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DEVELOPMENT OF *IN VITRO* PLANT REGENERATION PROTOCOL OF BANANA (*MUSA* SP.) CV. GRAND NAINE USING SUCKER EXPLANT

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ABSTRACT

Banana is an important fruit crop belongs to the family *Musaceae*. This has more demand for it multifarious uses like food, medicinal as well as industrial values. The present study was carried out to develop micropropagation protocol for large scale production of banana cv. Grand naine using sucker explant. Sucker explants were inoculated on Murashige and Skoog's (1962) (MS) basal medium and MS basal medium supplemented with different types and concentrations and combination of plant growth regulators. Highest mean number of shoots (10.2) per explant having mean shoot length 5.2 cm was observed on MS medium supplemented with 4.0 mg/L BA, 2.0 mg/L Z, 1.0 mg/L NAA, and 3.0 mg/L ADS. For large scale production of shoot, *in vitro* regenerated shoots were harvested, cut into small pieces and inoculated on the optimum medium for multiple shoot proliferation. In this way, more than thousand numbers of *in vitro* shoots were regenerated from a single explant at six month of culture. *In vitro* regenerated shoots were excised and rooted on ½ MS medium supplemented with 1.0 mg/L IBA. Finally *in vitro* regenerated plants were acclimatized and subsequently transferred to field with zero mortality. This protocol helps to meet the demand of the farmers.

***Keywords*:** Banana, Fruit crop, Grand Naine, Micropropagation

INTRODUCTION

Banana is one of the oldest, popular, and important fruit crop in different countries in the world including India (Lassoudière, 2007; Singh *et al*., 2016). This plant is a monocotyledonous, perennial, giant herb, belongs to the Family Musaceae. Commonly banana plant is growing or propagated vegetatively through rhizome (Fu *et al.,* 2018; Ranjha *et al.,* 2020). Banana is generally grown in Tropical and less grown in subtropical region. According to FAOSTAT report in south and south East Asia and the pacific nearly 37%, in Tropical Africa 30%, in south and Central America and Carribien 26% and rest 7% grown in elsewhere (Fu *et al*., 2018). Nearly about 15% of total global production of banana produced in India only. This plant is well known for its food, medicinal as well as industrial values (Kumar *et al.,* 2012; Fu *et al.,* 2018). In the world market, its ranking is next to rice, wheat, and maize based on food value (Singh *et al.,* 2016). Economically, banana possesses fifth rank as agricultural food crop in the global market, after coffee, cereals, sugar, and cacao as well as an important fruit crop in the world after grapes, citrus, fruits, and apples (Aurore *et al.,* 2009; Singh *et al.,* 2016). It is eaten raw as a dessert or as a staple food by cooking or eaten by processing in different form like juice, puree, chips, drinks, sausages, and flour (Singh *et al.,* 2016). Various parts i.e., rhizome, pseudo stem, leaf, flower, fruits of this plant have been used in traditional systems in medicine for the treatment of various diseases including ulcer, stomach disorder, burn and wound, diarrhoea, arthritis, anaemia, kidney stone, migraine headache, haemorrhage, epilepsy, and neurodegenerative disease (Singh *et al.,* 2016; Ranjha *et al*., 2020). Banana pulp have possesses a number of bioactive compounds including phenolic acids and flavonoids with high antioxidant, anti-tumour activity, anti-depression, anti-bacterial, anti-hypertensive, anti-ulcer genic, urolithiasis, laxatives, and anti-helminthic (Crpnauer and Krikorian, 1984; Ribeiro *et al*., 1988; Kailash *et al.*, 1993; Alarcon-Aguilara *et al.*, 1998; Cidian, 2005; Borges *et al.*, 2014). High quantity of potassium present in banana, which is beneficial for the muscles (Singh *et al.*, 2016). Due to its high iron content, banana is mainly suggested for anaemia patients

and was also proven to be beneficial in controlling blood pressure as it has low salt and high potassium content. Serotonin is also present in banana, which helps to overcome or prevent depression by changing mood and relaxing the body. Besides banana whole plant, leaf as well as fruit (both raw and ripen type) is widely used in many festivals in different state of India including Odisha. Banana is broadly classified in to two categories, one is Cooking type and another is dessert type. The ripe Banana is belongs to dessert type and for the cooking starchy Banana are boiled, deep-fried and rousted before eat. For its nutritional, industrial, and medicinal values, banana is most popular and has potential to combat against malnutrition in different developing countries in worldwide.

Banana plant generally propagated through conventional method by using rhizome or corms, large and small suckers, and sword suckers (Cronauer and Krikorian, 1984; Arias, 1992), which is slow and time consuming process. Therefore, this traditional process of propagation could not able to produce large number of plant in a short duration of time to meet the demand of farmers (Behera *et al.*, 2019). Cultivation and production of banana through traditional is also hampered by several risk factors like pets and diseases (weevil borers, nematodes, sigatoka complex, fusarium wilt, moko disease as well as viruses) resulting in the implementation of excess pesticides with serious impact to environment (Stover and Simmonds, 1987; Ganry, 1990). The foresaid problems are overcome by using plant tissue culture (Behera *et al.*, 2015; Behera *et al.*, 2018). There are many different variety of banana are developed among them G9 or Grand naine variety popular for its disease resistance capacity and for high yielding. This banana variety has many fruits in long Ghani. For large scale production of banana the farmers are encouraged by The Prime Minister's Agriculture Modernization Project in Nepal (https://kathmandupost.com/money/2020/06/29/g9-banana-more-disease resistant-but-less-marketable-farmers-say). Throughout the world production, more than 40% of the banana production is Grand Naine. In addition, this variety dominant in banana export market (Dale *et al.*, 2017). Therefore, the present study was conducted to develop micropropagation protocol for large scale production of banana plant cv. Grand Naine using sucker explant.

MATERIALS AND METHODS

Collection, preparation and surface sterilization of explant

Healthy banana cv. Grand Naine suckers as explants source were collected from Raghunathpur, Jagatsinghpur, Odisha, India. The selected suckers carefully removed from mother plants, was taken to laboratory and thoroughly washed in running tap water to remove soil particles and debris. The scally leaves and roots were removes carefully and the pseudo stem (about 10 cm length) taken in the beaker containing 2.0% (v/v) liquid detergent (Tween @ 20; Hi-media, India) and dipped for 20 min followed by treated with 3.0% (w/v) Bavistin (Fungicide) for 10 min. Then the explants were washed in double distilled water for 3 to 4 times. Surface sterilisation was done by 0.1% (w/v) mercuric chloride (Hi-media, India) solution for about 10 min. Then the explants were washed several times (3-5) with sterile double distilled water and both end of the explant was trimmed (about 3.5 cm length explant) before inoculation on culture medium for shoot multiplication.

Culture medium and Culture conditions

The surface sterilized explants were inoculated on culture mediums i.e., Murashige and Skoog's (1962) (MS) basal medium and MS basal medium supplemented with different concentrations and combinations of plant growth regulators such as N^6-Benzyladenine (BA) (1.0-5.0 mg/L), Kinetin (KIN) (1.0-5.0 mg/L), Zeatin (Z) (1.0-5.0 mg/L) α-Napthaleneacetic acid (NAA) (0.5-1.5 mg/L), and Adenine sulphate (ADS) (1.0-5.0 mg/L) for multiple shoot proliferation. After shoot multiplication medium optimization, the *in vitro* regenerated shoots were harvested and cultured on the same optimization medium (MS + 4.0 mg/L BA + 2.0 mg/L Z + 1.0 mg/L NAA + 3.0 mg/L ADS) for large number *in vitro* shoot regeneration within short time duration. *In vitro* regenerated shoots were harvested from multiple shoot culture and inoculated on MS basal medium, half strength of MS basal medium, and half strength MS basal medium augmented with different concentrations of Indole-3-butyric acid (IBA) (0.5-4.0 mg/L). All medium containing 0.7% (w/v) agar and 3% table sugar respectively. The pH level of the medium was maintained 5.8±0.1 before autoclave. All cultures (shoot multiplication and rooting culture) were maintained in the culture room temperature at 24±1 ^{0}C with photoperiod of 16 h day /8 h night and light intensity was maintained 3000 lux by cool white fluorescent tube (Philips, India). All chemicals were purchased from Hi-media, India for this experiment.

Acclimatization of *in vitro* regenerated plantlets

In vitro regenerated plantlets were carefully taken out from the culture tube and agar medium were removed from roots under running tap water. The plantlets were planted on the small pot containing potting substrate i.e., autoclaved sand, soil, and vermin-compost (1:1:1) and potting substrates were moistened with tap water, and potted plantlets were covered with polyethylene bag to maintain humidity and kept in the culture room. Plantlets were watered as per the requirement. After 15 days of hardening plantlets were taken out from culture room and kept in shade condition. After 18 days of acclimatization, the polyethylene bags were removed from plantlets and water sprayed three to four times per day up to three days. Then all acclimatized plants were kept in shade condition and subsequently transferred to full sun and then established in the field.

Data collection and statistical analysis

All these experiments were conducted three times. One explant or *in vitro* regenerated shoot per culture vessel having ten replicas for both shoot multiplication and rooting experiment respectively. The data (explant response percentage, mean shoot per explant, mean shoot length, rooting percentage, mean roots per *in vitro* regenerated shoot, and mean root length (cm) was taken at regular interval. Data were analysed using analysis of variance (ANOVA) for a completely randomized design (CRD) and Duncan's multiple range tests (DMRT) was used to separate the mean values for significant effect as per the method described by Gomez and Gomez (1984).

RESULTS

Effect of cytokinins on multiple shoot proliferation

Aseptic sucker explants were inoculated on MS basal medium alone and MS medium supplemented with different types and concentrations of cytokinins (Table 1; Fig. 1 A, B). It was observed that the explants were started to initiate shoot bud on the all tested medium after three weeks of culture. The percentage of explants response for multiple shoot proliferation was varies in the entire tested medium (Table 1). Shoot multiplication was observed after five weeks of culture (Fig. 1 C). About 70.0% of explants responded on MS medium Supplemented with 4.0 mg/L BA. Whereas, 63.3% of explants were responded for shoot formation on MS medium supplemented with 4.0 mg/L KIN and 66.7% explants responded for shoot formation on MS medium supplemented with 3.0 mg/L Z. Out of all these above said tested types and concentrations of cytokinins, maximum per cent of explant response for shoot multiplication was found on MS basal medium augmented with 4.0 mg/L BA. On this medium highest number of shoots per explant (6.5) were found having mean shoot length 4.5 cm (Fig. 1 D). Highest 6.2 numbers of mean shoots per explant having mean shoot length 4.5 cm was found on MS basal medium supplemented with 3.0 mg/L Z (Table 1; (Fig. 1 E). With increasing or decreasing the concentration of cytokinin the per cent of explant response for multiple shoot proliferation and the number of shoot regeneration was also influenced.

While the explants were inoculated on optimum shoot multiplication medium (i.e., MS medium supplemented with 4.0 mg/L BA) supplemented with different type and concentrations, and combinations of plant growth regulators (KIN, Z, NAA, ADS), the per cent of explant response for shoot multiplication was increased. Highest 96.7% of explants was responded on MS medium supplemented with 4.0 mg/L BA and 2.0 mg/L Z, 1.0 mg/L NAA, and 3.0 mg/L ADS among the entire tested medium (Table 2). Maximum numbers of shoot (10.2) with highest length of shoot (5.2 cm) were observed on MS medium supplemented with 4.0 mg/L BA, 2.0 mg/L Z, 1.0 mg/L NAA, and 3.0 mg/L ADS (Table 2; Fig. 1 F). For large number of shoot production, *in vitro* regenerated shoots were harvested, cut into small pieces and inoculated on the optimum medium (MS + 4.0 mg/L BA + 2.0 mg/L Z + 1.0 mg/L NAA + 3.0 mg/L ADS) for multiple shoot proliferation. In this way, more than thousand numbers of *in vitro* shoots were regenerated from a single explant at six month of culture.

Rooting of *in vitro* regenerated shoots

In vitro regenerated shoots were harvested from the mother explants and inoculated on full strength MS medium, half strength MS medium and half strength MS medium supplemented with different concentrations of 0.5-4.0 mg/L IBA. From *in vitro* shoots, roots were started initiation on all medium after one week of culture on rooting medium (Fig. 1 G). Maximum per cent (73.3%) of *in vitro* regenerated shoots were responded for rooting on half strength MS medium supplemented with 1.0 mg/L IBA. Only one root was observed on MS medium without any growth regulator. About 3.2 numbers of roots having root length 3.5 cm were formed on half strength MS medium. Highest numbers of roots (6.5) having root length 4.7 cm were produced on ½ MS medium supplemented with 1.0 mg/l IBA (Table 3; Fig. 1H)

Acclimatization

In vitro regenerated plantlets were carefully taken out from the culture tube and acclimatized on the small pot containing potting substrate i.e. autoclaved sand, soil, and vermin-compost (1:1:1) and potting substrates were moistened with tap water, and kept in the culture room. All plantlets were successfully acclimatized with zero mortality (Fig. 1 I). Subsequently theses acclimatized plantlets were transferred to the field.

DISCUSSIONS AND CONCLUSION

For the development of successful *in vitro* plant regeneration protocol requires to evaluate different factors like explant, medium, plant growth regulators, etc (Mohanty *et al.*, 2013). Out of all, selection of suitable explant is one of the most important factors for development of successful protocol. The type of explant and pathway of plant regeneration play key role for production of genetically stable *in vitro* regenerated plant (Mohanty *et al.*, 2013). Generally meristematic explant is used for the production of genetically uniform plants, due to less chance of genetic instability. Thus, in this study sucker was used as explant for *in vitro* plant regeneration of Banana var. Grand Naine. This explant was also used by various worker for *in vitro* plant regeneration of Banana (Muhammad *et al.*, 2004; AI-Amin *et al.*, 2009; Jing-Yan *et al.*, 2011; Aremu *et al.*, 2012; Mondal *et al.*, 2012; Ramachandran and Amutha, 2013; Rahman *et al.*, 2013; Ahmed *et al.*, 2014).

For development of *in vitro* plant regeneration protocol, selection of basal medium and plant growth regulators were also important. In this study, MS medium used as basal medium. MS medium without any growth regulators failed to produce shoot. However, sucker explant was inoculated on MS medium supplemented with cytokinins i.e. BA, Z, NAA, and ADS for multiple shoot proliferation and found that MS medium supplemented with BA (4.0 mg/L), Z (2.0 mg/L), NAA (1.0 mg/L), and ADS (3.0 mg/L) showed maximum number of multiple shoot proliferation. Whereas, Ahirwar *et al.* (2012) were reported that they found highest frequency of shoot regeneration (52.25), number of shoots regenerated per explant (3.25) and shoot length (4.69 cm) on MS medium supplemented with 7.5 mg/L BA and 0.3 mg/L NAA. Strosse *et al.* (2008) were also reported that highest number of multiple shoot proliferation on MS medium supplemented with 5.0 mg/L BA and 0.5 mg/L NAA. Analogical results obtained by Sipen and Davey (2012) in *Musa spp.* Pisang Nangka on MS medium supplemented with 5.0 mg/L BA and 0.2 mg/L IAA. Vuylsteke and De Langhe (1985) and (Bairu *et al.* (2008) were also reported that 5.0 mg/L BA was the optimum concentration for most banana cultivars. While, Rahman *et al.* (2013) were obtained maximum number of shoots each explants (5.9) on MS medium supplemented with BA (4.0 mg/L). However, Rai *et al.* (2012) were found best shoot multiplication on MS medium augmented with BA (2.0 mg/L) and NAA (0.5 mg/L) with average of 7.5 shoot/explants. While Akbar and Roy (2006) were cultured banana explants on MS medium supplemented with 0.5 mg/L of BA, KIN and NAA and they were found that addition of 10% coconut water to the medium resulted in increased number of differentiated shoots per culture. Different results obtained by different authors might be due to differences of genotypes and explants used. So, the multiplication rate was decreased with decreasing the concentration of BA in MS

medium because less bud formation and also the multiplication rate decreasing with increasing the concentration of BA in MS medium due to abnormality development of the buds.

For plantlet formation, *in vitro* regenerated shoots were harvested from the culture vessel and inoculated on rooting medium. The addition of auxins on basal medium is required for the induction and development of roots from *in vitro* regenerated shoots derived from sucker explant. The optimum concentration of auxin is required to provide sufficient stimulus for root initiation (Yoeman, 1986). Maximum percentage of root formation, maximum number of roots per *in vitro* regenerated shoot and maximum root length was found on ½ MS medium supplemented with IBA 1.0 mg/L. Result of this studies agree with the results of rooting has been reported earlier by Al-Amin *et al.* (2009), Vasane *et al.* (2010), Roy *et al.* (2010), Rahman *et al.* (2013). Babylatha (1993) has been observed maximum rooting on half MS medium supplemented with 5.0 mg/L IBA. However, the maximum percentage of adventitious root formation has been reported on half MS medium supplemented with IBA 1.5 mg/L and NAA 1.0 mg/L (Govindaraju *et al.,* 2012). Ahirwar *et al.* (2012) were reported significantly increased frequency of root regeneration, number of root regenerated per shoot and shoot length on the medium containing 1.0 mg/L NAA.

Acclimatization is one of the most essential steps for *in vitro* regenerated plant, because *in vitro* regenerated plant material is not adapted to natural environmental conditions (Brainerd and Fuchigami, 1981). *In vitro* regenerated plants are very poorly adopted to resist the low humidity, higher light levels and more variable temperature prevailing outside (Wainwright, 1988). Thus, light, temperature and relative humidity are the three major factors to be controlled during acclimatization to natural environment. Physiochemical and biological properties of potting substrate mixture are also important factors in the establishment of micropropagated plant. In this study, *in vitro* regenerated plantlets were acclimatized on small pot containing autoclaved sand, soil, and vermin-compost (1:1:1) and all plants were successfully acclimatized. Subsequently, all these acclimatized plants were transferred to field with zero mortality. Rai *et al.* (2012) were used different potting substrate like soil, sand, and coco peat (1:1:1); soil, sand, and farmyard manure (1:1:1) and mixture of coco peat and sand (2:1) for acclimatization of *in vitro* rooted plantlets of banana var. Grand Naine (G9) in portray and they were observed that the mixture of Coco peat and sand (2:1) showed maximum (96%) survival of acclimatized plantlets. Shiv Shankar *et al.* (2014) found the survival rate of the plantlets in coconut coir pith to be 84.44% during primary hardening. All the plantlets were subjected to the secondary hardening with garden soil, sand and red soil in the ratio of 1:1:1 in polybags and all the plantlets showed 100% survivability.

Banana is an important horticultural fruit plant, which is widely as food and medicinal purposes. This plant is largely cultivated in worldwide. But, this plant is generally cultivated by vegetative propagation using rhizomatic sprout. This process is very slow and time consuming process and unable to produce large number of plant throughout the year. In this study, highest number of shoot produced on MS medium supplemented with BA (4.0 mg/L), Z (2.0 mg/L), NAA (1.0 mg/L), and ADS (3.0 mg/L). These *in vitro* regenerated shoots were rooted ½ MS medium supplemented with 1.0 mg/L IBA. This protocol may be useful to produce large number of plant regeneration from sucker explant for supply of large number of healthy planting material to farmers.

Table 1: Effect of cytokinins on *in vitro* shoot proliferation

MS basal medium supplemented with plant growth regulators (mg/L)			Shooting (%)	Shoots/ explant	Shoot length (cm)
BA	KIN	Z			
-	-	-	0.00 ± 0.0^{j}	0.0 ± 0.0^{p}	0.0 ± 0.0^{j}
1.0	-	-	46.7 ± 1.7^{g}	3.2 ± 0.2^{ij}	$2.8\pm0.1^{f-h}$
2.0	-	-	53.3 ± 0.6^{e}	3.8 ± 0.5^{h}	3.0 ± 0.3^{fg}
3.0	-	-	60.0 ± 1.7^{d}	5.4 ± 0.1^{c}	3.6 ± 0.1^{de}
4.0	-	-	70.0 ± 0.8^{a}	6.5 ± 0.5^{a}	4.5 ± 0.3^{a}
5.0	-	-	66.7 ± 1.1^{b}	5.0 ± 0.3^{d}	4.5 ± 0.2^{a}
-	1.0	-	36.7 ± 0.2^{i}	2.2 ± 0.1^{o}	$2.5\pm0.3^{g-i}$
-	2.0	-	43.3 ± 0.6^{h}	2.5 ± 0.1^{mn}	$2.8\pm0.4^{f-h}$
-	3.0	-	50.0 ± 0.0^{f}	3.0 ± 0.4^{jkl}	3.2 ± 0.3^{ef}
-	4.0	-	63.3 ± 0.3^{c}	3.4 ± 0.2^{i}	$4.0\pm0.4^{a-c}$
-	5.0	-	60.0 ± 1.0^{d}	3.1 ± 0.1^{ijk}	$3.8\pm0.3^{b-d}$
-	-	1.0	53.3 ± 0.6^{e}	2.6 ± 0.2^{m}	3.6 ± 1.0^{de}
-	-	2.0	60.0 ± 1.5^{d}	4.3 ± 0.3^{fg}	3.6 ± 0.3^{de}
-	-	3.0	66.7 ± 0.3^{b}	6.2 ± 0.3^{b}	4.5 ± 0.3^{a}
-	-	4.0	63.3 ± 0.8^{c}	4.7 ± 0.2^{de}	4.5 ± 0.2^{a}
-	-	5.0	60.0 ± 0.8^{d}	4.5 ± 0.3^{ef}	4.2 ± 0.3^{ab}

In a column, different letters in superscripts represent statistically significant difference between the means (P≤0.05; Duncan's new multiple range test; DMRT).

Table 2: Effect of cytokinins in combination with cytokinins, auxin, and growth additives on *in vitro* shoot proliferation

MS basal medium supplemented with plant growth regulators (mg/l)					Shooting (%)	Shoots/ explant	Shoot length (cm)
BA	KIN	Z	NAA	ADS			
4.0	1.0	-	-	-	70.0±2.0^{i}	6.4±0.2^{j-l}	4.5±0.2^{cd}
4.0	2.0	-	-	-	76.7±1.7^{g}	7.1±0.1^{f-h}	4.8±0.5^{a-c}
4.0	3.0	-	-	-	73.3±0.6^{h}	6.8±0.4^{h-j}	4.3±0.1^{de}
4.0	4.0	-	-	-	73.3±0.6^{h}	6.5±0.2^{jk}	4.5±0.2^{cd}
4.0	-	1.0	-	-	76.7±1.1^{g}	7.0±0.3^{f-i}	4.3±0.3^{de}
4.0	-	2.0	-	-	83.3±0.5^{e}	7.4±0.4^{e-g}	4.5±0.2^{cd}
4.0	-	3.0	-	-	80.0±2.6^{f}	7.0±0.5^{f-i}	4.5±0.3^{cd}
4.0	-	4.0	-	-	76.7±0.5^{g}	6.5±0.5^{jk}	4.0±0.4^{ef}
4.0	-	2.0	0.5	-	86.7±0.2^{d}	7.9±0.4^{de}	4.5±0.3^{cd}
4.0	-	2.0	1.0	-	90.0±0.9^{c}	8.5±0.2^{bc}	5.0±0.3^{ab}
4.0	-	2.0	1.5	-	86.7±0.7^{d}	8.0±0.3^{cd}	4.8±0.4^{a-c}
4.0	-	2.0	1.0	1.0	83.3±0.6^{e}	8.0±0.9^{cd}	5.0±0.4^{ab}
4.0	-	2.0	1.0	2.0	86.7±1.1^{d}	8.7±0.3^{b}	4.8±0.3^{a-c}
4.0	-	2.0	1.0	3.0	96.7±0.6^{a}	10.2±0.3^{a}	5.2±0.3^{a}
4.0	-	2.0	1.0	4.0	90.0±1.0^{c}	8.5±0.3^{bc}	4.5±0.9^{cd}
4.0	-	2.0	1.0	5.0	93.3±0.6^{b}	7.5±0.3^{d-f}	4.5±0.7^{cd}

In a column, different letters in superscripts represent statistically significant difference between the means (P≤0.05; Duncan's new multiple range test; DMRT).

Table 3: Rooting of *in vitro* regenerated shoots

Medium for rooting	Rooting (%)	Roots/ shoot	Root length (cm)
MS	40.0±2.6^{g}	1.0±0.0^{f}	2.3±0.3^{e}
½ MS	53.3±2.3^{f}	3.2±0.2^{e}	3.5±0.2^{d}
½ MS + 0.5 mg/L IBA	63.3±0.6^{d}	4.3±0.3^{d}	4.5±0.3^{ab}
½ MS + 1.0 mg/L IBA	73.3±1.3^{a}	6.5±0.5^{a}	4.7±0.3^{a}
½ MS + 2.0 mg/L IBA	70.0±1.7^{b}	6.0±0.4^{ab}	4.5±0.2^{ab}
½ MS + 3.0 mg/L IBA	66.7±1.1^{c}	6.0±0.3^{ab}	4.0±0.1^{c}
½ MS + 4.0 mg/L IBA	60.0±1.7^{e}	5.4±0.7^{c}	4.0±0.0^{c}

In a column, different letters in superscripts represent statistically significant difference between the means (P≤0.05; Duncan's new multiple range test; DMRT).

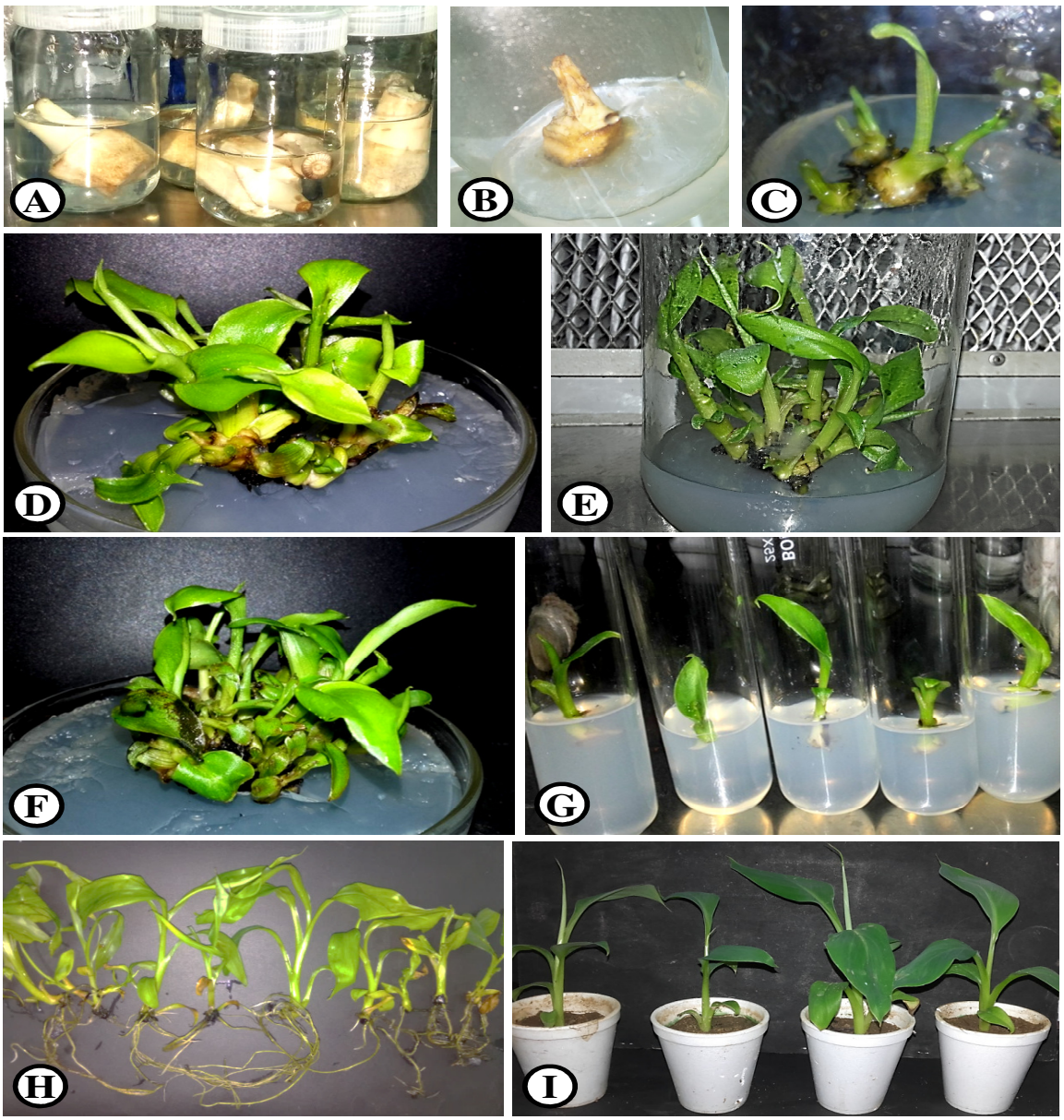

Fig. 1 (A) Banana sucker explant; (B) Aseptic explant inoculated on culture medium; (C) Shoot multiplication after five weeks of culture; (D) Multiple shoot proliferation on MS medium + 4.0 mg/L BA; (E) Multiple shoot proliferation on MS medium supplemented with 3.0 mg/L Z; (F) Multiple shoot proliferation on MS medium supplemented with 4.0 mg/L BA + 2.0 mg/L Z + 1.0 mg/L NAA + 3.0 mg/L ADS; (G, H) Rooting of *in vitro* regenerated shoots on ½ MS medium supplemented with 1.0 mg/L IBA; (I) Acclimatization of *in vitro* regenerated plantlets on small pot containing autoclaved sand, soil, and vermin-compost (1:1:1).

CONFLICTS OF INTEREST

Authors declared that there are no conflicts of interest.

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