.00)	5-7	6.20 (± 0.21)	-	-
1	0.0	79.16(± 3.40)	4-6	7.30 (± 0.32)	-	-
1.5	0.0	91.66 (± 6.80)	3-5	8.00 (± 0.32)	-	-
1.5	0.5	87.50 (± 5.89)	3-5	7.41(± 0.34)	60-90	1.03 (± 0.12)

Discussion

The combined concentrations of cytokinin and auxin were found positive and suitable for the shoot and root initiations in test plant. The *in vitro* studies of the species of *Allium* are little bit hard as since the responding rate of species is low on MS medium. The high concentrations of cytokinins and auxins as growth regulators shown the shoots and callus formation in *Allium* (Mehrabi *et al*., 2012; Tiwari *et al*., 2007). For shoot proliferation growth regulators especially cytokinins are one of the most important promoters to affect the responses (Lane, 1979; Bhojwani, 1980; Garland and Stolz, 1981). Our study supports the earlier findings on the reliability of cytokinins (BA) for shoot proliferation (Table 1; Figure I, G, H). A wide range of cytokinins like kinetin, BA, 2-isopentenyl adenine (2iP) and Zeatin have been employed in shoot proliferation (Bhojwani and Razdan, 1982). Murashige (1974) described 2iP as more effective than either kinetin or BA.

The effectiveness of the combination of BA and IBA for *in-vitro* shoot formation with root induction was also demonstrated by several studies (Sanatombi and Sharma, 2008; Nongdam and Tikendra, 2014; Gantait *et al*., 2009; Mohamed *et al*., 1994; Pandey *et al*., 1992). Studies on *Allium* species also suggested that the modified media, supplemented with cytokinin and auxins were found better than the medium without growth regulators (Roksana et al., 2002; Seabrook, 1994; Nagakubo *et al*., 1993). In the present study, the application of BA (0.5 mg/l to 1.5mg/l) induced the shoot growth. The auxins (IBA) have been used for *in-vitro* rooting in explants of *Allium* species (Mehrabi and Fezeli-nasab, 2012; Tiwari, *et al*., 2007; Wawrosch *et al*., 2001). The findings of present investigation also support that IBA is a good root inducer for *A. hookeri* as root initiation was not observed on MS and MS supplemented with BA. It may be concluded that BA was effective only for shoot initiation and worked as a root inducer on its application with the IBA, furthermore, only combined application of BA and IBA act as a root inducer.

The species is endemic to North-East parts of India as well as rate of propagation *in vivo* and *in vitro* for present and others species of *Allium* is very slow that's why the species of *Allium* are facing a load of harvesting in the localities. Therefore, there is a need to develop conventional techniques for *in vitro* and *in vivo* cultivation of the species, to conserve and cultivate them. In the present investigation, the phytohormones (BA and IBA) show a better response in propagation of the species may be helpful to propagate and cultivate this endemic species *A*. *hookeri* widely.

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Authors' contributions: *Harsh Kumar Chauhan produced and collected the data in laboratory as well as helped in interpretation of data and Anil Kumar Bisht designed the research and manuscript. Lokendra Singh corresponding author write and communicate the manuscript.*

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