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Research Article

In Vitro Multiplication and Acclimatization of Banana (*Musa* spp.) Plantlets

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Abstract: Banana (*Musa* spp.) is a horticulturally important fruit crop of economic significance, particularly in tropical and subtropical regions of the world. Vegetative propagation of banana via suckers is hindered by a low multiplication rate and high susceptibility to diseases. The current study optimized efficient protocols for large-scale *in vitro* multiplication and acclimatization of two commercially valued banana cultivars (William and Grand Naine) initially propagated from shoot tip explants. Clusters of shoot buds (1-2 cm) were multiplied on the Murashige and Skoog (MS) medium consisted of different concentrations (0.5, 1.0, 2.0, and 3.0 mg l^{-1}) of benzyl adenine (BA). Medium comprised of different concentrations of activated charcoal (0.5, 1.0, 1.5, and 3.0 mg l^{-1}) was used for healthy shoot and root development. Acclimatization of the plantlets (40 cm) in the greenhouse was carried out on different soils, i.e., peatmoss, river sand, clay soil, hill sand, and desert sand. Results revealed that significantly highest shoot bud multiplication (Avg. 21.2), and plantlet formation was obtained in cv. Grand Naine on the MS medium consisted of 3.0 mg l^{-1} BA. Significantly highest leaf number (4 leaves), leaf length (10.7 cm), root number (12 roots), and root length (13 cm) were noted on the MS medium consisted of 3.0 mg l^{-1} activated charcoal, 0.1 mg l^{-1} naphthalene acetic acid (NAA). Plantlets (15 cm) developed healthy shoot and roots during *in vitro* growth were shifted in the greenhouse for acclimatization and further growth. Significantly higher survival rate (96%) of the acclimatized plantlets in the greenhouse and healthy growth was noted on the soil medium consisted of river sand in cv. Grand Naine after 1M (month). Protocols optimized for *in vitro* and *ex vitro* growth of the two elite banana cultivars (William and Grand Naine) will benefit to the farmers for large-scale cultivation in the area and across the world.

Keywords: Acclimatization, *Ex vitro* Growth, Greenhouse, *In vitro* Plantlets, Shoot Tip Explants.

1. INTRODUCTION

Banana (*Musa* spp.) is one of the important fruit crops belongs to the family Musaceae is the crossbreed of *Musa balbisiana* and *Musa acuminata* [1-3]. Over 1000 banana cultivars and varieties are cultivated across the world [4]. Banana word originated from the Arabic word "banan" meaning the finger [5]. Banana plant is herb, perennial, monocot, and is cultivated in tropical regions of the world in more than 130 countries [6]. Banana is a rich source of vitamin A, C, and B6, as well as proteins, carbohydrates, and minerals including calcium, magnesium, manganese, and potassium [7, 8]. Banana is propagated commercially via tissue culture provides mass propagation, rejuvenation of older varieties, disease elimination, conserving genetic resources, and management of biotic and abiotic stresses [9-11]. Tissue culture-derived plants yield high quality true-to-type fruits, and remain free from pests and diseases [12]. *In vitro* propagation of banana varieties has been tested in many countries as an alternative propagation method to naturally occurring suckers [13-15]. For many years, banana was traditionally propagated through suckers and diploid seeds' germination [16]. However, such efforts remained limited due to diseases, genetic variability and lack of

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uniformity [17]. Recently, different treatments of plant growth regulators (PGRs) were tested for large-scale multiplication of banana plantlets [18]. Banana is cultivated worldwide, and is among the important cash crops being a rich source of important nutrients [19]. Micropropagation at large scale always remains problematic due to the high cost of inputs required, microbial contamination, and lack of protocols to be used on various banana varieties [20]. It was demonstrated that the banana was effectively propagated *in vitro* using shoot tip explants obtained from healthy suckers [21]. Recent studies also reported the micropropagation protocols of banana through shoot tip explants, *in vitro* multiplication and acclimatization [22]. Corms and suckers are used as conventional propagating materials for fruit production [23, 24]. Suckers can be the source of transfer of diseases and pests, and also have low multiplication rate has rendered the interest of farmers to propagate the banana via tissue culture [25, 26]. Plant tissue culture is an efficient and reliable method of propagating the rare and endangered plants on commercial scale in a short time [27].

Micropropagation plays significant role for getting disease-free plants of the fruit crops for economic growth [28]. *In vitro* propagation through shoot tip explants may overcome disease problems because of the aseptic conditions followed during culture process [29]. Banana fruits have high nutritional value, and is low priced fruit making it familiar in developing countries [26]. Micropropagation of banana involves several *in vitro* and *ex vitro* growth stages such as, sterilization of explants, initiation, shoot multiplication, rooting and acclimatization in the greenhouse [16]. Cell division, cell elongation and differentiation can be induced by manipulation of plant growth regulators in the media [30]. Preferred and widely used explants of banana for tissue culture are shoot tips obtained from suckers [16]. Shoot tip explants of banana remain free from the viruses and other pathogens due to occurrence of small vacuoles inside the cells and high nucleocytoplasmic ratio which restricts the pathogen invasion [31]. Several banana varieties previously were micropropagated via tissue culture through shoot tip explants [32]. Sivakumar and Visalakshi [33] conducted study on *in vitro* propagation of banana cv. Poovan using shoot tip explants. Singh *et al.* [26] described the effect of different plant growth regulators on *in vitro* propagation of banana cultivar Grand Naine using shoot tip explants. Several studies described successful *in vitro* regeneration protocols, shoot multiplication, rooting and acclimatization of banana plantlets using shoot tip explants [34-41].

Khairpur district of Pakistan is suitable area for banana cultivation with appropriate soil and climate; therefore, the current study is aimed to optimize protocols for *in vitro* multiplication and further growth of plants in the greenhouse, and in the open field. The present study is expected to establish efficient protocols for shoot bud multiplication initially obtained from immature shoot tip explants, shoot elongation and rooting, and acclimatization of the micropropagated plantlets on different soil types for two commercially valued banana cultivars William and Grand Naine.

2. MATERIALS AND METHODS

2.1. Plant Material

Clusters of shoot buds (initially obtained from shoot tip explants) were cultured on the media consisted of different concentrations of PGRs. Small plantlets (7-8 cm) were isolated from the clusters during each subculture (1-2 months interval) and cultured in jars for further growth and rooting for 1-2 subcultures.

2.2. Media Preparation

Little shoot bud clusters of banana cultivars were cultured on the Murashige and Skoog (MS) media consisted of different concentrations of benzyl adenine (BA), i.e., 0.5, 1.0, 2.0 and 3.0 mg l^{-1} used for shoot bud multiplication and plantlets' development (4-8 cm in length) for a single subculture. Long shoots were detached easily, whereas the remaining buds were recultured on the same medium for multiplication and plantlet formation. Plantlets (7-8 cm in length) were grown on the MS media consisted of different concentrations of activated charcoal, i.e., 0.5, 1.0, 1.5 and 3.0 g l^{-1} in addition to 0.1 mg l^{-1} naphthalene acetic acid (NAA) for the healthy root and shoot development for 1-2 months for a single subculture.

2.3. Culture Conditions

Clusters of shoot buds during multiplication and plantlet formation stage were kept in growth room under temperature 24 °C ± 2 (16 hours photoperiod), whereas, the plantlets during rooting stage were kept under temperature 27 °C (16 hours photoperiod) for the better growth of root and shoot.

2.4. Acclimatization

Plantlets (15 cm long) were acclimatized in the greenhouse on different soil mixtures obtained from local sources, i.e., river sand, desert sand, peatmoss, clay soil and hill sand. Plantlets were shifted to greenhouse in closed vessels, followed by careful washing with 2 g l^{-1} systemic fungicide (Carbendazim) for 1-2 minutes. Later the plantlets were transplanted on different soil types and covered completely with polyethylene plastic sheet for a week. Plantlets in the greenhouse were ventilated regularly after one week until complete removal of the plastic sheet after four weeks. Fertilizer treatment was started after two months of acclimatization in the greenhouse. Spray of fungicide (Copper Oxychloride) was carried out after every15 days to keep the plants intact from any fungal infection during the growth in the greenhouse.

2.5. Data Analysis

Data were based on three replicates from each cultivar on different treatments used throughout all *in vitro* and *ex vitro* growth stages, and each replicate was based on 20 randomly selected plantlets/ cultures (*in vitro*/ *ex vitro*). Data were subjected to two-way analysis of variance (ANOVA) followed by LSD (≤ 0.05) using the software (IBM SPSS Statistics version 30.0) as per the method of Steel and Torrie [42].

3. RESULTS AND DISCUSSION

3.1. Effect of Different Treatments of BA on Shoot Bud Multiplication and Plantlet Formation

Data in Table 1 show that significantly highest shoot bud multiplication (Avg. 21.2 buds) was noted in cv. Grand Naine followed by cv. William (Avg. 18.5 buds) after one month on the medium consisted of 3.0 mg l^{-1} BA. Medium consisted of 2.0 mg l^{-1} BA induced avg. 15.7 small shoots in cv. Grand Naine and avg. 13.3 small shoots in cv. William after one month (Figure 1(a)). Further, decrease in the BA concentration, i.e., 1.0, 0.5 mg l^{-1}, reduced significantly the average shoot formation in one month in both banana cultivars. Results of the two-way ANOVA showed significant differences, revealed that treatment effect was highly significant ($p < 0.001$) than cultivar ($p < 0.041$), and combined effect of cultivar and treatment ($p < 0.006$). Nevertheless, the high cultivar-treatment interaction shows that the reaction of each cultivar differed according to the level of PGR, with Grand Naine being relatively more responsive to all treatments. The findings clearly indicate that the growth response measured in both banana varieties, William and Grand Naine in the presence of higher concentration of PGRs was greatly increased. The same trend was recorded between 0.5 and 3.0 mg l^{-1} showing that the effect of concentration of PGRs on *in vitro* shoot buds multiplication was strong. Both cultivars had little response at the lowest concentrations (0.5 mg l^{-1}). Nonetheless, at 1.0 and 2.0 mg l^{-1}, a considerable change was noticed, indicating that moderate levels of PGRs are best to induce cell division and multiplication

Table 1. Effect of different concentrations of benzyl adenine (BA) on shoot bud multiplication of banana cultivars (William and Grand Naine).

PGRs (mg l^{-1})	William	Grand Naine
0.5	4.11 ± 0.57d	6.21 ± 0.78d
1.0	8.21 ± 0.11c	9.82 ± 0.45c
2.0	13.3 ± 0.31b	15.7 ± 0.61b
3.0	18.5 ± 1.11a	21.2 ± 0.77a
LSD (0.05%)	0.002	0.001
Variability		
Cultivar	0.041	
Treatment	0.001	
Cultivar × Treatment	0.006	

Mean values in columns with standard error denoted with different letters show significant levels at $p \leq 0.05$.

of the shoot. Optimum response was observed at 3.0 mg l^{-1} at which point Grand Naine performed better than William suggesting cultivar variation in responsiveness. The observed differences are further proven by statistical grouping (a-d) which shows that all levels of treatments were significantly different at ($p < 0.05$), in this case, the results are reliable. In general, the findings indicate that the concentration of 3.0 mg l^{-1} PGR results in the optimum growth in both cultivars, especially in Grand Naine. These results are in line with earlier researches that have cited improved shoot proliferation in banana with higher levels of cytokinin probably because of higher meristematic activity and nutrient mobilization. Current results report shoot multiplication agrees with Shukla *et al.* [8] who obtained shoot proliferation on MS medium consisted of 5 mg l^{-1} benzyl adenine for *Musa acuminata* var. hazari, *Musa paradisica* var. sanikol, and *Musa paradisica* var. kashkol; whereas, shoot proliferation of *Musa acuminata* var. hondakol was achieved on MS medium consisted of 3 mg l^{-1} BA. Generally, in several banana species, adenine-based cytokinins were used for *in vitro* propagation [43-47]. Al-amin *et al*. [48] tested BAP and NAA for *in vitro* regeneration and shoot multiplication of banana. Several studies observed positive effect of benzyl aminopurine (BAP) on shoot initiation and multiplication in different banana cultivars [49-54]. Khalil *et al*. [55] obtained multiple shoots on MS medium with 5 mg l^{-1} BAP, while MS medium with 1 mg l^{-1} NAA, 0.2 mg l^{-1} BAP produced longest shoots after 45 days. Wong [29] reported that increase in BAP concentration resulted in promoting number of shoots per explant. Backiyarani *et al.* [56] analyzed the effect of BA (1, 2, 3, 4, 5, and 6 ppm) on shoot induction, observed that 6 ppm of BA had a significantly positive effect on shoot formation. Another study conducted on the effects of different BA concentrations on different banana cultivars were noted as reproducible [57]. Nguyen *et al.* [58] conducted study on the effects of various levels of BA (0, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 ppm) on shoot multiplication in cv. Grand Naine observed the positive effects of BA. Previous studies described that BA is one of the synthetic cytokinins which assumed tolerance in different developmental forms in the plants promoting the growth [59, 60]. Generally, high frequency shoot bud multiplication of banana cultivars was related to appropriate BA concentrations added in the medium. Shoot bud cultures responded well on the

Fig. 1. (a) Cluster of little shoots of banana, (b) Banana plantlets with well-established leaves and roots ready for shifting in the greenhouse, (c) Shifting of 15 cm long plantlets of banana in the greenhouse, (d) Six months old plants of banana in the greenhouse, (e) 40 cm long healthy banana plant in the just prior to cultivate in the open field, and (f) 40 cm long plant shifted in the open field for further growth.

media supplemented with higher treatments of BA indicate high requirement of cytokinins, i.e., BA or BAP as used in banana tissue culture by several workers. On the contrary, there is risk of somaclonal variations generally when cultures are treated with higher doses of synthetic PGRs during *in vitro* growth. Hence, the attention should be paid to find out the PGRs treatments promoting suitable *in vitro* growth and to avoid any somaclonal variations by applying moderate levels of PGRs throughout all *in vitro* growth stages.

3.2. Effect of Different Treatments of Activated Charcoal on Plantlets' Growth and Rooting

Data in Table 2 indicate that significantly highest leaf number (4 leaves), leaf length (10.7 cm), root number (12 roots) and root length (13 cm) were recorded on the MS medium consisted of 3 g l^{-1} activated charcoal, 0.1 mg l^{-1} NAA in banana cv. Grand Naine (Figure 1(b)). MS medium consisted of 1.5 g l^{-1} activated charcoal, 0.1 mg l^{-1} NAA significantly reduced the leaf number (4 leaves), leaf length (9 cm), root number (7 roots) and root length (10.6 cm) in cv. Grand Naine and in cv. William. Further reduction in all growth parameters was recorded in both cultivars with further reduction in activated charcoal, i.e., 1.0 and 0.5 g l^{-1} with 0.1 mg l^{-1} NAA, however, significantly lowest values were recorded on the medium comprised of 0.5 g l^{-1} activated charcoal after one month. Results of two-way ANOVA showed overall significant effect of cultivar ($p < 0.003$), treatment ($p < 0.002$) and combined effect of cultivar and treatment ($p < 0.005$). The current findings showed that activated charcoal with 0.1 mg l^{-1} NAA is a good option in promoting shoot growth and rooting in banana plantlets. Leaf length, root number, and root length were found to increase steadily with the increase in AC concentration and the highest performance was found to be at 3.0 g l^{-1} in the both cultivars. Although there was minimal difference in the number of leaves, and a significant enhancement in the growth and rooting factors, especially the root number and root length which showed that AC favors rhizogenesis and enhances general plant vigor during *in vitro* growth. This increased performance at the high levels of AC can be credited to its capability to absorb the inhibitor compounds thus stimulating the effectiveness of NAA in the root induction. The existence of genotype differences between William and Grand Naine further underscores cultivar-specific physiological reactions with Grand Naine being more sensitive, especially in root proliferation and elongation. This could be explained by hormonal variations, uptake rate, or metabolic sensitivity to exogenous auxin and absorption via AC. The higher AC concentration (3.0 g l^{-1}) indicates that an ideal ratio between the uptake of inhibitory compounds and the maintenance of auxin concentration is important in terms of *in vitro* shoot development and rooting. Such a combination of treatments can lead to physiological efficiency resulting in the production of vigorous plantlets with enhanced acclimatization potential, which is essential in large-scale micropropagation systems. Several studies [61, 62] utilized indole butyric acid (IBA)

Table 2. Effect of different concentrations of Activated Charcoal + 0.1 mg l^{-1} NAA on plantlet formation and rooting in banana cultivars (William and Grand Naine).

AC (g l^{-1})	William				Grand Naine			
	LN	LL (cm)	RN	RL (cm)	LN	LL (cm)	RN	RL (cm)
0.5	3.0 b	7.3d	3.2d	5.2d	3.0b	6.3d	4.0d	7.1d
1.0	4.0a	7.5c	4.2c	5.8c	3.0b	6.5c	5.0c	7.3c
1.5	4.0a	8.2b	6.0b	10.2b	4.0a	9.0b	7.0b	10.6b
3.0	4.0a	10.2a	9.0a	12.0a	4.0a	10.7a	12.0a	13.0a
LSD (0.05%)	0.06	0.002	0.001	0.001	0.042	0.001	0.001	0.002
Variability								
Cultivar		0.003						
Treatment		0.002						
C × T		0.005						

LN = leaf number, LL = leaf length, RN = root number, RL = root length, C = cultivar, T = treatment. Mean values in columns denoted with different letters show significant levels at $p \leq 0.05$.

Table 3. Effect of different soil types on the survival percentage of *in vitro* grown banana plantlets of cvs. William and Grand Naine during acclimatization in the greenhouse.

Soil medium	Survival %			
	William		Grand Naine	
	1M	6M	1M	6M
River sand	95 ± 1.2a	94 ± 1.1a	96 ± 0.8a	94 ± 0.7a
Desert sand	87 ± 1.0b	84 ± 0.6b	89 ± 0.7b	85 ± 0.5b
Peatmoss	80 ± 0.7c	77 ± 1.2c	83 ± 0.5c	78 ± 0.7c
Clay soil	67 ± 0.5d	60 ± 1.3d	65 ± 1.2d	59 ± 1.1d
Hill sand	62 ± 1.0e	59 ± 0.7e	60 ± 1.1e	57 ± 1.3e
LSD (0.05%)	0.000	0.001	0.000	0.002
Variability				
Cultivar		0.005		
Treatment		0.001		
C × T		0.004		

Mean values in columns with standard error denoted with different letters show significant levels at $p \leq 0.05$.

for rooting in *in vitro* grown plantlets of banana. However, in this study different concentrations of IBA were tested in addition to NAA for rooting. Deo *et al*. [43] observed that NAA was more effective than indole acetic acid (IAA) for *in vitro* rooting in banana plantlets. Banerjee and De Langhe [63] used IBA for root induction in different banana cultivars. Kelta *et al*. [54] observed highest rooting on the MS medium consisted of activated charcoal 200 mg l^{-1}, IBA 1.0 mg l^{-1}. Selvakumar and Parasurama [41] reported that half strength MS medium containing activated charcoal 200 mg l^{-1} and 20 mg l^{-1} adenine sulfate supported rooting in banana cultivars Grand Naine and Elakkia. Deo *et al*. [43] observed healthy rooting in *in vitro* grown banana plantlets using NAA. Activated charcoal has been exploited extensively due to its positive effects on the *in vitro* shoot growth and rooting [64]. Activated charcoal previously was used in the medium to enhance the *in vitro* shoot multiplication and rooting [65-67]. AC also absorbs polyphenols affecting the root growth [68-70]. Similarly, current study observed positive effects of activated charcoal on growth of the plantlets of banana cultured *in vitro*. In addition to activated charcoal, NAA also supported the growth of the roots used in combination with activated charcoal. NAA is widely utilized PGR for *in vitro* rooting in wide variety of plant species. Besides developing dark green colour of the leaves of banana plantlets, the AC also decreases the necrosis in the banana leaves cultured on the medium with activated charcoal indicated normal photosynthesis and chlorophyll development in the plantlets during *in vitro* growth. Banana exhibits rapid *in vitro* growth, therefore subculture number during *in vitro* growth can be reduced, and rapid shifting in the greenhouse avoid genetic variations.

3.3. Effect of Different Soil Types on Survival Percentage of Plants in the Greenhouse after 1M and 6M

Data presented in Table 3 show that significantly highest survival percentage of the acclimatized plantlets in the greenhouse was noted in cv. Grand Naine transplanted on the river sand after 1M (96%) and 6M (94%). Results showed 4% mortality after 1M and further 2% mortality after 6M (total 6% mortality) was recorded in cv. Grand Naine. Better survival percentage of the plants in the greenhouse was also obtained on the desert soil after 1M (89%) and 6M (85%), however, total 15% mortality was recorded in cv. Grand Naine. Further, survival percentage of the plants in greenhouse was recorded on the different soil types after 1M and 6M, i.e., peatmoss (83% and 78% respectively in cv. Grand Naine), clay soil (67% and 60% respectively in cv. William) and hill sand (62% and 59% in cv. William). Results of the two-way ANOVA showed significant differences, with treatment effect ($p < 0.001$) more significant than the cultivar effect ($p < 0.005$), and combined effect of cultivar and treatment ($p < 0.004$). The cultivar-treatment interaction also suggests that cultivar response is different between soils, therefore it is important to maximize the substrate composition for a particular genotype. The findings clearly indicate that the type of soil played a significant role on the survival percentages of *in vitro* raised banana plantlets of cultivars (William and Grand Naine) over time 1M and 6M in greenhouse. Of all the treatments, plants on the river sand had the highest survival rates of 94-96%, and was statistically superior at 1M and 6M. This performance is due to its proper drainage, aeration which are important characteristics of the soils in successful acclimatization of the tissue cultured plantlets that have under developed root systems and are very sensitive to waterlogging. Plants on the desert sand on the other hand had moderate survival percentage (84-89%), although much lower than the plants were growing on the river sand. This may be because of low capacity of water retention and absence of necessary nutrients hindering the growth of plants in the long term after transplanting. Peatmoss resulted in intermediate survival (87-83%) even though it provides good moisture holding capacity, excess supply of water, and a reduced aeration, may have negatively affected root respiration during acclimatization. The lowest percentages of survival were found in clay soil (59-67%) and hill sand (57-62%). The low nutrient levels and irregular changes in moisture caused by poor aeration, compaction and waterlogging and the hill sand may have failed to allow the growth of the banana plants. Decrease in the survival percentage of treatments between 1M and 6M, demonstrated that the acclimatization stress in the long-term and the environmental variability continue to affect the plantlet establishment regardless of the time period since the initial transplanting. Vasane and Kothari [71] observed that soil, press mud cake, vermicompost (1:1:1) and soil, press mud cake, vermicompost (2:1:1) gave better results of plant growth and survival percentage of *in vitro*-derived banana cv. Grand Naine. Parkhe *et al.* [72] recommended several combinations, i.e., peat soil, farmyard manure (FYM), sand, vermicompost, sand and cocopeat alone and in combinations for banana cv. Grand Naine. Chamling and Bhowmick [73] recommended equal proportions of soil, compost, coir pith and sand for hardening the banana plantlets in the greenhouse. Medhane *et al.* [74] used 0.1% (w/v) Bavistin (Carbendazim) for 2-3 minutes for treating the *in vitro* grown plantlets of banana before transfer on soil; the soil was composed of autoclaved black soil, vermicompost and cocopeat (1:1:1). Similarly, in this study washing of whole the plantlets were carried out with fungicide solution (3 g l^{-1} Carbendazim) for 2-3 minutes before shifting on different soil types in the greenhouse for restricting any fungal infection during acclimatization stage.

Wijerathna and Kumarihami [75] obtained maximum survival rate of banana plants on river silt and desert sand (1:1 and 2:1) during acclimatization of banana plantlets in the greenhouse. Sivakumar and Visalakshi [33] transferred the rooted banana plantlets in plastic pots containing garden soil, farmyard manure and sand (2:1:1). Soil mixtures (soil, sand, manure in the ratio of 3:2:1, FYM, vermicompost, neem cake) used in greenhouse for hardening of tissue-cultured banana plantlets [76]. In contrast to previous studies described different soil mixtures for different banana varieties, current study is also an additional approach for getting maximum survival rate of the *in vitro* grown plantlets of the elite banana cultivars William and Grand Naine. Banana plantlets were carefully taken out of culture vessels and washed in a fungicide solution before to culture on different soils in the greenhouse (Figure 1(c)). Plants of both banana cultivars exhibited healthy growth in the greenhouse (Figure 1(d)). Healthy plants of banana (40 cm) were selected for transfer in the open field (Figure 1(e)). Plants were transferred in the open field showed 100% survival rate due to well-developed roots and shoot obtained during acclimatization stage in the greenhouse (Figure 1(f)). Fertilizer (NPK based on the ratio of 1:1:1) 3-4 grams per plant was applied when plants started to grow in the greenhouse. In addition, spray of 3 g l^{-1} fungicide (Copper Oxychloride) was carried out on the growing banana plants to restrict any fungal infection. Banana is rapidly growing plant; hence, elite and rare disease-free banana cultivars can be propagated via tissue culture on large scale in a shortest time and space.

4. CONCLUSIONS

The findings reinforced the critical role of the medium composition towards the successful micropropagation. The research will help improve the tissue culture protocols and will form a scientifically sound foundation upon which to scale up propagation systems in horticultural crops like banana. PGRs had significant contribution in all the *in vitro* growth stages. Activated charcoal with NAA was better to achieve healthy plantlets to be able to shift in the greenhouse for successful acclimatization. Banana plantlets exhibited rapid multiplication and plantlet formation, and rooting during *in vitro* growth. Plantlets successfully acclimatized in the greenhouse on different soil types showed better survival and growth with higher survival rate on river sand. Further, study is required to evaluate the plants in the field and to get fruiting in *in vitro* grown plants, and to study genetic stability of the plants obtained via tissue culture. Moreover, the studies should also be conducted to combine this approach with other growth regulators, and how it can be used under *ex vitro* conditions to further improve commercial relevance. Protocols devised in this study for two exotic banana cultivars will significantly improve to multiply the plants of the same and other banana cultivars on large scale cultivation in the area and across the world.

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6. CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

7. FUNDING

No funding was received to conduct this research.

8. AUTHORSHIP CONTRIBUTION

N.S. wrote the manuscript and analyzed the data, A.A. A-S. helped in experimentation and conceptualization, M.A.J. helped in editing and data analysis, A.A.M. helped in editing and data analysis, Z.A.J. helped in experimentation and editing, and G.S.M. helped in experimentation and methodology.

9. DECLARATION

Authors declare that: (i) the results are original, (ii) the same material is neither published nor under consideration for publication elsewhere, (iii) approval of all authors has been obtained, and (iv) in case the article is accepted for publication, its copyright will be assigned to the Pakistan Academy of Sciences.

10. ETHICAL STATEMENT

This work does not include any studies involving human or animal subjects.

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