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SOMACLONAL VARIATIONS INDUCED BY BENZYLAMINOPURINE TO ENHANCE THE FRUIT MORPHOLOGY OF HORN BANANA

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SUMMARY

Banana (*Musa paradisiaca*) is a delicious and popular fruit, rich with nutritional values that benefit human health. The presented study sought to evaluate the effect of plant growth regulator (PGR) benzylaminopurine (BAP) in enhancing shoot proliferation and its somaclonal variation effect on fruit morphological variation in bananas. The research proceeded with simultaneous steps, including in vitro culture of horn bananas, BAP treatment, and acclimatization. The shoots treatment with three different concentrations of BAP comprised 0 mg L^{-1} (control), 3 and 4 mg L^{-1}. Observations occurred on the number of shoots, the survival rate during acclimatization, and banana fruit productivity. The results revealed that BAP (4 mg L^{-1}) considerably enhanced the number of shoots in bananas. Explants treated with BAP (4 mg L^{-1}) were adaptive to the acclimatization stage, reaching a 100% survival rate. Bananas cultivated using BAP also exhibited morphological variations, with threefold enhanced fruit weight compared with the wild type, reaching 644.90 g. Physiological changes during in vitro culture stages revealed shoot initiation to acclimatization, resulting in morphological variations caused by somaclonal effects. These findings lead to understanding BAP as beneficial for crop improvement.

Keywords: Banana (*M. paradisiaca* L.), acclimatization, BAP, morphological variations, somaclonal effects, shoots, productivity

Key findings: Somaclonal effects induced by benzylaminopurine enhanced the morphological variations and fruit size in banana (*M. paradisiacal* L.).

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INTRODUCTION

The banana (*Musa paradisiaca* L.) is the most consumable fruit and belongs to the family Musaceae. Indonesia became the third largest producer of bananas after India and China, with a total output of 7.28 million tons, representing 6.28% of global production during 2022 (FAOSTAT, 2023). The entire parts of the banana tree could also have many uses. The banana leaf parts, peel, stem (petiole), bud, and rhizome/corm can serve various purposes. Processing the banana comprised the rhizomes into chips, banana buds into jerky, and banana stems (petioles) as raw material for recycled paper (Lestari *et al.*, 2012). Some essential nutrients in bananas include phenolic compounds, carotenoids, flavonoids, amino acids, vitamins B3, B6, B12, C, and E, and dietary fibers (Khoozani *et al.*, 2019).

Banana plant propagation can proceed conventionally through suckers and plantlets; however, plant seedling production requires a relatively long time, producing only 5–10 plant seedlings per year, with separated plantlets highly susceptible to disease transmission (Ratnasari *et al.*, 2016). Besides these limitations, vegetative propagation results in low diversity, as subsequent plant generations have the same genome as the parental stock (Moradi *et al.*, 2016). Tissue culture can enhance the number of shoots and genetic diversity by developing somaclonal variations in propagated explants. In tissue culture, the success of propagation is also a result of several factors, including the plant genotype, media formulation, nutrients, plant growth regulators (PGRs), and environment (Pasternak and Steinmacher, 2024).

Somaclonal variations occur because of the addition of PGRs, which affect the activity and cell cycle of in vitro plant cells (Krishna *et al.*, 2016). Additionally, the frequency of genetic variations may improve with the duration of in vitro culture and several subcultures, influencing the genetic stability that leads to somaclonal variations (Ferreira *et al.*, 2023). Somaclonal variations caused by genetic changes during in vitro culture enhance the genetic diversity and improve the plant quality. The occurrence rate of somaclonal variations in specific Cavendish bananas and plantains can reach up to 69% (Deepthi, 2018). The genetic diversity manifestations can be through phenotypic variations in plant structures, pigments, roots, stems, pseudostems, flowers, leaves, fruits, and seeds (Ferreira *et al.*, 2023). Somaclonal variations allow for morphological modifications, including variations in fruit size. The impact of these variations can be beneficial or detrimental, depending on the specific breeding and cultivation objectives.

PGRs have benefitted agriculture, horticulture, and other field crops in regulating flowering, managing plant responses to environmental stress, and enhancing overall yields. However, one should note that adding PGRs has limitations, including sensitivity to dosages that can also affect plant meristem tissues. PGRs can be effective at optimum concentrations; however, it is vital to consider the appropriate concentration to prevent toxicity to explants (Amoanimaa-Dede *et al.*, 2022).

The combined application of IAA and BAP (6 mg L^{-1}) was notable, with an increased percentage of emerging shoots in the 'kepok' banana, averaging 67.71% (Sadat *et al.*, 2018). Utilizing BAP (5 and 7 ppm) also enhanced the number of shoots in *M. textilis,* about 4–5 shoots per explant (Unsong *et al.*, 2022). Furthermore, the elevated concentration of BAP (5–6 mg L^{-1}) boosts the average number of banana shoots (Sipen and Davey, 2012). Applying NAA 0.5 mg L^{-1} and BAP 0.5 mg L^{-1} also raised the number of emerging shoots and their viability in rodent tubers (Sianipar *et al.*, 2015, 2017). The number of subcultures also affects shoot multiplication and genetic diversity through somaclonal variations. Sianipar *et al.* (2020) identified the differences between MV4 and MV5 generations of rodent tuber subcultures by the number of shoots and morphology due to genetic variations and the ability of shoot differentiation resulting from environmental factors.

Besides the optimal concentration, the success of generating new individuals through in vitro culturing can be distinct through the acclimatization stage. Acclimatization helps

reactivate the physiological processes and plant structures, enabling plants to perform photosynthesis, uptake nutrients, and function optimally, thus aiding in plant anatomical development (Chandra *et al.*, 2010). Therefore, the presented study sought to enhance the number of shoot plantlets through in vitro propagation with the addition of an optimal BAP concentration and identify the fruit morphological variations in somaclonal variants of horn banana.

MATERIALS AND METHODS

Sterilization and explant initiation

The explants came from horn banana in Bogor, West Java, Indonesia. The explants cutting and peeling reached the sizes of 1.5–2 cm. The explants sterilization subsequently soaked the banana seedlings in a detergent solution (1 g L^{-1}) for 5 min, fungicide solution (1 g L^{-1}) for 1 h, and bactericide solution (1 g L^{-1}) for an hour, repeating the process three times. Further soaking of explants in 30% Clorox solution took 30 min and 20% Clorox solution for 20 min, followed by rinsing with sterile distilled water. The final sterilization proceeded in a Laminar Air Flow Cabinet (LAFC) according to protocol, as described by Setyowati *et al.* (2022), by soaking the explants in 70% alcohol for 5 min and rinsing with sterile distilled water. The use of Murashige-Skoog (MS) medium had 30 g sucrose. The medium for explants initiation consisted of the MS medium and 4 mg L^{-1} BAP. The explants remained in the incubation room at 24 °C ± 10 °C for 16 h with a light intensity of 2000 lux for inducing shoot and root multiplication.

Shoot multiplication

Enhancing shoot multiplication of horn bananas transpired on the MS medium supplemented with three different concentrations of BAP (0, 3, and 4 mg L^{-1}). Each treatment had 10 replications. The observed variable was the number of shoots, counted from the second to the 16th week. Subsequently, the explants' sub-culturing continued on optimal media according to the concentration of BAP treatment. Sub-culturing of plant generated on MS + 4 mg L^{-1} progressed every six months for five subculture cycles, resulting in the 5th generation (MV_5). The MV_5 generation plantlets were samples used in the acclimatization stage.

Acclimatization

The acclimatization stages involved plantlets from in vitro cultures that had developed complete organs (roots, stems, and leaves). Six banana plantlets with uniform sizes were options, and those showing the highest number of shoots in the optimum BAP concentration (BAP 4 mg L^{-1}) sustained the acclimatization step. The acclimatization commenced by removing the plantlets from the in vitro medium and washing the roots. Cultivating plantlets in polybags ensued, containing a mixture of topsoil and organic fertilizer at a ratio of 1:1 (v/v), with each polybag containing one-banana plantlet, and then covered with glass bottles for three weeks. The acclimatization stages occurred in the greenhouse for eight weeks until the bananas reached a height of approximately 20 cm before transplantation to the field. The experiment employed a randomized complete block design with four replications. After transplanting the plants in the field, their maintenance included fertilization, weed control, sucker thinning, bunch covering, pests, and disease control tailored to the specific conditions and environment. The observed variables related to fruit production included the number of fruits, fruit wet weight, fruit length, and fruit diameter. Fruiting plants from induced explant received the labels TM1 and TM2.

Data analysis

Assessing the average number of shoots emerging in the sixth month and fruit production variables used Analysis of Variance (ANOVA). Post-hoc analysis using the Duncan test had a 5% significance level.

RESULTS AND DISCUSSION

BAP application effect

The average number of shoots was significantly different (Table 1). The highest average number of shoot primordia achieved in the 16th week was with the MS + 4 mg L^{-1} BAP, at 3.50±1.91 per explant. In addition, shoot primordia production appeared on the MS + 3 mg L^{-1} BAP with an average of 1.25. However, this value was nonsignificantly different from that produced on the MS medium without BAP (control), with an average of 1.00 shoot primordia. Based on these results, one can infer that adding 4 mg L^{-1} BAP to the medium enhanced the shoot primordia growth. It may indicate that cytokinin at higher concentrations contributes to the increase in shoot proliferation followed by higher shoot multiplication. These results were analogous to past findings that adding BAP (5.0 mg L^{-1}) increased the average shoot proliferation in *Prunus persica* L. (Eliwa *et al.*, 2024).

The growth of the banana shoot primordia with three different BAP concentrations resulted in varying developments from the second until the 16th weeks after the subculture. As shown in Figure 1, among the three BAP concentrations tested in the presented study, the MS + 4 mg L^{-1} BAP showed a substantial increase in shoot primordia production up to the 16th week. Closely at second was the 3 mg L^{-1} BAP, which began inducing the shoots in the second week. Utilizing BAP (4 mg L^{-1}) demonstrated significant optimal results in the formation and proliferation of banana shoot primordia growth (Figure 2). Manurung *et al.* (2021) also reported that an increased BAP concentration could enhance the shoot development in the banana cultivar Raja Bulu. Likewise, Muhie and Teshome (2023) reported that adding BAP (2.5 mg L^{-1}) accelerates the banana shoot formation. With media optimization through five subcultures, the study stopped, although there was still potential for exploring experimental variations. Optimal BAP provided an adequate understanding of the methods for increasing banana shoot primordia in crop plants (Wu *et al.*, 2021).

Shoot growth under optimal BAP has become crucial in plant development, especially in micropropagation. BAP, a cytokinin, stimulates cell division and shoot growth. At optimum concentration, BAP can trigger mitotic activity in shoot cells, resulting in rapid and balanced growth during the initiation stage in *Musa acuminata* (Jafari *et al.*, 2011). BAP can overcome apical dominance and induce lateral shoot proliferation to form sturdy and diverse shoots in *Stahlianthus thorelii* (Yen and Li, 2022). Optimization of BAP concentrations is vital to prevent toxicity that may inhibit growth and cause morphological abnormalities. However, the appropriate subculture treatments can induce new shoots in these nodules.

The BAP has positive effects on cell division and shoot development. Applying the BAP 4 mg L^{-1} can enhance the shoot growth compared with treatments without BAP in bananas. By promoting cell division, BAP significantly improves efficiency in forming new shoots. Wu *et al*. (2021) stated that cytokinin could enhance lateral shoot initiation by inducing cell elongation and division in crop plants. BAP also plays a primary role in overcoming apical dominance, where inhibiting the main shoot by certain hormones occurs in soybeans (*Glycine max* L.) (Mangena, 2022).

Table 1. Number of banana shoots with different concentrations of BAP at 16th week.

Medium	The Average Number of Shoots*
MS+0 mg L^{-1} BAP	1.00±0.00[a]
MS+3 mg L^{-1} BAP	1.25±0.50[a]
MS+4 mg L^{-1} BAP	3.50±1.91[b]

*Numbers in the same column followed by the same letter are not significantly different in the Duncan's Multiple Range Test at a 5% rate.

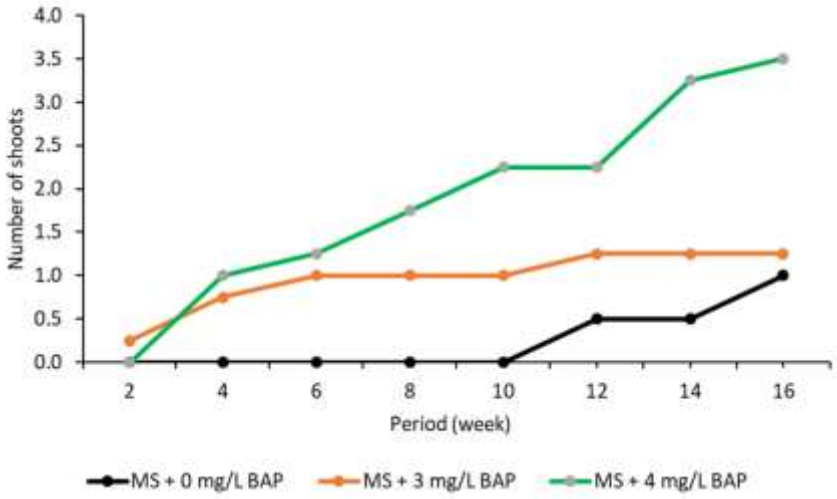

Figure 1. Growth of banana shoots at 16th week after subculture.

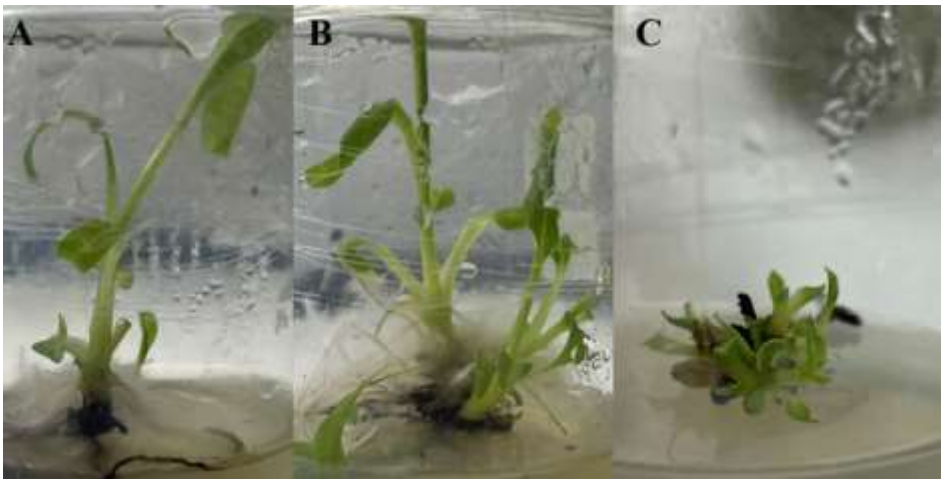

Figure 2. Observations for banana plant shoots induced by BAP at 16th week
A) MS+0 mg L^{-1}, B) MS+3 mg L^{-1}, C) MS+4 mg L^{-1}.

Survival rate at the acclimatization

During acclimatization, the viability percentage of the six banana seedlings was exceptionally high, reaching 100% (Figure 3). These seedlings originated from in vitro explants cultured in the MS medium supplemented with BAP (4 mg L^{-1}). The acclimatization process commenced with the maintenance of the initial culture before transplantation to the field, where the sterilized explants in a nutrient medium contained essential nutrients, growth hormones, and sugars. Four-month-old banana explants, robustly growing and undergoing several subcultures, proceeded their release to the natural environment. These processes also involved gradual adjustment to external environmental conditions, such as sunlight and humidity (Silva *et al.*, 2017). Banana plants resulting from in vitro culture exhibited well-developed roots and, during acclimatization, thrived well in conducive growth environments. Following acclimatization, carefully maintaining and monitoring banana plants ensured survival and healthy growth, enabling fruit production.

Figure 3. Acclimatization process of banana plants at two weeks after acclimatization.

Table 2. Comparison of banana fruit morphological-related traits between TM plants and their wild types.

Treatments	Fruits tree^{-1}	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)
Wild type	15±0.82^{a}	191.75±22.53^{a}	21.15±1.60^{a}	3.97±0.43^{a}
TM 1	20±1.63^{b}	644.90±62.29^{c}	30.27±1.66^{b}	6.26±0.32^{b}
TM 2	27±0.82^{c}	597.26±135.85^{b}	30.58±3.75^{b}	6.26±0.47^{b}

*Numbers in the same column followed by the same letter are not significantly different in the Duncan's Multiple Range Test at a 5% rate.

Acclimatization is one of the most crucial processes for adjusting plantlets before transplanting to achieve high survival rates and proper growth. This study acclimatized banana explants with the highest percentage (100%). The increased regeneration rates of acclimatized explants using media consisting of soil with compost were optimal. It was similar to the findings of Mekonen *et al.* (2021), where gradually acclimatizing banana seedlings (*Musa paradisiaca* L.) improved plant survival rates. Environmental conditions are one of the most vital factors during the acclimatization stages. The study covered the explants to support the plants' adaptation. The acclimatization process also requires adjusting to humidity, light intensity, and temperature and gradually exposing the plants to conditions resembling the growth environment after field planting (Irsyadi, 2021). Furthermore, the physiological conditions of in vitro plants have undergone good physiological adaptation, such as sufficient water content, photosynthetic capacity (including chlorophyll pigments and photosynthesis), and optimal stomatal function, which are essential for acclimatization success.

Productivity of bananas

Among the six banana plants resulting from acclimatization, only two trees ably produced their fruits, i.e., TM1 and TM2. Fruit production containing the following variables—weight, length, and diameter—was visible from the three banana samples, wild-type horn bananas, and two samples from previously in vitro cultured on MS + 4 mg L^{-1} BAP. Based on Table 2, it was evident that these plants were significantly different. The first notable difference after analysis was the significant disparity in fruit quantity. The horn bananas produced an average of only 15 fruits per bunch, while the two plants resulting from in vitro culture growth on MS medium supplemented with 4 mg L^{-1} BAP produced

more fruits, with an average of 20 fruits per bunch in TM1, and 27 fruits per bunch in TM2. The TM1 and TM2 banana plants had heavier fruits than the wild type. The average fruit weight of 644.90 g and 597.26 g was remarkable in TM1 and TM2 plants, respectively, heavier than the wild-type fruits at 191.75 g per fruit. This finding indicated that BAP stimulates cell division during the early stages of fruit development and initiates cell enlargement, resulting in higher fruit weight and size (Barać *et al*., 2022).

The TM1 and TM2 plants produced larger fruits than the typical horn bananas. The average fruit length was 30.27 and 20.58 cm in TM1 and TM2, respectively, with both more extensive than that in the wild type, with an average length of 21.15 cm. Similarly, TM1 and TM2 fruits had a diameter approximately twice as large as the wild type, with an average diameter of 6.26 cm, versus the wild type's average diameter (3.97 cm). The TM bananas exhibited fruit morphology distinct from their wild-type counterparts, indicating that applying BAP during the plant subculture can induce new diversity (Pereira *et al.*, 2018). The fruit weight in TM1 and TM2 reached 12.9 and 16.1 kg per plant, respectively. Figure 4 illustrates the TM banana plant induction process to obtain a better productive plant than their wild type. The development of TM bananas, differing from the typical horn banana, may refer to the genetic variations resulting from the combination of PGRs and the frequency of subcultures (Poerba *et al*., 2019; Gardoce *et al*., 2024; Pasternak and Steinmacher, 2024).

In the latest study, the BAP used as a plant growth regulator significantly influenced banana fruit size variations, determining fruit weight measurements. The results showed that the fruit weight of TM1 and TM2 banana plants was three times heavier than that of the wild-type horn bananas (Figure 5). The addition of BAP to plant growth media triggered a plant response that resulted in different fruit sizes compared with the control treatment. The research findings indicated that in vitro plants initiated with BAP (4 mg L^{-1}) appeared with significant increases in fruit size versus the

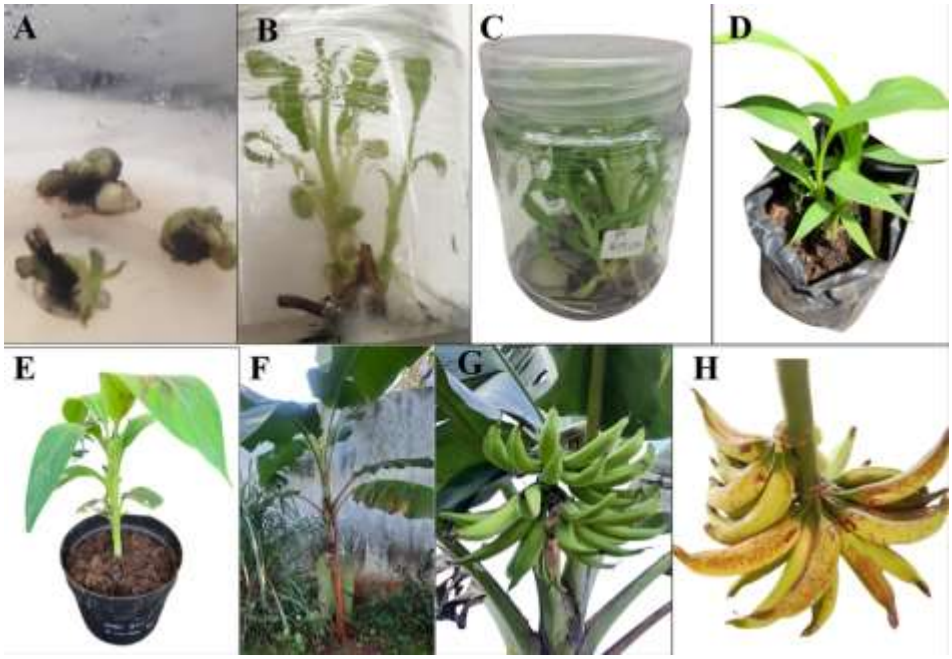

Figure 4. Mechanism of initiation of shoot explants to banana fruit production (A-H); (A) explant initiation, (B) shoots multiplication, (C) optimization of banana shoots at 4 mg L^{-1} for five months, (D) acclimatization stage, (E) 4th month after acclimatization, (F) one year old banana, (G) MV5 generation banana bearing fruit, and (H) ripe TM bananas.

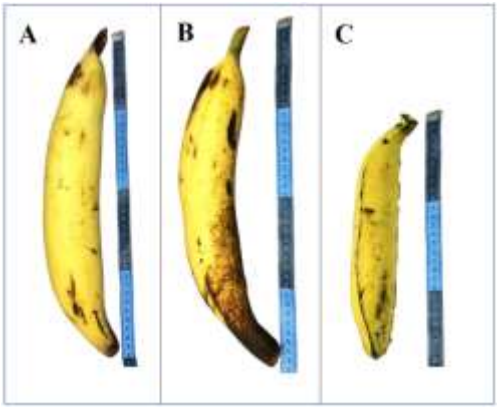

Figure 5. Comparison of banana fruits' size produced by TM plants and their wild types. A) TM 1, B) TM 2, and C) wild type.

control (without BAP). This variation may occur because BAP stimulates faster cell growth and affects cell differentiation in crop plants' fruit organs (Sharma, 2018). Additionally, variations in fruit size may coincide with the effect of BAP on the formation and development of lateral shoots, which can affect the resources used for fruit development in bananas (Razani *et al.*, 2020; Karuwal *et al*., 2024).

The TM bananas' fifth generation (MV_5) seemed to have undergone genetic variations, as evidenced by the morphological differences between TM and wild-type bananas. Sianipar *et al.* (2020) reported that the fifth generation of rodent tubers was notably with a decreased number of shoots. Multiple subcultures can significantly affect cell differentiation during plant development (Razani *et al.*, 2020). Each time cell replications happen, the potential for cell characteristics variations, both genetic and physiological, may increase. The repeated subculture process can trigger the genetic mutation with considerable variations in those cells in safed musli (*Chlorophytum borivilianum*) (Nakasha *et al.*, 2016).

The relationship between plant physiology and variations in fruit morphology is highly complex and interrelated. In crop plants, the physiological processes are chief in fruit formation and development, affecting the fruit morphology. Growth hormones, such as auxins, cytokinins, gibberellins, ethylene, and abscisic acid, regulate the various stages of fruit development. Plant physiology also influences photosynthesis, which provides the energy and organic substances needed for fruit growth and development. Cell differentiation and division during fruit development are crucial in shaping fruit structure and morphology, including color, size, and texture in tomato and melon (Monforte *et al.*, 2014). In this study, using BAP influenced the fruit formation of bananas, leading to enhanced banana fruit size.

PGRs play a crucial role in regulating various physiological processes in crop plants. Sharma *et al.* (2018) reported that PGRs influenced the size of fruits in the apple cultivar Scarlet Spur II. According to Milić *et al.* (2018), applying NAA and BA treatments could increase the size of blueberry fruits. A report stated adding BAP affected the number of soybean pods, which plays a primary role in raising the harvest yield. Soybeans sprayed with BAP (160 mg L^{-1}) produced more pods during the flowering stage than other BAP concentrations and the control treatment (Lian *et al.*, 2023). These results indicate that BAP is crucial in flower fertility, seed formation, and fruit development enhancement. Gan *et al.* (2022) also reported that cytokinin deficiency in tomato plants could reduce fruit weight and size. The presented study banks on the understanding that BAP could enhance plant physiology and become an alternative PGR to improving banana genetics.

In bananas, the genetic variations occurring through tissue culture are proposals to produce a better quantity and quality.

Micropropagation is a widely used technique to produce disease-free and genetically identical banana plants. Ensuring genetic stability is crucial to maintaining the desired traits and preventing unwanted genetic variations. Somaclonal effects characterized by genetic variations derived from tissue culture in crop plants result in further genetic modifications. These genetic variations can manifest in altered morphological parameters, different growth patterns, and even in the genetic makeup of the regenerated *Catharanthus roseus* plants (Alaakel and AL-Ammouri, 2023). Morphological variations in TM1 and TM2 indicate the genetic modification of horn banana treated with 4 mg L^{-1} BAP. Genetic variations through somaclonal effects can result in new traits contributing to plant improvement and adaptation; however, they may not be directly identifiable. Each cell will produce plant clones with unique DNA sequences, leading to genetic variation contributing to alterations in plant morphology within the induced population.

Genetic variations can occur at the chromosome level, specifically changes in chromosome number (aneuploidy or polyploidy), chromosome structure (deletion, duplication, insertion, and translocation), and DNA sequence level, mainly consisting of point mutations in the DNA sequence (Duta-Cornescu *et al.*, 2023). These mechanisms can lead to significant genetic variations within a plant population, including phenotypic variations. Some genetic variations may result in desirable traits, such as disease resistance and enhanced crop yields (Liu *et al.,* 2024). However, not all genetic variations accelerate the positive effects, and some may contribute to failure and deviation from desired traits. Therefore, a thorough understanding of the mechanism of genetic variations is crucial for developing better and more sustainable crops.

CONCLUSIONS

The optimal shoot number in bananas was notable with BAP (4 mg L^{-1}). The survival rates in the acclimatization stages were 100%. The TM1 and TM2 banana plants produced an average fruit weight of 644.90 and 597.26 g, respectively, with an average number of fruits per bunch of 20 and 27 fruits. The fruit weight per plant produced by TM1 and TM2 bananas reached 12.9 and 16.1 kg per tree, respectively. The difference in the wild-type (mother plant) with TM1 and TM2 bananas was, as expected, caused by optimal BAP application and repeated subculture processes, which subsequently influenced the banana morphology. Therefore, TM bananas offer promising opportunities as functional food cultivars.

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