Research Article

Effect of Plant Growth Regulators on Growth Traits of Banana Genotypes

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Abstract | *In-vitro* propagation is mostly used to create high-yielding banana plantlets. Therefore, the current research study was conducted to optimize the best concentration of BAP (cytokinin) and IAA (auxin) on shoot and root proliferation in selective banana varieties (W-11 and B-10). Additionally, this study determined the best tissue culture responsive variety. For shoot proliferation, 12 treatments were made using Murashige and Skoog (MS) basal media with combination of BAP (0, 2, 3.5, and 5 mg/l) and IAA (0, 0.5, and 1 mg/l). For root proliferation, 9 treatments were examined with BAP (0, 0.5, and 1 mg/l) and IAA (0, 1.5, and 2 mg/l). Completely randomized design (CRD) with 3 replications was used to conduct this experiment. The outcome of this study reported that the best result in both varieties were obtained when BAP and IAA were combined. For B-10 banana cv., the optimal concentration was found to be BAP 2 mg/l + IAA 0.5 mg/l for higher number of shoots (5.90), length of shoot (13.07 cm) and number of leaves (7.03). In contrast, for W-11 banana cv., the optimal concentration for higher number of shoot (4.87), shoot length (11.13 cm) and number of leaves (6.78) was BAP 5 mg/l + IAA 1 mg/l, BAP 2 mg/l + IAA 1 mg/l and BAP 3.5 mg/l + IAA 0.5 mg/l, respectively. The optimal concentration for number of roots (9.30) and length of roots (9.86 cm) in B-10 banana cv. was BAP 0.5 mg/l + IAA 2 mg/l while the optimal concentration for number of roots (6.80) and root length (7.60 cm) in W-11 banana cv. was BAP 1 mg/l + IAA 2 mg/l. Overall, the B-10 variety showed better results in all parameters as compared to W-11, indicating its better capability under the given tissue culture conditions. This can help improve the productivity of banana cultivation by ensuring that planting materials are disease-free, thereby minimizing the risk of crop damage from diseases and pests.

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Introduction

Banana (*Musa* spp.) belongs to the family Musaceae. The fruit of the banana is highly nutrient-dense, has a lot of health advantages, and provides a quick source of energy (Zafarullah *et al*., 2021). As a result, it is regarded as the ideal food for athletes and other people who engage in intense physical activity. This is due to its high caloric content, potassium-rich vitamins and minerals, as well as appealing sensory properties (Maertens *et al*., 2012). During digestion, the carbohydrates in bananas, approximately 20 g of carbohydrates per 100 g of fresh pulp, provide immediate yet enduring energy (Aurore *et al*., 2009). It is a major 4th staple food after rice, corn and wheat crop that supplies food and income for millions of people through trade both locally and globally (Singh *et al*., 2011).

In-vitro propagation method of banana (*Musa* spp.) offers many benefits. It ensures a rapid rate of multiplication, physiological consistency, year-round accessibility to disease-free plant material and uniformity of shoots. This method also shortens the harvest interval and quicker growth throughout the early stages of development (Vuylsteke, 1989). While traditional propagation techniques like corms, suckers, and sward suckers are unsuitable because they have poor phytosanitary qualities, slow growth rates, and the potential to harbour diseases, viruses, and nematodes (Sagi *et al*., 1998). In the modern era, plant tissue culture technologies have proven to be extremely beneficial for disease eradication, plant enhancement and large-scale industrial plant production (Ahmad *et al*., 2012; Ali and Mehmood, 2017; Ali *et al*., 2021; Jabeen *et al*., 2021; Jabeen *et al*., 2022).

Plant growth regulators (PGRs) are essential for the development of a specific growth mode of cultured tissues in banana tissue culture. This growth mode may be induced by an increase in particular biochemicals within the tissues. PGRs are applied in different ratios or combinations to promote shoot formation and growth (Madhulatha *et al*., 2004; Ikramul-Haq and Dahot, 2007). Cytokinins and auxins are commonly used phytohormones that influence banana shoot and root proliferation and elongation (Sipen and Davey, 2012). BAP is a cytokinin that is essential to initiate adventitious and axillary shoots in bananas from meristematic explants. It also inhibits the apical dominance in banana (Buah *et al*., 2010). Similarly, the best auxin is IAA (indole acetic acid), which is crucial for plant growth and cell differentiation. During *in vitro* propagation, IAA has been shown to promote and induce rooting in plants (Bohidar *et al*., 2008; Hussein, 2012).

Positive effects of combination of cytokinin with auxin have been reported in numerous studies (Shah *et al*., 2013; Shah *et al*., 2015; Jan *et al*., 2015). Dagnew *et al*. (2012) demonstrated that the highest shoot proliferation and elongation were noted at 3 mg/l BAP + 0.4 mg/l IAA concentrations for Dwarf banana cultivar. Al-Amin *et al*. (2009) reported that banana variety BARI Banana-I showed highest shoot proliferation at BAP 7.5 mg/l + NAA 0.5 mg/l. Moderate quantities of cytokinin increased the rate of shoot growth, but a very high concentration decreased both shoot elongation and multiplication (Gubbuk and Pekmzci, 2004), while high dose of auxin in shoot inducing media, suppressed the shoot development and growth (Buah *et al*., 2010).

Auxin and cytokinin combinations and concentrations in the nutrient media play an important role in the success of plant regeneration (North *et al*., 2010; Azam *et al*., 2019). Different cytokinin activity levels can be attributed to variations in the uptake rates found in various genomes (Muhammad *et al*., 2007). The goal of the current study was to enhance the *in-vitro* protocols of tissue culture for the mass propagation of the exotic banana cultivar B-10 and W-11. Therefore, in the current study the best concentration of BAP and IAA was optimized on shoot and root proliferation, as well as multiplication of selective banana varieties (W-11 and B-10). It also determined the best tissue culture responsive variety.

Materials and Methods

Plant material

Thirty-day-old suckers of high-yielding exotic banana varieties namely B-10 and W-11, which are in good health, were used as the source of explants for tissue culture. These suckers were sourced from the National Agricultural Research Centre (NARC) experimental fields in Tandojam, Sindh, Pakistan, and were employed to develop *in vitro* shoot tip cultures. The suckers, each with a single shoot tip, were peeled to a size of approximately 4 × 5 cm and then dipped in 20% ethanol. This study was conducted with the selected banana varieties in the Plant Tissue Culture

Laboratory of Allama Iqbal Open University, Islamabad, Pakistan.

Sucker sterilization

To get rid of any surface bacteria, the explants were properly washed in water, treated with 70% ethanol for 1 minute, and then treated with a 5.25% sodium hypochlorite solution (Clorox) diluted at 10%, 20%, 30 % and 40% (v/v) for 20 minutes. The explants were thoroughly cleaned in water that had been double-distilled to get rid of any sodium hypochlorite residue for 4 to 5 times. Afterward, the explants were transferred to simple MS media to optimize best Clorox concentration. For this purpose, observed the contamination and sprouting frequency.

Media preparation and culture condition for shoots

In order to shoot initiation and multiplication, aseptically disinfected explants were cultured into MS (Murashige and Skoog, 1962) basal media fortified with different concentrations of BAP and IAA as indicated in Table 1. Medium's pH was adjusting to 5.75 before adding Gellan gum powder. Keep shoot cultures in growthroom at 25±2°C under a 16/8-hour photoperiod and 3,000 lux intensity of light. The shoots were aseptically removed from the culture when they attained a length of approximately 3-5 cm and had three to six fully developed leaves. They were then split apart and cultivated once more on similar fresh medium. The shoots were subcultured on MS media every three weeks up to 3 cycles for multiple shoots. Data for number of shoots, length of shoot and number of leaves was recorded.

Table 1: *Treatments of BAP and IAA alone and its combination used for shoot multiplication.*

Treatments	BAP(mg/l) + IAA(mg/l)
T1	0 + 0
T2	2 + 0
T3	3.5 + 0
T4	5 + 0
T5	0 + 0.5
T6	0 + 1
T7	2 + 0.5
T8	3.5 + 0.5
T9	5 + 0.5
T10	2 + 1
T11	3.5 + 1
T12	5 + 1

4.43 g/l MS (Murashige and Skoog) basal media was fortified with BAP and IAA of different concentrations, 30 g/l sucrose and 2.25 g/l Gellan gum powder.

Root induction media

After *in vitro* multiplication, the individual shoots were removed from the explants and transferred to a newly made MS rooting medium and then treated with various amount of cytokinins and auxins as indicated in Table 2. Number of roots and root length were examined 6 weeks after shoots were inoculated to rooting media.

Table 2: *Treatments of BAP and IAA alone and its combination used for root proliferation.*

Treatments	BAP (mg/l) + IAA (mg/l)
T1	0 + 0
T2	0.5 + 0
T3	1 + 0
T4	0 + 1.5
T5	0 + 2
T6	0.5 + 1.5
T7	1 + 1.5
T8	0.5 + 2
T9	1 + 2

MS (Murashige and Skoog) basal media was fortified with of BAP and IAA of different concentrations, 30 g/l sucrose and 2.25 g/l Gellan gum powder.

Acclimatization and transplantation

Agar was removed from the roots of elongated plantlets by carefully washing the roots under running tap water. Individual plantlets were placed in small polybags that were filled with different sterile potting mixtures in a 1: 3 (v/v) ratio (sand to soils). For a week, the plants were kept in the greenhouse with high humidity levels (80 to 90%) to aid in hardening and acclimatization. Plantlets were kept outside the chamber and the humidity was gradually lowered. After that, the plants were moved to larger polybags that were filled with sand, manure, and forest soil (2: 1: 1). Eventually, the hardened plants were ready to move into the field.

Experimental design and statistical analysis

This experiment was carried out following complete randomized design (CRD). In this study, 12 treatments consisted of different concentrations of BAP and IAA were applied in three replications to two banana varieties (B-10 and W-11) for shoot proliferation and 9 treatments consisted of BAP and IAA of different concentrations for root proliferation. Shoots number per clump, length of shoots per plantlet, number of leaves per explants, length of roots and number of roots were considered as the main responsive variables

of this study. Number of leaves, roots and shoot were calculated by basic counting. The collected data were entered into computer and processed with Statistix (8.1 version) software for analysis. Two-way ANOVA (Analysis of various) technique at P≤0.05 were used to determine the significant difference in response variables between the treatments and varieties. LSD test were applied to evaluate the significant differences among means. To identify the most responsive banana variety, the mean response of each variety across all treatments was compared, and the overall mean of each variety was evaluated.

Result and Discussion

Sterilization

First essential step is surface sterilization that stop the microorganisms growth. The impact of various clorox (5.25% sodium hypochlorite) concentration was assessed for frequency of contamination and sprouting frequency of two banana cultivar (B-10 and W-11). Results demonstrated that the highest frequency of contamination in B-10 and W-11 was 31.67% and 23.33%, respectively, at a low concentration of 10% clorox, although it was progressively reduced as the concentration of clorox was raised. The highest efficient Clorox concentration was 40%, resulting in the lowest frequency of contamination (3.33 and 2.22%) in B-10 and W-11 (Table 3), but sprouting frequency was lower (19.9 and 6.48%) as compared to 30% clorox, which produced the greatest sprouting frequency (31.01 and 29.16%) in B-10 and W-11 respectively (Table 4). Within 3 weeks, all genotype explants began to sprout. Our finding is in contrast with Kumari and Misra (2016), who reported that a 40 % Clorox concentration was optimal for banana tissue culture. It may be due to difference in genotypes. The elimination of microorganisms in plant tissue culture procedures has great importance because microorganism easily grow on the tissue and affect the viability of tissue and regeneration capacity (Yildiz *et al*., 2012).

Effect of BAP and IAA on shoots

Table 5 displays the mean number of shoots for both genotypes. For both banana types, the data show that the control group without PGRs does not show the shoot formation. Treatment of 5 mg/l BAP alone gave 4.00 ± 0.91 and 4.50 ± 1.00 number of shoots in B-10 and W-11 banana variety, respectively. Alone concentration of IAA resulted in the lowest number

Table 3: *Assessment of different clorox (v/v) concentration on contamination frequency (%) in both B-10 and W-11 banana cultivars.*

Clorox concentration (v/v) used	Contaminated plants		Contamination frequency (%)	
	B-10	W-11	B-10	W-11
10%	9.50	7.00	31.67^{a}±0.5	23.33^{ab}±3.00
20%	8.83	4.33	29.44^{a}±1.25	14.44^{bc}±1.52
30%	2.33	1.33	7.78^{cd}±2.08	4.44^{d}±1.15
40%	1.00	0.67	3.33^{d}±1.00	2.22^{d}±0.57

The mean of three replicates is represented by each data. Significant differences are indicated by the mean values that follow the different letters (p ≤ 0.05). LSD value was 2.8431 at p ≤ 0.05. The standard deviation (n = 3) is shown by the values after the ± sign. 60 explants use for each treatment for both banana varieties.

Table 4: *Assessment of Clorox (30 and 40%) on sprouting frequency % in two banana cultivars.*

Clorox concentration (v/v) used	No of sprouting explants		Sprouting frequency %	
	B-10	W-11	B-10	W-11
30%	11.17	10.5	31.01^{a}±0.76	29.16^{a}±0.5
40%	7.17	2.33	19.9^{b}±2.75	6.48^{c}±1.52

The average of three replicates is shown in each set of data. Significant differences(p ≤ 0.05) are indicated by the mean values displayed with different letters. 66 explants were used for each treatment for both banana varieties.

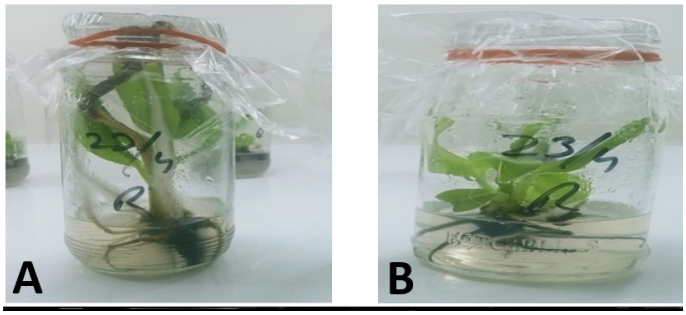

Figure 1: *Shoots formed in both varieties under the treatment with combination of BAP and IAA. (a) Shoots produced in B-10 banana variety (b) Shoots produced in W-11 banana variety.*

of shoots in both banana cultivars. It was observed that the combined effect of BAP and IAA enhanced shoot proliferation (Figure 1). The number of shoots significantly (p≤ 0.05) increased at 2 mg/l BAP + 0.5 mg/l IAA (T7) and 5 mg/l BAP + 1 mg/l IAA (T12) in B-10 (5.90 ± 0.36) and W-11 (4.87± 0.41) banana variety, respectively. The present study demonstrate that BAP was most effective when it combined with IAA, for optimum shoot proliferation of B-10 and W-11 banana cultivars. B-10 showed the highest shoot length of 13.07 ± 0.23 cm with treatment consist of 2

mg/l BAP and 0.5 mg/l IAA. For W-11, the highest shoot length was 11.13 ± 2.24 cm with treatment consist of 2 mg/l BAP and 1 mg/l IAA (Table 6). These findings demonstrate that shoot length is significantly ($p \leq 0.05$) affected by the combination of BAP and IAA, with different treatments providing the best results for different varieties. Alone effect of BAP and IAA reduced the outcomes of the number of leaves in both varieties. The highest mean number of leaves observed in B-10 was 7.03 ± 4.02 and in W-11 was 6.80 ± 1.48 at 2 mg/l BAP + 0.5 mg/l IAA (T7) and 3.5 mg/l BAP + 0.5 mg/l IAA (T8), respectively (Table 7). These results indicated that BAP and IAA together have a significant influence on number of shoots, length of shoot and leaf count, with different treatments working best for different varieties. The mutual beneficial effect of BAP and IAA for shoot multiplication has been investigated earlier for different banana verities, and the finding of the current study are in line with those reported by other researcher who induced multiple shoots of different banana genotypes. Dagnew *et al.* (2012) demonstrated that 3 mg/l BAP + 0.4 mg/l IAA, 3 mg/l BAP + 0.2 mg/l IAA and 4 mg/l BAP + 0.4 mg/l IAA for Dwarf, Poyo and Giant respectively, were best for shoot proliferation and elongation.

Table 5: *Effects of various BAP and IAA concentrations on the number of shoot in B-10 and W-11 banana cultivars.*

Treatments	Numbers of shoots	
BAP (mg/l) + IAA (mg/l)	**B-10**	**W-11**
T1 (0 + 0)	$0.00^{j} \pm 0.00$	$0.00^{j} \pm 0.00$
T2 (2 + 0)	$2.83^{gh} \pm 0.57$	$1.97^{hi} \pm 0.15$
T3 (3.5 + 0)	$3.43^{fg} \pm 0.97$	$3.40^{fg} \pm 0.52$
T4 (5 + 0)	$4.00^{def} \pm 0.91$	$4.50^{bcde} \pm 1.00$
T5 (0 + 0.5)	$1.87^{i} \pm 0.11$	$1.60^{i} \pm 0.69$
T6 (0 + 1)	$2.03^{hi} \pm 0.05$	1.77i ± 0.75
T7 (2 + 0.5)	$5.90^{a} \pm 0.36$	$4.07^{def} \pm 0.40$
T8 (3.5 + 0.5)	$4.57^{bcd} \pm 0.60$	$4.60^{bcd} \pm 0.85$
T9 (5 + 0.5)	$3.63^{efg} \pm 0.75$	$4.67^{bcd} \pm 0.28$
T10 (2 + 1)	$5.00^{abc} \pm 0.50$	$4.00^{def} \pm 0.00$
T11 (3.5 +1)	$4.67^{bcd} \pm 0.41$	$4.40^{cde} \pm 0.52$
T12 (5 + 1)	$5.40^{ab} \pm 0.52$	$4.87^{bcd} \pm 0.41$
Mean	3.6111^{a}	3.3194^{b}

The average of three replicates is shown in each set of data. Significant differences ($p \leq 0.05$) of mean values have been shown with the different alphabet letters. The standard deviation (n = 3) is shown by the values after the ± sign. 180 total number of explants of B-10 and W-11 were inoculated in MS shooting media. LSD was computed at $p \leq 0.05$ (Varieties=0.2679; Treatments= 0.6562; Interaction = 0.9281).

Table 6: *Effect of different concentration of BAP and IAA on shoot length in B-10 and W-11 banana cultivars.*

Treatments	Shoot length	
BAP (mg/l) + IAA (mg/l)	**B-10**	**W-11**
T1 (0 + 0)	$0.00^{m} \pm 0.00$	$0.00^{m} \pm 0.00$
T2 (2 + 0)	$6.3^{fghij} \pm 1.15$	$4.77^{hijkl} \pm 1.25$
T3 (3.5 + 0)	$5.33^{ghijk} \pm 1.15$	$4.43^{ijkl} \pm 2.00$
T4 (5 + 0)	$4.50^{ijkl} \pm 1.64$	$2.68^{kl} \pm 0.45$
T5 (0 + 0.5)	$2.77^{kl} \pm 0.58$	$2.63^{lm} \pm 0.15$
T6 (0 + 1)	$4.13^{ijkl} \pm 1.27$	$3.90^{jkl} \pm 0.96$
T7 (2 + 0.5)	$13.07^{a} \pm 0.23$	$8.20^{def} \pm 1.93$
T8 (3.5 + 0.5)	$11.37^{ab} \pm 1.53$	$9.77^{bcd} \pm 2.12$
T9 (5 + 0.5)	$10.93^{abc} \pm 1.52$	$8.60^{cdef} \pm 1.53$
T10 (2 + 1)	$9.13^{bcde} \pm 3.59$	$11.13^{abc} \pm 2.24$
T11 (3.5 +1)	$6.47^{fghij} \pm 2.51$	$7.67^{defg} \pm 0.91$
T12 (5 + 1)	$6.77^{efghi} \pm 2.14$	$7.17^{defgh} \pm 1.52$
Mean	6.7306^{a}	5.9128^{b}

The average of three replicates is shown in each set of data. Significant differences ($p \leq 0.05$) of mean values have been shown with the different alphabet letters. The standard deviation (n = 3) is shown by the values after the ± sign. 180 total number of explants of B-10 and W-11were inoculated in MS shooting media. LSD was computed at $p \leq 0.05$ (Varieties = 0.7666; Treatments = 1.8777; interaction = 2.6555).

Table 7: *Effect of different concentration of BAP and IAA on number of leaves in B-10 and W-11 banana cultivars.*

Treatments	Number of leaves	
BAP (mg/l) + IAA (mg/l)	**B-10**	**W-11**
T1 (0 + 0)	$0.00^{e} \pm 0.00$	$0.00^{e} \pm 0.00$
T2 (2 + 0)	$2.50^{bcd} \pm 0.86$	$2.00^{bcde} \pm 0.57$
T3 (3.5 + 0)	$2.37^{bcd} \pm 0.55$	$2.07^{bcde} \pm 0.55$
T4 (5 + 0)	$4.10^{b} \pm 1.01$	$2.10^{bcde} \pm 1.01$
T5 (0 + 0.5)	$2.23^{bcde} \pm 0.72$	$0.47^{de} \pm 0.72$
T6 (0 + 1)	$2.24^{bcde} \pm 0.25$	$0.97^{cde} \pm 0.25$
T7 (2 + 0.5)	$7.03^{a} \pm 4.02$	$3.03^{bc} \pm 4.02$
T8 (3.5 + 0.5)	$3.73^{b} \pm 1.48$	$6.80^{a} \pm 1.48$
T9 (5 + 0.5)	$4.07^{b} \pm 0.90$	$2.30^{bcd} \pm 0.90$
T10 (2 + 1)	$3.53^{b} \pm 1.28$	$3.00^{bc} \pm 1.24$
T11 (3.5 +1)	$3.23^{bc} \pm 0.85$	$2.13^{bcde} \pm 0.23$
T12 (5 + 1)	$3.07^{bc} \pm 0.90$	$2.27^{bcde} \pm 0.94$
Mean	3.1756^{a}	2.2617^{b}

The average of three replicates is shown in each set of data. Significant differences ($p \leq 0.05$) of mean have been values shown with the different alphabet letters. The standard deviation (n = 3) is shown by the values after the ± sign. 180 total number of explants of B-10 and W-11were inoculated in MS shooting media. LSD was computed at $p \leq 0.05$ (Varieties= 0.6610; Treatments= 1.6190; Interaction= 2.2896).

Table 8: *Evaluate the effect of different concentration of BAP and IAA on number of roots of both banana cultivars.*

Treatment	Number of roots	
BAP (mg/l) + IAA (mg/l)	**B-10**	**W-11**
T1 (0 + 0)	$0.00^{e} \pm 0.00$	$0.00^{e} \pm 0.00$
T2 (0.5 + 0)	$2.03^{de} \pm 0.45$	$1.70^{de} \pm 0.81$
T3 (1 + 0)	$3.00^{cd} \pm 0.20$	$2.00^{de} \pm 0.20$
T4 (0 + 1.5)	$3.40^{cd} \pm 0.34$	$2.73^{cd} \pm 0.23$
T5 (0 + 2)	$3.80^{cd} \pm 0.26$	$2.90^{cd} \pm 0.65$
T6 (0.5 + 1.5)	$4.83^{bc} \pm 3.03$	$2.83^{cd} \pm 1.45$
T7 (1 + 1.5)	$4.23^{bcd} \pm 2.67$	$3.16^{cd} \pm 2.55$
T8 (0.5 + 2)	$9.30^{a} \pm 0.43$	$3.66^{cd} \pm 2.63$
T9 (1 + 2)	$5.03^{bc} \pm 3.16$	$6.80^{ab} \pm 0.91$
Mean	3.9593^{a}	2.8667^{b}

The average of three replicates is shown in each set of data. Significant differences (p≤0.05) in mean values have been shown with the different letters. The standard deviation (n = 3) is shown by the values after the ± sign. Total 135 shoots of both varieties were inoculated in MS root induction media. LSD was computed at p ≤ 0.05 (Varieties = 0.8691; Treatments= 1.8437; Interaction= 2.6074).

Effect of BAP and IAA on roots

Table 8 show the response of two banana cultivars (B-10 and W-11) to varying BAP and IAA concentrations in terms of root count. In the B-10 variety, the treatment with 0.5 mg/l BAP + 2 mg/l IAA (T8) resulted in the highest number of roots (9.30 ± 0.43), followed by T9 with 1 mg/l BAP + 2 mg/l IAA (5.03 ± 3.16). T1 as a control group (0 mg/l BAP + 0 mg/l IAA) resulted no roots. Similarly, W-11 variety yielded the highest number of roots (6.80 ± 0.91) at T9 with 1 mg/l BAP + 2 mg/l IAA (Figure 2). These data show the significant impact of the combined BAP and IAA treatments on root induction. Table 9 show the response of two banana cultivars (B-10 and W-11) to varying BAP and IAA concentrations on root length. For the B-10 variety, the highest root length (9.86 ± 1.24 cm) was recorded with the treatment of 0.5 mg/l BAP + 2 mg/l IAA (T8). Second highest root length (5.83 ± 0.41 cm) was observed with treatment consist of 1 mg/l BAP + 1.5 mg/l IAA. No roots were produced by the control group (0 mg/l BAP + 0 mg/l IAA). W-11 banana variety treated with 1 mg/l BAP + 2 mg/l IAA (T9) gave the highest root length (7.60 ± 0.52 cm), followed by T8 with 0.5 mg/l BAP + 2 mg/l IAA (4.66 ± 2.88 cm). Similarly, no roots were observed by the control group (Table 7). These findings show that combined BAP and IAA treatments significantly increased root elongation. Similar, higher number of roots at combine effect of BAP and IAA were reported by Khan *et al.* (2021) in Pisang banana variety. Hussein (2012) also reported that BAP and IAA improved banana growth during tissue cultured experiments. Maximum roots per plant (9.25±2.08) was reported by Shah *et al.* (2020) for bananas grown in MS media at 2.0 mg/l IAA + 0.5 mg/l BAP.

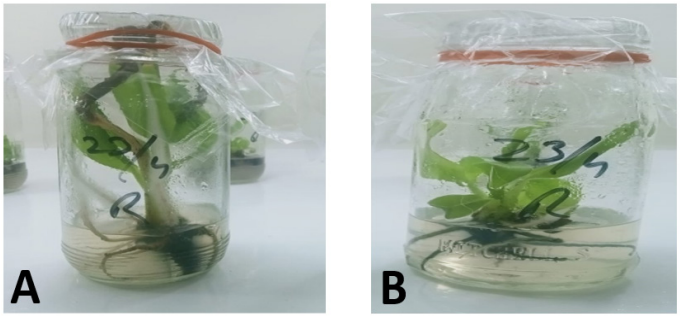

Figure 2: *Root formation in both banana varieties (a) root formation in B-10 banana variety (b) roots formation in W-11 banana variety.*

Table 9: *Evaluate the effect of different concentration of BAP and IAA on root length (cm) of both banana cultivars.*

Treatment	Root length (cm)	
BAP (mg/l) + IAA (mg/l)	**B-10**	**W-11**
T1 (0 + 0)	$0.00^{f} \pm 0.00$	$0.00^{f} \pm 0.00$
T2 (0.5 + 0)	$2.86^{de} \pm 1.18$	$2.03^{ef} \pm 1.34$
T3 (1 + 0)	$2.13^{ef} \pm 0.60$	$1.73^{ef} \pm 1.56$
T4 (0 + 1.5)	$4.03^{cde} \pm 2.50$	$3.00^{de} \pm 2.50$
T5 (0 + 2)	$4.86^{cd} \pm 2.47$	$3.53^{cde} \pm 1.95$
T6 (0.5 + 1.5)	$4.10^{cde} \pm 1.87$	$3.10^{de} \pm 1.87$
T7 (1 + 1.5)	$5.83^{bc} \pm 0.41$	$3.96^{cde} \pm 1.66$
T8 (0.5 + 2)	$9.86^{a} \pm 1.24$	$4.66^{cd} \pm 2.88$
T9 (1 + 2)	$5.66^{bc} \pm 0.28$	$7.60^{ab} \pm 0.52$
Mean	4.3741^{a}	3.2926^{b}

The average of three replicates is shown in each set of data. significant differences (p≤0.05) in mean values have been shown with the different letters. The standard deviation (n = 3) is shown by the values after the ± sign. Total 135 shoots of both varieties were inoculated in MS root induction media LSD was computed at p≤0.05 (Varieties = 0.8269; Treatments = 1.7542; Interaction = 2.4808).

Highly responsive banana variety

Comparison between two varieties presented in Tables 5 to 9 across different tissue culture parameters, including the number of shoots, shoots length, number of leaves, number of roots, and roots length, to identify the best tissue culture-responsive banana variety under the given conditions. Results demonstrate that the B-10 variety performed

better than the W-11 variety, consistently achieving higher mean values across all measured parameters. Specifically, B-10 showed higher number of shoots (3.6111), shoot length (6.7306 cm), leaves count (3.1756), roots count (3.9593), and root length (4.3741 cm) compared to W-11 banana variety. Because of its consistent performance across all parameters, the B-10 variety is a better option for mass propagation because it responded better under given tissue culture conditions. This study was in line with Dagnew *et al.* (2012), who reported an overall higher mean of the *Poyo banana* cv. (3.54) as compared to dwarf *Cavendish banana* cv. (3.25) in number of shoots. Similarly, the Wiallium-8818 hybrid was observed to be a higher responsive banana variety as compared to the Pisang banana variety across all the parameters under the given tissue culture conditions (Khan *et al.*, 2021).

Table 10: *Analysis of variance (ANOVA) for number of shoots, shoot length and number of leaves.*

Sources of variance	Mean sum of square for shoots			
	DF	No. of shoots	Shoot length	No. of leaves
treatments	11	15.3971**	72.2757**	12.8117**
variety	1	1.5313*	12.0377*	15.0335**
Treatments × varieties	11	0.8058*	4.8798^{ns}	4.0504*
Error	48	0.3196	2.6165	1.9452
Total	71	-	-	-

*Significant at $p \leq 0.05$ = *; highly significant at $p \leq 0.05$ = **; non-significant at $p \leq 0.05$ = ns.*

Table 11: *Analysis of variance (ANOVA) for roots number and length (cm).*

Sources of variance	Mean sum of square for roots		
	DF	No. of roots	Roots length
Treatments	8	23.2137**	30.9546**
Variety	1	16.1157*	15.7896*
Treatments × varieties	8	5.9278*	5.3317*
Error	36	2.4793	2.2444
Total	53	-	-

*Significant at $p \leq 0.05$ = *; highly significant at $p \leq 0.05$ = **

Conclusion

To sum up, the B-10 variety showed a higher shoot and root growth, compared to W-11, indicating its better suitability for tissue culturing. The treatments demonstrated significant improvements in shoot multiplication, quantity of leaves, roots numbers and length of roots, demonstrating their effectiveness for banana tissue culture procedures. High-yielding banana varieties can be quickly multiplied through tissue culture, potentially increasing farmers' access to disease free planting materials. This can help to increase the productivity of banana cultivation and raise the income of farmers. Cultivation of planting materials free of disease help to minimize the risk of crop damage due to diseases and pests.

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Novelty Statement

This study established an optimized in-vitro propagation protocol for banana varieties B-10 and W-11 under local conditions. It identified the most effective BAP and IAA combinations using two-way ANOVA and revealed B-10 as the superior cultivar for large-scale, disease-free plant production.

Author's Contribution

The experiment, data collection, and manuscript preparation were all done by Huma Fatima. Khalid Mehmood supervised the study and provided direction for the data analysis. Technical assistance and laboratory support were given by Sabir Hussain Shah. Naveed Iqbal Raja and Amjad Rashid Kayani helped with the manuscript revision and data interpretation. The final version was read and approved by all authors.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technology was used in the writing, data analysis, or preparation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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