



Development of an Efficient Micropropagation System for Four Commercially Important Banana Genotypes of Bangladesh

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Abstract

Under this study, four important commercial banana genotypes, such as Grand Naine (G-9), Sabri, Meherasgor and Jahaji, were considered, and experiments were carried out to investigate the effects of different cytokinins (BAP, KIN) and auxins (IAA, IBA, NAA) on shoot initiation and multiplication. Excised shoot-tips were used as explants and cultured on MS medium supplemented with various concentrations and combinations of BAP, IAA, and NAA to evaluate regeneration efficiency. The highest response of explants (78%) and maximum number of shoots per explant (5.04) were observed with 3.5 mg/L BAP + 1.5 mg/L IAA in G-9. In Sabri, the highest response of explants (65%) and maximum number of shoots per explant (5.50) were observed with 2.5 mg/L BAP + 1.0 mg/L Kin. The variety Meherasgor showed the highest response in on callus induction and regeneration (67%) and maximum number of shoots per explant (6.22), where 4.5 mg/L BAP + 2.0 mg/L IAA were used. The Jahaji showed the maximum number (72%) of shoots per explant (6.24). G-9 showed the highest number of roots (5.75) with 2.0 mg/L of IAA in MS medium. In Sabri, 5.81 roots were observed with 1.50 mg/L of IBA. In Meherasgor, the maximum number of roots (6.66) was recorded at 1.50 mg/L of IBA. Similarly, in Jahaji, 6.69 roots were observed with 1.00 mg/L of IBA. The regenerated healthy rooted plantlets were transferred from the culture bottle to soil in small plastic pots and were kept at outdoor environment, the survival rate was 88.89%. After proper acclimatization, the well-rooted plants were successfully transferred and hardened. This technology presents significant prospects for advancing commercial banana production, particularly by providing researchers with a reliable platform for large-scale propagation and genetic improvement.

Keywords: Micropropagation, MS medium, *Musa* sp., Sucker, PGRs.



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Introduction

Banana (*Musa* sp.) is the fourth world's most important food crop globally after rice and wheat (Karule et al. 2016). It is a staple food and an export commodity which contributes to the food security of millions of people in the developing world, and when traded in local markets, provides income and employment to the rural population, supplying both rural and urban communities with an important source of nutrition and energy (Mia et al. 2025). The fruits eaten as dessert without cooking are called bananas, whilst the starchier types, which need cooking before they can be used as vegetables, are called plantains (Ali et al. 2013). In Bangladesh, bananas, which are rich in carbohydrates, minerals, phosphorus, calcium, potassium, and vitamin C, are widely consumed due to their year-round availability, plentiful production, and high customer acceptability. It is a nutritious food, rich in carbohydrates, vitamin C, potassium, and iron (Patel and Sahay 2025).

The cultivation of tissue-cultured banana plants has been increasing and providing higher income to farmers, and it has led to a banana revolution in Bangladesh (Al-Amin et al. 2009, Hossain et al. 2009). It is probably the cheapest fruit available throughout the year. The main trouble in the inconsistent planting of bananas is the difficulty in getting high-quality, disease-free planting materials through field propagation (Shukla et al. 2025). The farmer-produced suckers are good transmitters of insect pests and diseases (Saranya et al. 2024). Propagation of banana through vegetative propagation is easy, but it takes a longer duration for the large-scale mass propagation of planting material, and vegetative propagation serves as the potential source of transmission of fungal, bacterial and viral diseases, which may affect yield (Medhane et al. 2023). Traditionally,

classical tissue culture techniques have been applied to many plant species. These methods are mainly used to produce seedlings and saplings through micropropagation. *In vitro* micropropagation through the plant tissue culture technique is the best alternative for producing healthy and disease-free planting materials of banana (Hossain et al. 2009, Medhane et al. 2023). Banana and plantain plants are susceptible to a wide range of diseases and pests. As a result, banana productivity decreases, and the yield becomes very poor. Only 2 to 4 suckers can be obtained from a plant per year in the conventional method, whereas in traditional clonal propagation method appears unable to satisfy the increase in demand for disease-free and healthy planting materials of banana (Khatun et al. 2017). Therefore, the present investigation was undertaken to determine the suitable concentrations of BAP and NAA on *in vitro* regeneration of banana cultivars, to allow for the rapid multiplication of uniform and high-quality clonal planting materials, and to supply planting material in large quantities, especially for large-scale cultivation.

Each banana represents a finger, and a bunch of bananas represents a hand. It is the oldest fruit crop to mankind (Subrahmanyam and Gantait 2022). Production of banana plants is done in two major ways, e.g. conventional or tissue culture system. The former method is not feasible for large-scale banana production due to the slow rate of multiplication (4-5 suckers per year), transportation of bulky suckers, and disease and pest infection. Micropropagation overcomes all these insufficiencies of the conventional method in the following ways: planting material produced is at a higher rate, uniform and disease-free (Erol et al. 2023). It also makes the production process season-independent, season independent as with the help of micropropagation, planting materials can be produced round the year (Singh et al. 2023). The conventional method for multiplication depends on the number of suckers, which is time-dependent. To get healthy and high-yielding planting material, *in vitro* multiplication of banana has proved to be a successful modern technique throughout the world (Hossain et al. 2016, Batool et al. 2020). The current study was undertaken to minimize the above-mentioned problems with the major objective of developing a protocol for *in vitro* micropropagation, followed by the effect of plant growth regulators (PGRs) and standardizing the surface sterilization protocol. This study was conducted to find out the best combination of growth regulators and to optimize their concentration for a better regeneration rate of the most important commercial banana varieties in Bangladesh.

Materials and Methods

Stock solution and culture medium

Stock solutions of the components of MS basal medium (Murashige and Skoog 1962) were prepared by dissolving the appropriate and recommended amount of macronutrients, micronutrients and organic supplements in double-distilled water. In the same way, plant growth regulators, a stock solution was prepared using a proportion of 1 mg/1 ml and stored in a refrigerator at 4°C. The iron stock solution was protected from light by storing the solution in a black bottle covered with aluminium foil. Sucrose (30 g/l) was added to the medium solution before pH adjustment. The stock solutions were mixed properly using a magnetic stirrer. The pH of the medium was adjusted to 5.80 with 0.1 N HCl and 0.1 N NaOH, followed by the addition of 6.5 g/l of agar. The medium was autoclaved at 121°C, 15 psi for 15 min.

Collection and surface sterilization of plant material

Two-month-old young and disease-free sword suckers (40-100 cm height) of G-9, Sabri, Meheresagor and Jahaji were used as explants in this study. The plants (explant sources) were collected from the research field of the Institute of Biological Sciences, University of Rajshahi. The pseudo-stems at the lower parts of the suckers containing meristems were used as explants. The sword suckers containing meristems were carefully detached from the mature banana plants. After removing the outer sheaths of the suckers were thoroughly washed with tap water for 40 min, followed by a detergent soup solution for 20 min to remove adherent soils. Then the explants were washed thoroughly with sterile distilled water three times so that no chemical residues remain. The explants were then surface sterilized in a combination of fungicide (Autostin, Halal Agro Techm Bangladesh) for 20 min,

followed by soaking in 2% NaOCl with two drops of Tween 20 for 15 min. Furthermore, the sword sucker tips were treated with 70% alcohol in a laminar air flow chamber for 2 min. Finally, the surface-sterilized shoot-tip explants were reduced to a 2 cm size by removing the outer layers of corm and leaf sheaths using sterilized forceps and scalpel and inoculated under clean conditions.

Inoculation of the explants in PGRs

After sterilization, the excised shoot tips were inoculated to shoot initiation media comprising of MS basal medium supplemented with different concentrations of BAP (1.5, 2.5, 3.5, 4.5 and 5.5 mg/L) in combination with same amount of IBA (indole-3-acitic acid- 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L); Naphthalacetic acid (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L), Kinetin (IAA) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) and IBA for *in vitro* regeneration of G-9, Sabri, Meherasgor and Jahaji variety of Banana. MS basal medium without plant growth regulator (PGR) was used as a control. Therefore, the total number of treatments used at this stage was 5, with 3 replications arranged randomly. The cultures were then kept in a dark room for three days to prevent the blackening of the initiated sucker produced during the stepwise removal of the covering leaf primordia. Then the initiated cultures were incubated and maintained for four weeks with a photoperiod of 16/8h light/dark using cool white, fluorescent lamps (photon flux density, 40 μ mol m⁻² s⁻¹ irradiance), at a temperature of 25±2°C and relative humidity of greater than 75% in a growth room. Finally, the initiation response, initiated shoot numbers per explant and average days taken for shoot initiation were recorded.

Data recording and statistical analysis

The experiment was arranged in a completely randomized design, and data were subjected to one-way analysis of variance (ANOVA) using the SPSS software packages (version 25), and the significant differences among the treatment mean values were evaluated using Fisher's least significant differences (LSD) at 5% level of significance.

Results and Discussion

Effects of different concentrations of PGRs on shoot initiation

In the present study, the properly surface-sterilized shoot tips obtained from the sword suckers of banana cv. G-9, Sabri, Meherasgor and Jahaji were directly inoculated on full-strength MS medium supplemented with different concentrations of BAP, NAA, IAA and IBA for *in vitro* shoot initiation. Results showed that the percentage of shoots initiated per explant was significantly ($p<0.001$) varied between treatments, with PGRs treatment at all concentration levels significantly surpassing values observed in the control (Table 1). It was observed that the multiplication rate is influenced by both the concentration of cytokinin and the specific genotype. Typically, shoot tips from cultivars possessing two or more genomes generate a cluster of numerous shoots and buds during each sub-culture cycle (Kumar et al. 2024). The effect of BAP on shoot proliferation and elongation from the shoot tip of G-9, Sabri, Meherasgor and Jahaji varieties was investigated by adding different concentrations of BAP. The highest response of explants (78%) was observed, and the maximum number of shoots per explant (5.04) was observed with 3.5 mg/L BAP + 1.5 mg/L IAA in G-9. Similar type of research was done by Mekonen et al. (2021), Kumar et al. (2024) and Kumar et al. (2022) that supports the result of this study, as they obtained the best shoot formation response (94%) using banana shoot tip meristem explant cultured on MS medium fortified with 1.0 mg/L BAP. In Sabri, the highest response of explants (65%) and maximum number of shoots per explant (5.50) were observed with 2.5 mg/L BAP + 1.0 mg/L Kin. The variety Meherasgor showed the highest response of explants (67%), and the maximum number of shoots per explant (6.22) was observed with 4.5 mg/L BAP + 2.0 mg/L IAA, and Jahaji indicated (72%), and the maximum number of shoots per explant (6.24) was observed with 5.5 mg/L BAP + 2.5 mg/L IAA. The highest per cent response of explants (84%) was observed in 5.0 mg/L BAP. The second highest (72%) was observed at 4.0 mg/L BAP, while the lowest (44%) was observed in the control mg/L. (Khatun et al. 2017). *In vitro* culture of shoot tip result's green globular structure with a hard coating within 10-15 days of inoculation in media containing different concentrations of BAP and a combination of IAA, NAA and IBA. The response of colour change of the explants of G-9, Sabri, and Jahaji varied in

respect of time and concentrations of BAP used (Table 1). The explants of G-9 and Jahaji became dark green at 3.5 mg/L BAP and 1.5 mg/L IAA after 15 DAI. A ball-like structure was observed in regeneration media containing different concentrations of BAP and NAA after 15-20 days of inoculation, as reported by Khatun et al. (2017). Shoot or meristem tip cultures are suitable not only for the large-scale production of uniform and vigorously growing propagules for field establishment, but also for germplasm conservation. For banana species which are predominantly propagated vegetatively, *in vitro* techniques are thus complementary to field conservation (Stross et al. 2001).

Table 1: Effect of different concentrations of BAP, IAA, NAA, IBA and KIN in MS medium on *in vitro* culture of G-9, Sabri, Mehersagor and Jahaji banana genotypes for shoot regeneration.

| PGRs | Concen. (mg/l) | Response of explants (%) | Length of the shoot (M \pm SEM) | | | |
|-----------|-------------------|-----------------------------------|--------------------------------------|-------------------|-------------------|--------------------|
| | | | G-9 | Sabri | Mehersagor | Jahaji |
| Control | - | 56 | 1.36 \pm 0.021 | 1.02 \pm 0.042 | 1.01 \pm 0.015 | 1.06 \pm 0.016 |
| BAP + IAA | 1.5+0.5 | 45 | 2.80 \pm 0.180c | 3.19 \pm 0.125c | 2.84 \pm 0.122d | 3.94 \pm 0.044c |
| | 2.5+1.0 | 65 | 3.10 \pm 0.206c | 5.37 \pm 0.152a | 3.16 \pm 0.109d | 4.21 \pm 0.108c |
| | 3.5+1.5 | 78 | 5.04 \pm 0.116a | 3.45 \pm 0.157c | 4.32 \pm 0.135c | 3.82 \pm 0.214c |
| | 4.5+2.0 | 67 | 4.19 \pm 0.077b | 4.32 \pm 0.193b | 6.22 \pm 0.088a | 5.77 \pm 0.217b |
| | 5.5+2.5 | 72 | 3.78 \pm 0.131b | 3.34 \pm 0.136c | 5.26 \pm 0.135b | 6.24 \pm 0.094a |
| BAP + NAA | 1.5+0.5 | 70 | 2.15 \pm 0.056c | 2.56 \pm 0.103c | 3.02 \pm 0.048c | 3.39 \pm 0.049c |
| | 2.5+1.0 | 55 | 2.30 \pm 0.094c | 5.12 \pm 0.056a | 3.09 \pm 0.032c | 3.45 \pm 0.079c |
| | 3.5+1.5 | 65 | 4.12 \pm 0.056a | 4.04 \pm 0.063b | 4.93 \pm 0.105b | 3.52 \pm 0.048c |
| | 4.5+2.0 | 50 | 4.20 \pm 0.065a | 4.13 \pm 0.064b | 6.05 \pm 0.015a | 6.07 \pm 0.075a |
| | 5.5+2.5 | 70 | 3.80 \pm 0.027b | 2.44 \pm 0.109c | 4.95 \pm 0.103b | 5.65 \pm 0.060b |
| BAP + KIN | 1.5+0.5 | 62 | 2.49 \pm 0.118a | 2.85 \pm 0.066d | 3.03 \pm 0.078c | 3.12 \pm 0.080c |
| | 2.5+1.0 | 65 | 3.71 \pm 0.064b | 5.50 \pm 0.113a | 3.12 \pm 0.099c | 3.42 \pm 0.119bc |
| | 3.5+1.5 | 62 | 4.75 \pm 0.065a | 3.58 \pm 0.064b | 4.22 \pm 0.094b | 3.52 \pm 0.112b |
| | 4.5+2.0 | 65 | 3.86 \pm 0.033b | 3.66 \pm 0.068b | 5.66 \pm 0.104a | 5.62 \pm 0.113a |
| | 5.5+2.5 | 55 | 3.76 \pm 0.026b | 3.21 \pm 0.090c | 3.15 \pm 0.146c | 3.26 \pm 0.105bc |
| BAP + IBA | 1.5+0.5 | 65 | 1.52 \pm 0.080c | 2.22 \pm 0.058c | 2.42 \pm 0.081c | 2.62 \pm 0.075c |
| | 2.5+1.0 | 50 | 1.60 \pm 0.103c | 4.13 \pm 0.123a | 2.52 \pm 0.093c | 3.62 \pm 0.071b |
| | 3.5+1.5 | 70 | 3.11 \pm 0.070a | 3.42 \pm 0.113b | 4.21 \pm 0.093b | 4.46 \pm 0.103a |
| | 4.5+2.0 | 75 | 2.90 \pm 0.090b | 3.49 \pm 0.083b | 5.81 \pm 0.031a | 3.66 \pm 0.086b |
| | 5.5+2.5 | 65 | 2.74 \pm 0.040b | 2.27 \pm 0.066c | 4.25 \pm 0.063b | 3.52 \pm 0.062b |

PGRs = Plant growth regulators, values represent mean \pm S.E. Each treatment was repeated three times. Means in a column with different letters are significantly different according to the least significant difference at $p<0.05$ levels.

Table 1 provides data on the response of different explants (G-9, Sabri, Mehersagor, and Jahaji) to various concentrations of plant growth regulators (PGRs), specifically combinations of BAP with IAA, NAA, KIN and IBA. The data consists of the explants' shoot lengths (M \pm SEM) and their response percentage. The response percentage increased significantly with BAP + IAA treatments, especially at higher concentrations. At the highest concentration of 5.5 + 2.5 mg/l, explants such as G-9 and Jahaji showed improved shoot lengths, with the G-9 explants showing a length of 3.78 \pm 0.131 cm and Jahaji reaching 6.24 \pm 0.094 cm as found by Mekonen et al. (2021). The shoot length varied between explants, with Jahaji generally showing the highest growth compared to the other explants across most concentrations. Like, BAP + IAA, BAP + NAA treatments enhanced shoot growth. The highest response (70%) was observed at 1.5 + 0.5 mg/l in the G-9 explant. The concentration of 4.5 + 2.0 mg/l, particularly for the Mehersagor and Jahaji explants, produced the most substantial growth, with shoot lengths of 6.05 cm for Mehersagor and 6.07 cm for Jahaji. The response in the G-9 explants, however, decreased at higher concentrations. For BAP + KIN, the response rates and shoot lengths varied. The highest growth was

observed for G-9 explants at $3.5 + 1.5$ mg/l, where a shoot length of 4.75 cm was achieved. As with other treatments, Jahaji explants showed consistent and high growth across the PGR concentrations, reaching up to 5.62 cm at $4.5 + 2.0$ mg/l. The response percentage remained relatively high, especially at $3.5 + 1.5$ mg/l, where the explants G-9, Mehersagor, and Jahaji had shoot lengths of 3.11 cm, 4.21 cm, and 4.46 ± 0.103 cm, respectively. The concentration of $2.5 + 1.0$ mg/l also yielded notable results, particularly in the Sabri explants, which showed the highest growth of 4.13 cm. BAP + IAA and BAP + NAA showed the most significant increases in response percentage and shoot length, particularly in Jahaji and Mehersagor explants. The effectiveness of the PGR combinations varied by explant, with some explants showing more robust growth at higher concentrations, especially in the Jahaji variety. BAP + KIN and BAP + IBA also contributed positively to shoot elongation but were generally less effective than BAP + IAA and BAP + NAA treatments. Jahaji explants consistently showed better growth across all treatments, suggesting that this variety may be more responsive to the applied PGRs compared to the other explants. In conclusion, the data indicate that higher concentrations of BAP in combination with IAA or NAA promote better shoot elongation in various explants, with the Jahaji explant particularly benefiting from these treatments. This information is crucial for optimizing growth conditions in plant tissue culture for specific explants.



Fig. 1(a-t): Shoot formation of the variety G-9 and Sabri (a,k = inoculation of explant; b,l = explant after 3 weeks; c,m = growing stage after 4 weeks; d,n = growing stage at the end of 5 weeks; and e,o = stage after 6 weeks) and formation of root and acclimatization (f-j; p-t) after 6-7 weeks of culture.

Effects of different concentrations of plant growth regulators on root initiation

For root induction, the *in vitro* developed healthy banana shoots were separated and transferred individually to half-strength MS medium supplemented with 30 g/L of sucrose and various concentrations of IBA, NAA, IAA, and Kin mentioned previously. Activated charcoal at 0.2 g/L for each rooting treatment was also added (Corozo et al. 2021). Half-strength MS medium devoid of any plant growth regulator was used as a control. The total number of treatments that were used in the rooting stage was 5, with 3 replications arranged randomly. The inoculated cultures were then incubated and maintained for four weeks in a growth room at a temperature of

25±2°C and 16/8h photoperiod provided by cool-white, fluorescent tubes. After four weeks, the rooting percentage, the number and length of the regenerated roots were recorded. The explants were grown in plastic cups with lids containing 30 ml of culture medium and 8 explants per glass for 30 days. The percentage of rooted shoots, number of leaves, plant height (cm), pseudostem diameter (mm), and maximum root length (cm) were recorded (Fig. 2). In root development stage, well grown shoots with expanded leaves were separated and transferred singly to fresh rooting MS medium with different concentrations of NAA, IAA, or IBA mentioned previously. Root growth and development were assessed 6 weeks after shoots were transferred to the rooting medium. Ten observations were recorded for several roots developed after a month of culturing, followed by two subsequent subcultures. Root initiation was carried out for all four treatments by applying IAA at a concentration of 0.1 mg/l and activated charcoal (Fig. 2). The maximum number of roots (5.75) was observed in 2.0 mg/L of IAA in G-9 (5.81) was observed in 1.50 mg/L of IBA in Sabri (6.66) was observed in 1.50 mg/L of IBA in Mehersagor and (6.69) was observed in 1.00 mg/L of IBA in Jahaji (Fig. 2).

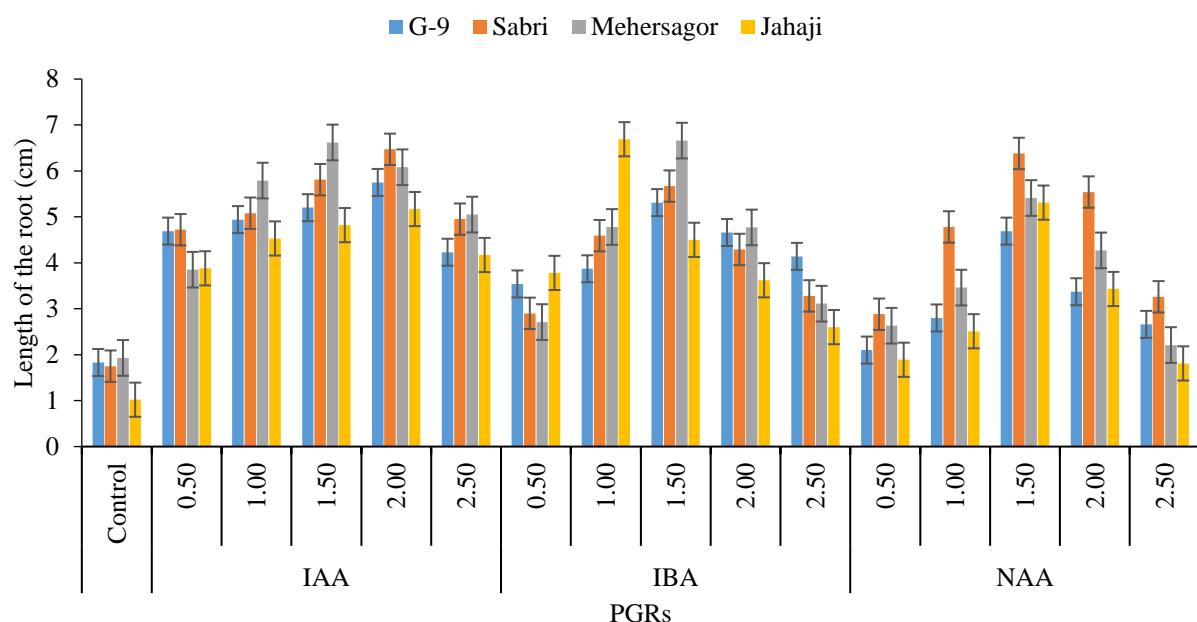


Fig. 2: Effect of different concentrations of IAA, NAA and IBA in $\frac{1}{2}$ MS medium on *in vitro* rooting of G-9, Sabri, Mehersagor and Jahaji banana genotypes.

Acclimatization and field transfer of plants

The *in vitro* regenerated and rooted banana plantlets were carefully removed from the culture bottles using sterilized forceps to prevent any damage to their root systems. Following extraction, the roots were thoroughly rinsed with slightly warm running tap water to eliminate any residual agar and medium. Immediately after washing, the banana plantlets were covered with damp newspaper and transported to the IBSc, RU shade house for primary hardening. They were then carefully transplanted using sharp wooden sticks into trays filled with moist coco peat, which were covered with transparent plastic bags to maintain high humidity levels (80 to 90%) and reduce light intensity for seven days. After this period, the plastic covers were removed, and humidity was gradually lowered to 50-60%, while light intensity was adjusted to normal levels and temperatures were increased to $27 \pm 2^\circ\text{C}$ over the course of 40 days. The survival rate of the successfully acclimatized plants during primary hardening was recorded. Subsequently, the plantlets were moved to larger plastic pots for secondary hardening, which were filled with a mixture of forest soil, sand, and manure in various ratios. Finally, the percentage of banana plantlets that survived from the total number transplanted in the greenhouse was calculated.

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Author's contribution: SMSI designed the experiment, supervised the study, and corrected the manuscript. MAS collected conducted experiments, data collection and analysis and wrote the original draft of the manuscript. Both authors have read and approved the final manuscript.

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