In vitro Regeneration of Ginger (Zingiber officinale Roscoe)

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Abstract
An efficient and reproducible in vitro regeneration protocol was established for two varieties of ginger (Z. officinale Roscoe) namely, BARI Ada-1 and Chinese ginger accession number SG876. In case of BARI Ada-1 best result was obtained on MS supplemented with 2.0 mg/l BAP, 0.5 mg/l Kn and 0.5 mg/l NAA. In this combination, 95% rhizome bud explants responded within 6 - 8 days and mean number of shoots per explant was 8.79 ± 0.42. On the other hand, Chinese ginger showed best (90%) shoot regeneration response from the same explants on the same medium and hormonal combinations but in exchange of 0.25 mg/l NAA. In this hormonal composition shoot initiation started within 7 - 8 days of culture and mean number of shoots/explant was 6.83 ± 0.71 after 24 - 27 days of culture. Maximum root induction (90 and 80%) was found on MS supplemented with 0.5 mg/l IBA and 0.5 mg/l NAA in case of BARI Ada-1 and Chinese ginger, respectively. The in vitro regenerated plantlets were successfully transplanted into the soil after acclimatization.

Introduction
Ginger (Zingiber officinale Roscoe) is a rhizomatous monocotyledon belongs to Zingiberaceae (Kambaska and Santilata 2009). It is a well known spice, produced from the rhizome of the tropical herbaceous plant. It was discovered in China as early as 400 BC and has a long history of medicinal application dating back to 2500 years in China and India (Bhargava et al. 2012).

It is widely used in manufacturing a number of food products like curry powder, certain curried meats, table sauces, confectionary and ginger ale. Fresh ginger contains

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80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibers and 12.3% carbohydrates (Zadeh and Kor 2014). It has pharmacological applications including antitumor, antioxidant, anti-inflammatory, antiapoptotic, cytotoxic, anti-proliferative and anti-platelet activities (Shukla and Singh 2007).

The global production of ginger was 3.3 million tons in the year 2016 (FAO 2017). Bangladesh is now the eighth largest ginger producing country in the world and ranked third in terms of consuming of ginger globally (FAO 2017). During 2001-2002, national yield of ginger in Bangladesh per acre was only 56913 metric tons. The productivity trend has been increasing since 2003 - 2004 and in 2017 - 2018 productivity reached 83004 metric tons per acre. Growing area of ginger increased from 18533 acres of land in 2001 - 2002 to 25246 acres in 2017 - 2018 (BBS 2018). Major ginger producing areas of Bangladesh are Rangpur, Nilphamari, Tangail, Rangamati, Bandarban, Khagrachari and Chittagong district (Choudhury et al. 1998). Ginger is vegetatively propagated through the underground rhizomes but unfortunately, multiplication rate using conventional breeding is very low due to poor flowering and seed setting. Moreover risk of systematic infections by root knot nematodes, bacterial wilt from the propagules is very high (Kavyashree 2009) and the rhizomes are not available in the crop season (Lincy and Sasikumar 2010).

It is estimated that three-folds increase in rhizome production could be possible by effective control of diseases and pests (Hosoki and Sagawa 1977). So, an efficient *in vitro* regeneration protocol is considered to be the best to produce disease free plant propagules for the continuous supply of plantlets for commercial uses (Hamirah et al. 2010). Therefore, an attempt has been made to establish an efficient, reliable and reproducible *in vitro* regeneration protocol for *Zingiber officinale* Roscoe available in Bangladesh in order to help large scale clonal propagation with better survival rate.

### Materials and Methods

Rhizomes of BARI Ada-1 (previous accession number was G001) were collected from BARI. Rhizomes of Chinese ginger accession number SG876 were collected from local supplier which are occasionally grown in Bangladesh. These were maintained in the garden of BCSIR, Dhaka.

Explants (rhizome buds and shoot tips) collected from the garden were washed thoroughly under running tap water and soaked in liquid detergent (Surf excel) for 20 min and then rinsed four to five times with distilled water. The explants were then pretreated with fungicide (Babystin) for 3.0 min and rinsed three times with distilled water. After transferring the explants in autoclaved flask, final surface sterilization was done with ethanol (70% v/v) for 30 sec and 0.1% HgCl₂ for 9 - 10 min inside the laminar flow cabinet. During this period, the flask was agitated continuously. Then the explants were washed five times with sterilized distilled water.
Rhizome bud and shoot tip explants were excised with the help of a pair of sterilized forceps and scalpel on a sterile Petri dish. The culture establishment and proliferation of shoot tip and rhizome bud explants and their subsequent plantlet production on MS with BAP, Kn, NAA and IAA (used singly or in combination). All *in vitro* grown cultures were maintained according to the protocol described by Banu et al. (2017). After three weeks, the explants showing the sign of initiation of multiple shoot regeneration were sub-cultured on the same fresh medium. When shoots were produced in adequate number, elongated shoots were separated and cultured in the rooting medium for root formation. The plantlets with sufficient root system were taken out from the culture vessels and cleaned the agar on root surface under running tap water. The plantlets were then transplanted to small pots containing sterilized soil for further development.

**Results and Discussion**

The regeneration experiments were mainly conducted using the rhizome bud and shoot tip explants from BARI Ada-1 and Chinese ginger. Rhizome buds and shoot tips are commonly used as explants for micropropagation of pathogen free propagules on a large scale (Lincy and Sasikumar 2010).

Sterilization of rhizome buds was done using 0.1% HgCl$_2$ solution for 10 - 20 min to establish aseptic cultures in ginger (Suma et al. 2008, Kambaska and Santilata 2009, Khatun et al. 2016), turmeric (Rahman et al. 2004, Bharalee et al. 2005) and black thorn (Rahman et al. 2005). Between the two explants, rhizome bud was found to be the most responsive in terms of percentage of shoot regeneration as well as the number of shoots per explant in both the varieties (Table 1).

In BARI Ada-1, best result (95%) was found on MS with 2.0 mg/l BAP, 0.5 mg/l Kn and 0.5 mg/l NAA from rhizome bud explants. In this combination, shoot initiation started within 6 - 8 days and mean number of shoots per explant was $8.79 \pm 0.42$ (Table 1). Response towards shoot induction, multiple shoot formation and elongation of shoots in case of rhizome buds are presented in Fig. 1 (A-C). On the other hand shoot tip explants of BARI Ada-1 showed highest mean number of shoots ($6.72 \pm 0.49$) on MS with 2.0 mg/l BAP, 0.5 mg/l Kn and 0.25 mg/l NAA. Fig. 1 (D-F) shows the various stages of shoot regeneration from shoot tip explants of BARI Ada-1. Rhizome bud explants also produced higher mean number of shoots ($7.29 \pm 0.47$) on this combination of BAP, Kn and NAA. Kambaska and Santilata (2009) reported that medium containing 2.0 mg/l BAP and 0.5 mg/l NAA showed best response for shoot multiplication (7.5 shoots per rhizome bud) from rhizome bud explants of ginger.

Khatun et al. (2003) found best response from shoot tip explants of ginger on MS with 2.5 mg/l BAP and 0.5 mg/l Kn. But in the present study, when different concentrations of BAP and Kn were applied to examine their effect on shoot multiplication adequate response was recorded in MS supplemented with 2.0 mg/l BAP.
and 0.5 mg/l Kn from rhizome bud explants of BARI Ada-1. In this combination shoot tip explants produced comparatively lower mean number (3.93±0.70) of shoots (Table 1). Islam et al. (2004) observed that BA (12.0 μM) and NAA (0.3 μM) were suitable for the induction of in vitro micro-rhizomes in case of Curcuma longa. Hazare et al. (2005) reported that MS with 2.0 mg/l BAP and 2.0 mg/l Kn proved superior for shoot multiplication from rhizome bud explants of turmeric.

Table 1. Effect of different concentrations and combinations of BAP, Kn, NAA and IAA on shoot regeneration from rhizome buds and shoot tips of BARI Ada-1 and Chinese ginger.

| Varieties | Explants | BAP (mg/l) | Kn (mg/l) | NAA (mg/l) | IAA (mg/l) | % of responsive explants | Initiation of shoots (days) | Mean no. of shoots/explant (±SE) |
|-----------|----------|------------|-----------|------------|------------|--------------------------|-----------------------------|--------------------------------|
| BARI Ada-1 | Rhizome buds (RB) | 3.0 - - - | 80 | 7-8 | 5.63±0.72 |
|           |          | 3.0 - 0.5 - | 90 | 8-9 | 5.94±0.73 |
|           |          | 2.0 0.5 - | 85 | 8-10 | 6.06±0.66 |
|           |          | 2.0 0.5 0.5 | 95 | 8-10 | 8.79±0.42 |
|           |          | 2.0 0.5 0.25 | 85 | 7-9 | 7.29±0.47 |
| Shoot tips (ST) | 3.0 - 0.5 - | 85 | 8-9 | 4.94±0.90 |
|           |          | 2.0 0.5 - | 75 | 10-11 | 3.93±0.70 |
|           |          | 2.0 0.5 0.5 | 85 | 8-9 | 5.53±0.72 |
|           |          | 2.0 0.5 0.25 | - | 90 | 6.72±0.49 |
|           |          | 2.0 0.5 - | 75 | 9-10 | 4.40±0.51 |
|           |          | 4.0 - - | 80 | 8-9 | 5.50±0.52 |
|           |          | 3.0 - 0.5 - | 80 | 7-9 | 5.44±0.51 |
|           |          | 2.0 0.5 - | 75 | 8-10 | 5.20±0.68 |
|           |          | 2.0 0.5 0.5 | - | 85 | 8-9 | 5.82±0.62 |
|           |          | 2.0 0.5 0.25 | - | 90 | 7-8 | 6.83±0.71 |
|           |          | 2.0 0.5 - | 80 | 8-10 | 5.13±0.62 |
|           |          | 4.0 - - | 80 | 8-9 | 5.25±0.45 |
|           |          | 3.0 - 0.5 - | 75 | 8-9 | 4.67±0.49 |
|           |          | 2.0 0.5 - | 80 | 9-10 | 4.19±0.40 |
|           |          | 2.0 0.5 0.5 | - | 80 | 8-9 | 5.31±0.48 |
|           |          | 2.0 0.5 0.25 | - | 85 | 8-9 | 5.88±0.70 |
|           |          | 2.0 0.5 - | 75 | 8-10 | 4.53±0.52 |
Some reports represented that increasing dose (6.0 - 8.0 mg/l) of BAP decreased the rate of shoot multiplication of ginger (Rout et al. 2001). In the present study similar results were observed. MS with different concentrations of BAP (1.0 - 5.0 mg/l) were applied to show their effect on regeneration of shoots. Among these combinations, maximum mean number of shoots (5.63 ± 0.72) was observed from rhizome buds of BARI Ada-1 on MS containing 3.0 mg/l BAP (Table 1). Higher concentration of BAP (5.0 mg/l) decreased the rate of shoot multiplication. Naz et al. (2009) observed the similar types of results in turmeric.

**Fig. 1(A-I).** Different stages of *in vitro* shoot regeneration, formation of roots and acclimatization of BARI Ada-1. A. Initiation of shoots from rhizome buds (RB). B. Multiple shoots formation from RB on same medium as mentioned in Fig. A. C. Elongation of multiple shoots from RB on same medium as mentioned in Fig. A. D. Initiation of shoots from shoot tips (ST). E. Multiple shoots formation from ST on same medium as mentioned in Fig. D. F. Elongation of multiple shoots on same medium and explants as mentioned in Fig. D; G. Initiation of roots from the base of excised regenerated shoots. H. Regenerated plantlets on soil in small plastic pots. I. Mature plantlets on large pot.
In case of Chinese ginger, best response (90%) was observed on MS supplemented with 2.0 mg/l BAP, 0.5 mg/l Kn and 0.25 mg/l NAA from rhizome buds within 7 - 8 days. Mean number of shoots per explant was 6.83 ± 0.71 (Table 1). Different stages of shoot regeneration in case of rhizome buds are shown in Fig. 2A-C. Almost 85% of the shoot tip explants showed shoot regeneration on MS supplemented with 2.0 mg/l BAP, 0.5 mg/l Kn and 0.25 mg/l NAA. About 8-9 days were required for initiation of multiple shoots and mean number of shoots per explant was 5.88 ± 0.70 (Table 1). Effects of shoot initiation, multiple shoot formation and elongation of shoots in case of shoot tips are presented in Fig. 2D-F. Mollika et al. (2011) reported that the highest percentage of
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responsive explants towards the regeneration of shoots was obtained in MS with 2.0 mg/l BAP, 0.5 mg/l Kn and 0.2 mg/l NAA in case of BARI Sarisha-11 and BARI Sarisha-16.

Beside this, MS supplemented with 4.0 mg/l BAP showed better response towards shoot multiplication in both the explants of Chinese ginger (Table 1). Abbas et al. (2011) recorded the highest percentage of shoot multiplication on MS containing 4.5 mg/l BAP for sprouting bud explants of ginger. BAP and NAA supplemented medium showed maximum response (80%) of both the explants of Chinese ginger in MS with 3.0 mg/l BAP and 0.5 mg/l NAA (Table 1). Sathyagowri and Seran (2011), Yesmin et al. (2015) reported that 3.0 mg/l BAP and 0.5 mg/l NAA were best for shoot multiplication in ginger varieties.

It was observed that 90% shoots of BARI Ada-1 were found to form roots on MS supplemented with 0.5 mg/l IBA (Fig. 1G). On the other hand, MS with 0.5 mg/l NAA were optimum for root induction in Chinese ginger where 80% shoots were found to form roots (Fig. 2G). Bhagyalakshmi and Singh (1988) stated that IBA is more effective compared to NAA for root formation in meristem culture of ginger. In contrary, NAA was more effective than IBA for induction of rooting in ginger (Kambaska and Santilata 2009). After sufficient development of roots, plantlets of both the varieties were successfully transplanted in small plastic pots (Figs 1H and 2H). Three weeks after transplantation, when the regenerated plants were fully established in the small pots, then they were transferred to larger pots for further growth and development (Figs 1I and 2I). In here, the survival rate of the transplanted plantlets was found to be 95 and 85% in BARI Ada-1 and Chinese ginger, respectively.

Based on the above discussion it may be concluded that regeneration protocol developed in the present investigation can successfully be used for large scale clonal propagation of Zingiber officinale. This will reduce the high importing cost of ginger and plays an important role in the economy of Bangladesh as well.

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