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Optimization of BAP Concentration for *In vitro* Mass Multiplication of G9 and Agnishwar Banana (*Musa* spp.) Varieties

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Key words: Grand Naine (G9), Agnishwar, BAP, Quantitative data, Banana micropropagation

Abstract

Banana (*Musa* spp.) is an economically important fruit crop cultivated globally, including Bangladesh. Efficient *in vitro* propagation is crucial for large-scale production of disease-free planting materials. This study aimed to optimize the concentration of 6-benzylaminopurine (BAP) for mass micropropagation of two banana cultivars, the red banana (Agnishwar) and the G9 (Grand Naine) Cavendish cultivar. Shoot tip explants were cultured on MS medium supplemented with 1.0 mg/l 1-naphthaleneacetic acid (NAA) and varying concentrations of BAP (1.0-6.0 mg/l). The effect of BAP on shoot multiplication, shoot elongation, leaf number and root induction were assessed at 60 days after culture (DAC). Results showed that shoot multiplication was significantly influenced by BAP concentration, while shoot elongation and leaf number exhibited significant results for genotype × BAP interactions. The highest shoot length (27.88 cm) was recorded in G9 at 6.0 mg/l BAP, whereas Agnishwar attained a maximum shoot length of 13.04 cm at 3.0 mg/l BAP. Leaf number was significantly affected at 1% level, with G9 producing more leaves than Agnishwar. Root induction efficiency remained unaffected by BAP concentrations, though genotype-specific responses were observed. *Ex vitro* acclimatization of micropropagated plantlets demonstrated high survival rates (83.33% for G9 and 81.25% for Agnishwar), confirming the effectiveness of the optimized protocol. The BAP concentration plays a critical role in shoot multiplication, shoot elongation and leaf development, with genotypic variations influencing response to *in vitro* conditions. The optimized protocol can facilitate large-scale propagation of high-quality planting materials for sustainable banana production in Bangladesh.

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Introduction

Banana (*Musa* spp.) is a highly important fruit crop belonging to the Musaceae family. It is extensively cultivated in developing countries due to its economic significance, high nutritional value and role in food security. Globally, banana ranks as the second most widely produced fruit crop, following citrus fruits in terms of total production (Madhulatha et al. 2004). Edible bananas serve as one of the essential foods for both rural and urban populations in tropical and subtropical countries, providing a vital source of nutrition and energy. In addition to their dietary importance, banana cultivation and trade significantly contribute to the livelihoods of rural communities, serving as a key source of income for smallholder farmers and agricultural laborers. It is one of the most important fruit crops in Bangladesh, contributing significantly to food security, nutrition and the agricultural economy. Among the commercially cultivated varieties, Agnishwar and Grand Naine (G9) are highly valued for their superior yield, fruit quality, and market demand. Bananas are popular because of their low price and are used as both vegetables and dessert fruits. They are high in carbohydrates and contain a variety of vitamins, including vitamin B. They also contain high levels of potassium, phosphorus, calcium and magnesium. Regular using of banana can reduce the risk of heart disease and is advised for people with high blood pressure, arthritis, ulcers, gastroenteritis, and renal diseases. Due to the year-round availability, popularity and production, the banana is considered to be the number one fruit in Bangladesh. Bananas grow practically everywhere in the country throughout the year.

The red banana, commonly known as Agnishwar, is a distinct triploid cultivar (AAA group). The botanical name is *Musa acuminata*. This type of banana is smaller in size and has a distinct red to purple peel compared to traditional yellow bananas like Shagar and Sabri (Kumar 2012). Additionally, it has a delicate and creamy texture and a somewhat pink tint. The red bananas have a delicious strawberry aroma and a crimson color caused by beta-carotene (Kadam et al. 2023). The G9 banana is a high-yielding and Cavendish banana cultivar known for its flavor, disease resistance and nutritional value. G9 bananas have long fruits on pretty short plants. When immature, they are solid green, but when mature, they turn yellow. This banana cultivar was supposed to be introduced to Bangladesh via India (Genewin Biotech 2024).

The banana is usually propagated by suckers or offshoots from mature plants. While this method is simple and widely used, it has disadvantages, including a low multiplication rate, time consuming, a limited supply of disease-free planting material and non-uniformity in growth, yield and fruit quality (Tumuhimbise and Talengera 2018, Agbadje et al. 2021). Micropropagation offers a remedy for the drawbacks of traditional methods and has great promise for the large-scale production of red and G9 bananas. Banana plantlets produced through tissue culture exhibit several advantages over conventionally propagated plants. The absence of diseases in *in vitro*-grown plantlets ensures the establishment of healthy and disease-free plantations. It produces genetically

identical plants, ensuring consistency in fruit quality, growth and yield. Micropropagation is not influenced by seasonal constraints, ensuring a consistent supply of planting material (Abdalla et al. 2022). They have a higher survival rate, require lower pest and disease management costs, demonstrate more vigorous growth and reach maturity faster, leading to earlier harvesting and greater economic benefits (Rout et al. 2022). Compared to suckers, tissue-cultured banana plantlets are lighter, particularly when using a light potting substrate and their growth rate is higher than that of the typical suckers. Moreover, tissue-cultured plantlets flower faster, provide a consistent harvest and have a 20-50% increase in fruit output (Kumar et al. 2024). Micropropagation has played a crucial role in banana and plantain breeding programs worldwide, enabling large-scale production of disease-free and genetically uniform plantlets (Kumar et al. 2024).

In banana micropropagation, plant growth regulators (PGRs) are crucial because they regulate and improve important processes like bud proliferation and root development, differentiation, cell division, and organogenesis. The success of *in vitro* propagation relies heavily on the precise type, concentration and combination of PGRs in the culture medium (Kaur et al. 2022, Pasternak and Steinmacher 2024). In shoot tip culture, cytokinins play a crucial role in promoting bud growth, shoot initiation and overall shoot development (North et al. 2012, Sosnowski et al. 2023). Among various cytokinins, 6-benzylaminopurine (BAP) is widely used due to its synergistic effect, which enhances shoot proliferation when combined with other growth regulators. Several researchers have successfully employed BAP in their studies to improve *in vitro* shoot multiplication and development (Al-Amin et al. 2009, Jafari et al. 2011, Sipen and Davey 2012, Ngomuo et al. 2013, Padmavathi et al. 2023).

Plant tissue culture technology has become a fundamental tool in modern biotechnology, significantly contributing to crop improvement and conservation. In this context, the present study aims to optimize the concentration of the cytokinin BAP for the rapid *in vitro* multiplication of two banana cultivars such as red banana (Agnishwar) and Grand Naine (G9) to enhance propagation efficiency and facilitate the successful establishment of micropropagated plants in open-field conditions.

Materials and Methods

Healthy, three-month-old sword suckers of the red banana (Agnishwar) variety and Grand Naine (G9) were selected as explants and collected from disease-free mother plants cultivated at the Horticulture Center, Dept. of Agricultural Extension, Doulatpur, Khulna. These explants served as the starting material for *in vitro* propagation.

The outer sheaths of the collected sword suckers were carefully removed and the suckers were cut into smaller segments (approximately 8 to 10 cm). These sucker segments were first rinsed under running tap water for 30 min. This was followed by immersion in a solution containing a few drops of Tween-20 for 10 min to remove surface

contaminants. Next, the explants were treated with 0.1% Bavistin fungicide for 2 min to eliminate any pathogens. The explants were sequentially treated with 80% sodium hypochlorite, followed by 0.1% mercuric chloride and 80% ethanol to eliminate microbial contamination. After that, the explants were thoroughly rinsed 5 times with sterile distilled water to remove any residual chemicals. Finally, the explants were allowed to air dry for a few minutes before being trimmed to a final size of approximately 3 to 4 cm for further *in vitro* processing (Fig. 1).

The experiment was set up in a factorial Completely Randomized Design (CRD) where Factor A: Two banana varieties (Agnishwar and G9) and Factor B: Six variable doses of BAP along with one without growth regulator, where each treatment was replicated 5 times. Each replication consisted of 15 culture bottles (113 cm × 22 cm) where each bottle contained 50 ml medium. The medium was supplemented with 1.0 mg/l NAA, either with or without the addition of BAP (according to the treatments), 100 mg/l ascorbic acid, 8 mg/l agar and 30 mg/l sucrose. The pH of the media was adjusted to 5.6 before autoclaving at the temperature of 121°C and 15 psi pressure for 15 min.

The prepared explants were then aseptically transferred to above mentioned medium. The cultures were incubated in a growth chamber with a 16 hrs light/ 8 hrs dark photoperiod, under fluorescent lights providing a light intensity of 3000 lux. The temperature was maintained at 25 ± 1°C, with a humidity level of 50-70%. After every 4-week interval, the cultures were sub-cultured onto fresh medium of the same composition to ensure continued growth and development.

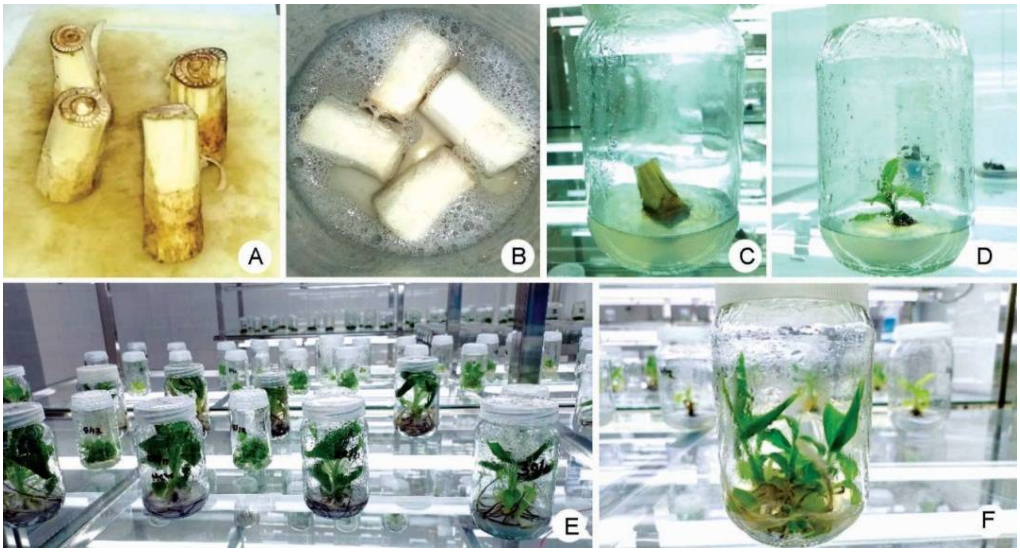

Fig. 1 (A-F). *In vitro* multiplication of banana from shoot tips: (A) Banana shoots tips used as the explant source, (B) Surface sterilization of the explants, (C) Culture of explants onto shoot induction medium, (D) Shoot initiation from the cultured explants, (E) Shoot proliferation in the culture medium, (F) Shoot multiplication from proliferated shoots.

For rooting, healthy *in vitro* shoots, measuring 4 to 5 cm in length, were transferred to half-strength MS medium, which was supplemented with 1.0 mg/l of indole-3-butyric acid (IBA) to promote root development.

Well-rooted, healthy plantlets were gently washed under running tap water and then treated with the antifungal solution Bavistin @ 0.1%. The plantlets were transferred to plastic pots (10 cm in length) containing pot mixtures (garden soil, sand, semi-decomposed rice husks and vermicompost= 2 : 1 : 1 : 1) and kept in a net house. They were frequently watered and kept under observation for 6 weeks. The established plants from small pots were transferred to the main field into 20 cm × 20 cm planting pits. Soils of each pit were mixed with 10 kg vermicompost, 100 g TSP, 100 g MoP, 10 g $ZnSO_4$ and 50 g gypsum and were filled with the pits (Fig. 2).

Data were collected at 4-week intervals after subculturing and recorded for various parameters, including the number of shoots explant^{-1}, shoot length (cm), the number of leaves per explant and the *ex vitro* survival rate of the plantlets. The data were statistically analyzed based on the mean values for each treatment using analysis of variance (ANOVA). The statistical analysis was performed using the R software package (R Core Team 2018), version 2.14.0.

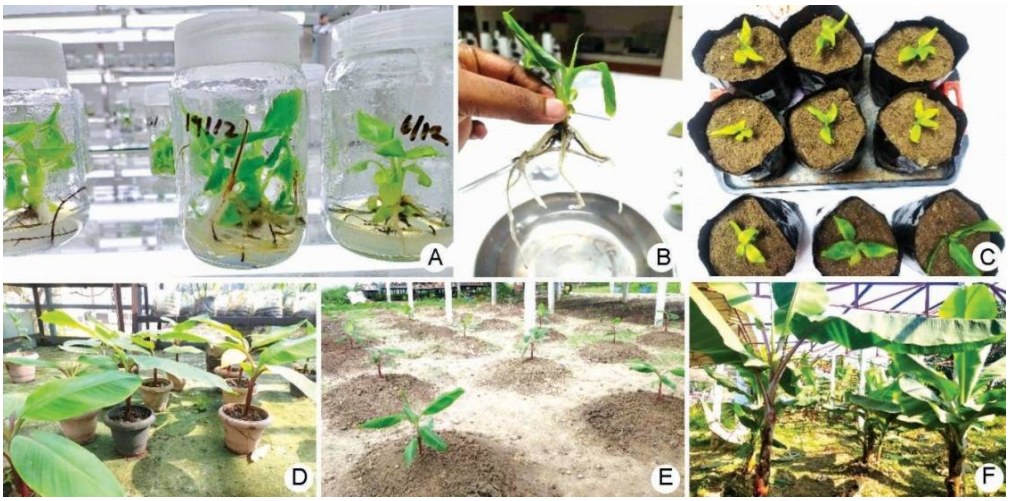

Fig. 2 (A-F). Root induction to the *in vitro* grown banana shoots and their *ex vitro* establishment: (A) Culture of regenerated shoots on root induction media, (B) Well rooted banana plantlets ready to *ex vitro* transfer, (C) *Ex vitro* transfer of regenerated plantlets, (D) Survived plantlets in net house condition, (E) Hardened and acclimatized plants in soil, (F) Established banana plants in field condition.

Results and Discussion

The analysis of variance demonstrated the impact of variety (G9 and Agnishwar), BAP concentration, and their combined effect on banana plant growth indices at 60 days after culture (DAC) (Table 1). There was no significant difference in the results based on the

banana variety or the BAP concentrations alone for any of the parameters measured. But the interaction effect of banana variety and BAP concentrations on growth indices were significant for shoot length and leaf numbers whereas non-significant effects were exhibited on shoot number and root number.

Table 1. Analysis of variance (ANOVA) table of the effect of BAP concentration on *in vitro* growth indices of two banana varieties.

Source of variance	df	Mean square			
		Shoot number	Shoot length (cm)	Leaf number	Root number
Variety	1	1.17^{NS}	493.87^{NS}	4.95^{NS}	53.25^{NS}
BAP conc. (mg/l)	6	18.69*	201.51^{NS}	5.17^{NS}	19.48^{NS}
Variety × BAP conc.	6	2.40^{NS}	297.87**	5.85**	11.40^{NS}
Error	56	7.27	125.44	3.49	13.56

NS = Not significant ($p > 0.05$), * = Significant at $p \leq 0.05$, ** =Significant at ≤ 0.01.

The effect of different concentrations of BAP along with 1.0 mg/l NAA on shoot multiplication was evaluated. The number of shoots showed a significant variation at the 5% level, with the highest shoot number (4.26) observed at 1.0 mg/l BAP (Table 2). Shoot length varied among the treatments. Shoot length varied between 6.60 cm and 15.35 cm. The number of leaves per plantlet also showed variation. The lowest leaf number (1.48) was recorded at 2.0 mg/l BAP, while the highest (2.83) was observed at 1.0 mg/l BAP.

Table 2. Effect of BAP levels on different morphological parameters of *in vitro* grown banana plantlets.

Level of BAP (mg/l)	Shoot number	Shoot length (cm)	Leaf number
0.0	1.43^{b}	13.62	1.97
1.0	4.26^{a}	12.90	2.83
2.0	2.26^{ab}	6.60	1.48
3.0	2.83^{ab}	10.50	2.63
4.0	2.30^{ab}	14.49	1.95
5.0	1.60^{b}	14.49	2.17
6.0	1.30^{b}	15.35	2.07
Level of significance	*	NS	NS

* = Significant at 5% level of significance; NS = Not significant.

Varying levels of BAP concentrations did not exhibit any significant effect on subsequent root induction efficiency of banana varieties when they were transferred to root induction media (Fig. 3). The highest (5.56) roots per plant was recorded for the

shoots obtained from media supplemented with 1.0 mg/l BAP, while lowest root count (2.79) was observed for the plantlets gained from 2.0 mg/l BAP containing shooting media. Overall, no significant differences were observed among treatments for shoot length, leaf number or root number.

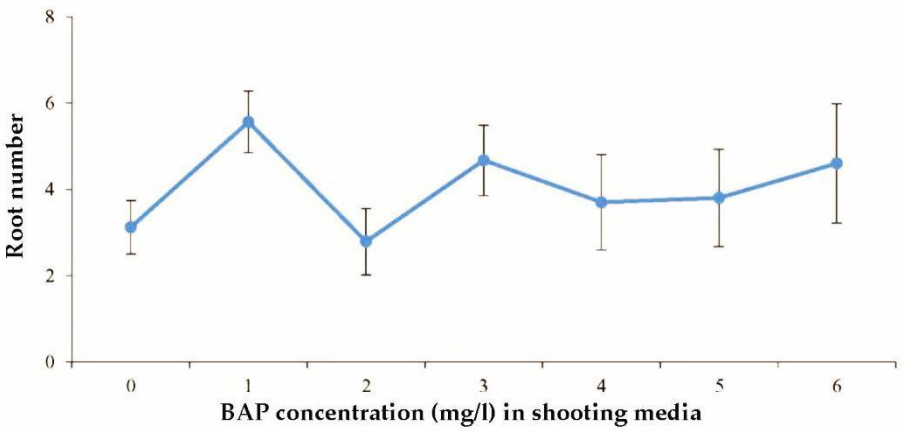

Fig. 3. Effect of BAP concentration of shooting media on root induction efficiency of banana in rooting media.

Variation in the concentration of BAP with 1.0 mg/l NAA exhibited a significant difference in shoot multiplication of two banana varieties was noted. According to the results of the study, the most suitable BAP concentration for efficient shoot multiplication was 1.0 mg/l in MS media supplemented with 1.0 mg/l NAA. Higher concentrations of BAP (5.0 and 6.0 mg/l) had inhibitory effects that may be attributed to disrupted hormonal balance leading to poor growth or partitioning to fewer shoots. Similar research done by Ali et al. (2011) validates the result of this study as they obtained optimal shoot formation response (100%) using banana shoot tip explant cultured on MS medium fortified with 1.0 mg/l BAP. Variation in the concentration of BAP exhibited no significant differences in the shoot length, leaf number and root number of the banana. The low level of variability makes it possible to state that, on the condition of the given experiment, BAP stimulation did not have a significant impact on shoot elongation, leaf number and root number. The longest shoots were recorded at 6.0 mg/l BAP. These results are in fair agreement with Ferdous et al. (2015) and Khatun et al. (2017) revealed that the higher concentration of BAP is the key factor for high shoot regeneration and shoot length. Cytokinins like BAP are essential in the culture to enhance the cell differentiation, proliferation of shoot and morphogenesis (Chugh et al. 2009, Justine et al. 2022, Adero et al. 2023). Suitable cytokinin concentrations in the medium inhibit apical dominancy and support the initiation of lateral shoots (Jafari et al. 2011). The highest root proliferation obtained at 1.0 mg/l is in consonant with the findings of Rahman et al. (2023), Sivakumar and Visalakshi (2021) and Amente et al. (2022) who reported that excess of BAP stimulates rooting in banana tissue cultures. High levels of BAP may be

detrimental to the process of rooting since they may compromise the ratio between auxin and cytokinin. Aremu et al. (2020) confirm that the regulatory growth stimuli need a correct ration of cytokinins and auxins in tissue culture medium.

No significant variation on shoot multiplication, shoot length, leaf number, and root number of the two banana varieties, G9 and Agnishwar, was observed (Fig. 4).

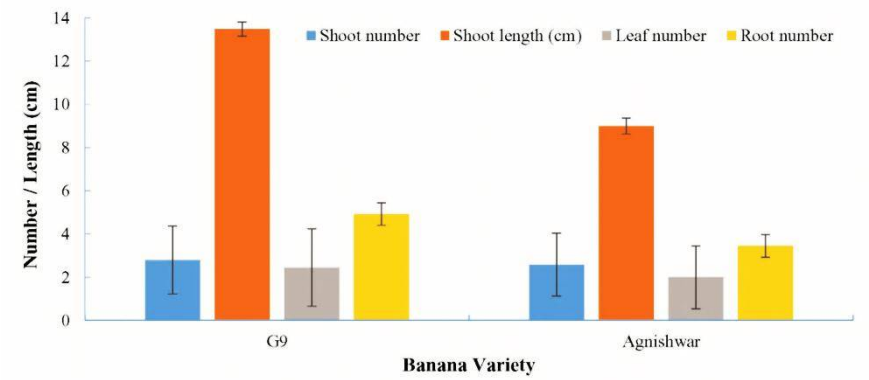

Fig. 4. Varietal effect of banana on *in vitro* shoot multiplication, shoot length, leaf number and root number.

Insignificant difference between shoots multiplication, shoot length, leaf number and root number of G9 and Agnishwar indicates that both varieties have similar response to culture conditions (Fig. 4). Slightly higher values in G9 can indicate a slightly better result but did not find the statistical significance of these results. Gübbük and Pekmezci (2004), Ngomuo et al. (2013) and Agbadje et al. (2021) suggested that apart from the genotype, shoot proliferation was also affected by exogenous cytokinin concentration in growth medium. The non-significant differences in shoot multiplication and shoot length between G9 and Agnishwar implies that genotypic influence on shoot proliferation was minimal under the given culture conditions. The primary role of BAP in stimulating cell division and shoot induction appears to be effective in both varieties, leading to uniform shoot growth.

The interaction between banana varieties and different BAP concentrations did not show a significant effect on shoot number or root number. However, a significant interaction effect was observed for shoot length. The longest shoots were recorded in G9 at 6.0 mg/l BAP, with shoot lengths of 27.88 cm (Table 3). In comparison, Agnishwar exhibited shorter shoot lengths across all BAP concentrations. The maximum shoot length for Agnishwar was 11.77 cm. These results indicate that shoot elongation is significantly influenced by both the genetic makeup of the variety and the BAP concentration used. A significant interaction effect was also observed for leaf number. The variation in leaf number between the two varieties across BAP levels was significant at the 1% level. This suggests that both the banana variety and the BAP concentration significantly impact leaf development over time.

Table 3. Interaction effect of variety and levels of BAP on *in vitro* shoot multiplication, shoot length, leaf numbers and root numbers of banana.

Variety	Level of BAP (mg/l)	Shoot number	Shoot length (cm)	Leaf number
G9	0.0	1.82	6.95^{bc}	2.36^{abc}
	1.0	4.39	15.38^{abc}	2.88^{ab}
	2.0	1.85	5.05^{bc}	1.14^{bc}
	3.0	2.92	11.32^{bc}	2.89^{ab}
	4.0	3.00	17.21^{ab}	2.30^{abc}
	5.0	2.00	18.01^{ab}	2.41^{abc}
	6.0	2.00	27.88^{a}	3.70^{a}
Agnishwar	0.0	1.50	7.33^{bc}	1.68^{abc}
	1.0	4.10	9.67^{bc}	2.76^{abc}
	2.0	2.80	8.62^{bc}	1.92^{abc}
	3.0	2.70	9.44^{bc}	2.31^{abc}
	4.0	2.60	11.77^{bc}	1.60^{abc}
	5.0	1.40	10.96^{bc}	1.93^{abc}
	6.0	1.20	2.81^{c}	0.43^{c}
Level of significance		NS	**	**

** = Significant at 1% level of significance; * = Significant at 5% level of significance; NS= Not significant.

Interaction effect of variety and BAP concentrations of shoot induction media was found insignificant in root induction efficiency of banana when they were transferred onto root induction media (half-strength MS medium supplemented with 1.0 mg/l of IBA). Variable results on root number were recorded with the increase of BAP concentrations in shoot induction media for both the varieties (Fig. 5). The maximum root number (6.8) was observed for the variety G9 which were obtained from the shoot induction media supplemented with 6.0 mg/l BAP followed by the plantlets obtained from the shoot induction media supplemented with 1.0 mg/l BAP for the same variety. Whereas the minimum root (2.05) was found for the plantlets of Agnishwar variety that were gained from the shooting media with no BAP.

The interaction between banana varieties and different BAP concentrations did not significantly affect shoot number. This suggests that under the tested conditions, both varieties responded similarly in terms of shoot proliferation and root initiation, indicating that genotypic variation had little influence on these parameters. However, Arinaitwe et al. (2000) reported that similar explants from different banana cultivars can respond differently to the same cytokinin concentration, reinforcing the idea that hormonal sensitivity is genotype-dependent. Furthermore, studies by Rahman et al. (2013), Iqbal et al. (2013) and Subrahmanyeswari et al. (2022) have demonstrated that the

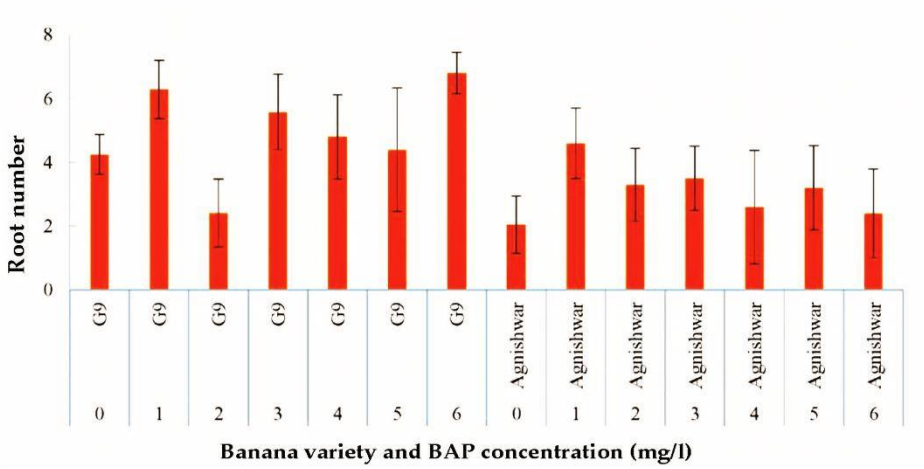

Fig. 5. Interaction effect of variety and levels of BAP of shooting media on root numbers of banana grown on rooting media.

in vitro shoot regeneration capability of bananas is highly genotype-specific and significantly influenced by cytokinin concentrations in shoot-inducing media. This suggests that while G9 and Agnishwar did not exhibit significant differences in shoot number or root number in this study, other genotypes may respond differently under the same conditions. The lack of significant variation in our results may indicate that the tested BAP concentrations were within an optimal range for both varieties, thereby reducing the observable differences in shoot and root development.

A significant interaction effect was observed for shoot length, indicating that the elongation response of the plantlets was influenced by the interaction between variety and BAP concentration. G9 showed greater shoot elongation, particularly at 6.0 mg/l BAP, with shoot lengths of 27.88 cm. In contrast, Agnishwar had shorter shoot lengths across all BAP concentrations, with maximum values of 11.77 cm (at 4.0 mg/l BAP). This significant difference highlights that genetic makeup plays a crucial role in shoot elongation, as different cultivars may have varying hormonal sensitivities, affecting their growth responses. The results suggest that G9 responds more favorably to higher BAP concentrations for shoot elongation, whereas Agnishwar may require a different hormonal combination for optimal growth. Similar findings have been reported by Maseko et al. (2024) and Singh et al. (2024) who observed that different banana genotypes exhibit variable shoot growth patterns in response to BAP levels, highlighting the importance of variety-specific optimization in micropropagation protocols.

Similarly, a significant interaction effect was observed for leaf number. The variation in leaf number between the two varieties across different BAP concentrations was significant at the 1% level. This indicates that both genotype and BAP concentration influence leaf production over time. The progressive increase in leaf number over time

suggests that the long-term exposure to BAP had a cumulative effect on leaf initiation and development, which could impact overall plant vigor and photosynthetic capacity. The findings of this study align with those of Ferdous et al. (2015), who reported that the highest leaf number in two banana cultivars was achieved in media containing 5.0 mg/l BAP. Correspondingly, Shiragi et al. (2008) observed significant variations in leaf number among different BAP concentrations, with the highest leaf count recorded at 3.0 mg/l BAP, which was comparable to results obtained with 5.0 mg/l BAP.

The results indicate that varying BAP concentrations did not have a significant impact on root induction efficiency when the banana varieties were transferred to rooting media. The lowest root count (2.79 roots per plantlet) was observed in plantlets that originated from 2.0 mg/l BAP-containing shoot induction media, while the highest root number (5.56 roots per plantlet) was recorded in plantlets derived from 1.0 mg/l BAP-containing media. The findings align with earlier reports by Rustikawati et al. (2021) and Bayhan and Yücesan (2024) which suggested that excessive BAP concentrations may inhibit root induction in banana tissue culture. High cytokinin levels, such as BAP, are known to promote shoot proliferation but can also negatively impact root development by disrupting auxin-cytokinin balance, which is critical for root initiation. The lower root number in G9 compared to Agnishwar across all BAP levels further suggests a genotype-specific response to BAP, consistent with previous studies by Gobena et al. (2018) and Khaskheli et al. (2021). Their findings emphasized that different banana varieties exhibit varying sensitivities to plant growth regulators, which can influence rooting efficiency.

The survival rates of *in vitro* regenerated G9 and Agnishwar plantlets were evaluated after their transfer to potting mixtures under *ex vitro* conditions (Fig. 6). The results indicate that both varieties exhibited high survival rates, with G9 achieving 83.33% survivability and Agnishwar showing a slightly lower survival rate of 81.25%. Out of 12 transferred G9 plantlets, 10 successfully acclimatized, whereas for Agnishwar, 13 out of 16 plantlets survived.

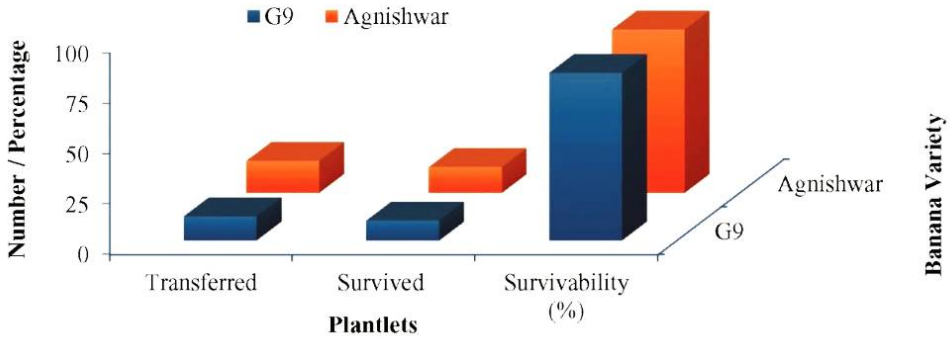

Fig. 6. Survivability of *in vitro* grown plantlets of two banana varieties to *ex vitro* condition.

Acclimatization of *in vitro*-regenerated banana plantlets is a critical phase in micropropagation, as it determines their ability to survive and grow under natural environmental conditions. The high survival rates for both G9 and Agnishwar indicate that the micropropagated plantlets successfully adapted to the transition from controlled *in vitro* conditions to soil-based environments, a crucial step for large-scale banana propagation. The slightly higher survival rate of G9 compared to Agnishwar could be due to genotypic differences in stress tolerance, root development or overall physiological adaptability during acclimatization. Similar findings have been reported by Nandwani et al. (2000), who observed a 90% survival rate in the banana cultivar Basrai and by Kelta et al. (2018), who recorded 82% and 88% survival rates for *in vitro*-regenerated plantlets of Poyo and Giant Cavendish cultivars, respectively.

The study was designed to evaluate the effect of different BAP concentrations along with 1.0 mg/l NAA on *in vitro* shoot multiplication and root induction of two banana varieties, G9 and Agnishwar to optimize micropropagation protocols. The findings revealed that BAP significantly influenced shoot elongation and leaf number, with G9 responding more favorably at higher BAP concentrations, whereas Agnishwar showed shorter shoot lengths. But, shoot number and root number were not significantly affected by BAP levels, indicating that both varieties had similar shoot proliferation and rooting responses under the tested conditions. However, genotypic differences in root induction efficiency, with G9 producing fewer roots than Agnishwar, highlighting the importance of variety-specific optimization for tissue culture protocols. The high survival rate of *in vitro* regenerated plantlets in *ex vitro* conditions demonstrates the feasibility of large-scale propagation of banana using micropropagation techniques.

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