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Comparative study on micropropagation techniques in Banana Cv. Nendran and Nanjangud Rasabale

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Abstract

The experiment were conducted to investigate the effect of different concentration of BAP, and Kinetin on *in vitro* shoot initiation and multiplication of two banana varieties Nendran and Nanjangud Rasabale. The rapid shoot initiation was obtained from MS medium fortified with 5 mgL⁻¹ BAP for Nendran and 6 mgL⁻¹ BAP for Nanjangud Rasabale varieties of banana. For multiplication, in Nendran and Nanjangud Rasabale were observed on MS medium supplemented with 6 mgL⁻¹ BAP and 5 mgL⁻¹ BAP respectively. For root induction, MS medium fortified with 2 mgL⁻¹ IBA (91.48%) and 2 mgL⁻¹ NAA (80.29%) was found effective for highest percentage of root regeneration in Nendran and Nanjangud Rasabale variety of banana respectively. The rooted plants transferred in potting mixture of Red soil, FYM and Sand present in equal proportion (1:1:1) recorded 89.57% and 88.55% survivability in Nendran and Nanjangud Rasabale, respectively.

Keywords: Nendran, Nanjangud Rasabale, Micropropagation, BAP, NAA

Introduction

The banana, or *Musa* spp., is a large perennial herbaceous monocotyledon plant is a member of the Scitamineae order of *Musa cecae* family. It is one of the first crops that people have cultivated. The two species from which modern edible banana varieties originated are *Musa acuminata* and *Musa balbisiana* (Simmonds, 1962) [21]. The Food and Agriculture Organization reports that there are more than a thousand different types of germplasms worldwide, which include both cultivated and wild species.

Among all the fruits bananas are a major food crop that are grown and consumed in more than 100 countries in the tropics and subtropics. India is the world's top producer of bananas, with an annual production volume of 29,124,000 tonnes, followed by China, Indonesia, Brazil and other nations. India contributes around 30.8% to the total production of banana globally (Anonymous, 2023) [3]. Andhra Pradesh is the largest banana producing state in India with a share of 16.27%, followed by Gujarat (14.54%) Maharashtra (13.69%), Tamil Nadu (10.42%), Uttar Pradesh (10.31%) and Karnataka (7.57%) (Anonymous, 2022) [2]. In India, the states which plant largest numbers of banana include Maharashtra, Gujarat, Karnataka, Andhra Pradesh, Tamil Nadu etc. where the single variety 'Grand Naine' is preferred. In Maharashtra state other important varieties of banana cultivated in different districts are *Dwarf Cavendish*, *Basrai*, *Robusta*, *Lal Velchi*, *Safed Velchi*, *Rajeli Nendran*, *Grand Naine*, *Shreemanti*, *Red Banana* (Patil *et al.*, 2012) [18]. While in Konkan region *Safed velchi*, *Red Banana*, *Lal velchi*, *Mutheli*, *Rajeli*, *Rajapuri*, *Bankel* and *Bhurkel* are mostly cultivated (Patil *et al.*, 2012) [18].

Nendran Banana, also known as 'Nendran Pazham' or 'Kerala Banana', is a popular cultivar originating from the Southern Indian state of Kerala. Nendran bananas are renowned for their firm texture, distinct ridges, and a mild sweetness when ripe. Agricultural significance of Nendran is due to its demand and unique flavour, Nendran banana holds economic importance in consistent quality and yield. Nanjangud Rasabale (silk subgroup) Banana, also known as 'Rasa Bale' or 'Gros Michel', is a well-known banana variety found primarily in parts of South India. Nanjangud Rasabale bananas are appreciated for their rich and distinctive flavour, with a sweet and creamy taste when ripe. They are often preferred for their dessert-like qualities. Agricultural significance of Nanjangud Rasabale bananas are valued for their flavour and aroma, which makes them sought after in local markets.

Nendran variety is susceptible to Banana Bract Mosaic Virus (BBMV), nematodes and borers. While, Nanjangud Rasabale variety is susceptible to bacterial and viral diseases reducing the area of cultivation to only 5 ha, and hence it is considered as an endangered cultivar (Anonymous, 2023) [3].

The traditional clonally propagation method is no longer able to meet the demand for new planting material due to the rapid rise in consumption of bananas and plantains caused by population increases and the development of new markets, particularly in Europe (Arias 1992) [4]. Recent advances in biotechnology for crop improvement have had a great impact on bananas and plantain cultivation. The micropropagation of shoot tips *in vitro* using growth medium with different combinations of plant growth regulators is the most common application of biotechnology in agriculture (Doyle and Doyle, 1990) [7]. Since most of the hybrid varieties are seedless, it is propagated vegetatively.

The main advantages of *in vitro* regeneration technique are rapid multiplication of plants with known desirable characters, free from pest and diseases, high survival rate during field establishment, vigorous growth, retention of healthy leaves, uniform growth, shortened harvesting period and higher yields. The disadvantage in Nanjangud Rasabale and Nendran micropropagation comes from inherent issues with them, such as poor multiplication rates, a higher degree of culture browning, *etc.*, under *in vitro* conditions. One of

these issues is phenolic browning, which is one of the main barriers to the *in vitro* regeneration of Nendran and Nanajangud Rasabale (Prabhuling *et al.*, 2017) [19].

An attempt has been made to standardize the protocol for *in vitro* multiplication of these two varieties. These explants upon repeated transfer to the multiplication media hardly showed any multiplication and hence the present study was undertaken to induce shoot proliferation using the different sources of cytokinin and root regeneration using various concentration of auxins.

2. Materials and Methods

2.1 Materials

2.1.1 Genotype

The present investigation was carried out with banana (*Musa* spp) var. Nendran and Nanjangud Rasabale. The experimental material, sword suckers, of the present investigation was collected from the Department of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist: Ratnagiri.

2.1.2 Explants

The Sword suckers of two to three months old were removed from a healthy, disease-free mother plant of both the varieties. Roots and outer sheaths of sucker were removed to prepare the shoot tips, which were then sterilized and used for the experiment.



Fig 1: Sword sucker used as explant

2.1.3 Culture Medium

A basal medium developed by Murashige and Skoog (1962) [16] was further enhanced with various concentrations and mixtures of antibiotics and plant growth regulators.

2.1.4 Experimental Conditions

All *in vitro* experiments were carried out in a laminar air flow chamber under aseptic conditions. The culture chamber in which the experiments were conducted was kept at a constant temperature of $25 \pm 0.5^\circ\text{C}$, and a 16/8-hour light/dark cycle was used to provide consistent illumination of light (1600 Lux) using fluorescent lamps with a 7200 K color temperature.

2.2 Method

2.2.1 Preparation of Explants

The sword sucker of *Musa* spp. var. Nendran and Nanjangud Rasabale that was collected from the field was thoroughly washed for 30 minutes under running water. Using autoclaved stainless-steel knife, the explants were reduced in size until

their length was between 6 and 7 cm and their diameter at the base of the leaf was between 4 and 5cm.

2.2.2 Surface Sterilization

These shoot tips were collected in a tray and kept in water for 5 min, then explants were pretreated with, 1% savlon for 10 minutes and 0.5% tween 20 for 10 minutes. Then explants were treated with 0.1% Carbendazim and Streptomycin 200 mgL^{-1} solution for 30 minutes. Transfer the explants to Cefotaxime 200 mgL^{-1} and Streptomycin 200 mgL^{-1} solution for 30 minutes. Then dipped in 70% Ethanol for 60 seconds. After this it was washed with distilled water and surface sterilized with 2% Sodium hypochlorite solution for 10 minutes with few drop of Tween 20, 0.1% HgCl_2 for 10 minutes.

2.2.3 Inoculation of explants

The standard dissection and disinfection procedure was followed, as described by Cronauer and Krikorian (1984). The

suckers were further cut down to their final size of 2-3 cm before being inoculated using the aseptic culture technique in bottles containing MS medium fortified with various concentration of BAP ranging from 2 mgL⁻¹, 2.5 mgL⁻¹ to 6 mgL⁻¹. The cultures were kept in a culture room for

incubation. The cultures were initially kept on the establishment medium for up to 21 days. Cultures were first incubated in the dark for 7 to 15 days before being transferred to light (1600 lux) for 15 days.

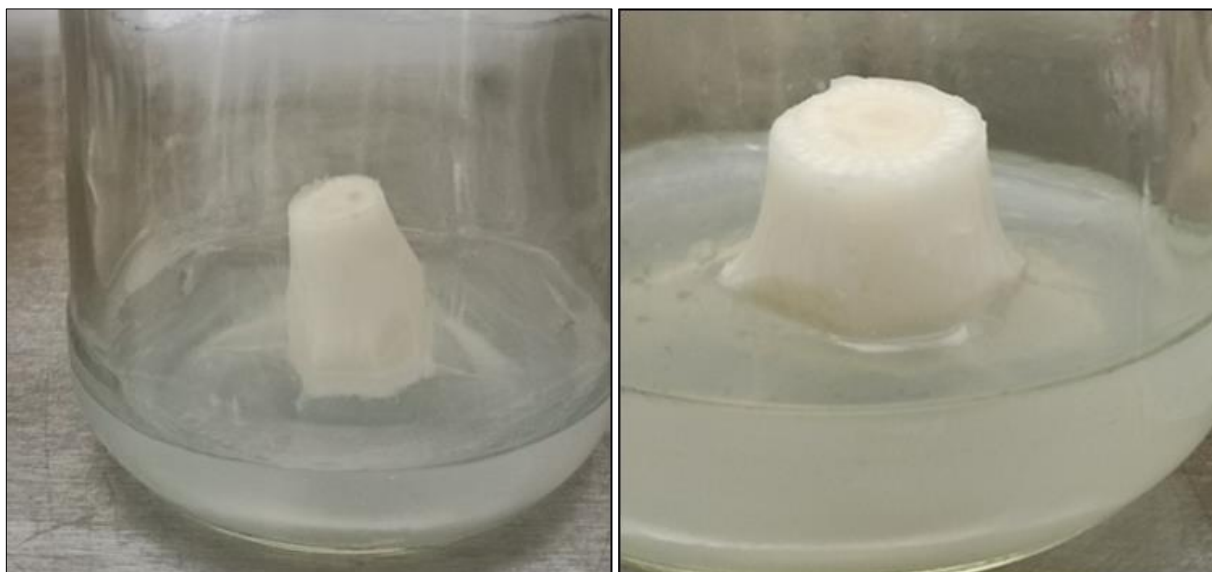


Fig 2: A. Inoculation of Nendran explant into establishment medium B. Inoculation of Nanjangud Rasabale explant into establishment medium

2.2.4 Shoot proliferation and multiplication

For the establishment and proliferation of shooting tips, MS medium fortified with BAP and Kinetin (1,2,3,4,5,6 mgL⁻¹ respectively) was used. Explants were subcultured every 15 days for the purpose of multiplication on new medium containing the same medium combinations.

2.2.5 Root regeneration

MS medium was utilized for the initiation of roots from regenerated shoots. Individual shoots were placed in rooting medium that was supplemented with IBA and NAA in varying concentrations ranging from 0.5 to 2.5 mgL⁻¹ respectively, for *in vitro* root development.

2.2.6 Statistical Analysis of Data

The data recorded from the experiments were conducted according to completely randomized design (CRD). Each experiment was replicated three times. Each replication contains 20 samples. The goal of the study was to find a significant difference between the treatment means. On the basis of critical difference, the treatment means were classified as significant or non-significant (CD) (Gomez and Gomez, 1984) [8].

3. Results and Discussion

3.1 Effect of surface sterilization

The shoot tips of both the varieties were collected in a tray, submerged in water for five minutes, and then explants were pretreated with 1% savlon for 10 minutes and 0.5% tween 20 for 10 minutes. Then, treated with different sterilants such as 0.1% carbendazim, streptomycin 200 mgL⁻¹, and cefotaxime 200 mgL⁻¹ treatment were given to the explant over a period of time.

For Nendran variety treatment consists of 70% ethanol (70 ml ethanol in 30 ml DDW) for 1 min + 10% NaOCl (10 ml

sodium hypochlorite in 90 ml DDW) for 10 min + 0.1% HgCl₂ (100 mg in 100 ml DDW) for 10 min showed highest aseptic percentage and survival rate (78.65%). For the Nanjangud Rasabale treatment combination, which consists of 70% ethanol for 1 minute, 10% NaOCl for 10 minutes, and 0.15% HgCl₂ for 5 minutes, showed the highest aseptic percentage and survival rate (69.48%). Similar results also reported by Ahmed *et al.*, (2014) [11]- got 90% aseptic culture when treated with 70% ethanol for 1 min, 10% NaOCl for 10 min followed by 0.1% HgCl₂ for 10 min which was slightly similar to the result which found best in this experiment.

3.2 Effect of dark and light condition on explants

Explants were inoculated using the aseptic culture technique after surface sterilization in bottles containing MS medium supplemented with various growth regulators. The cultures were grown in a dark environment for 7 to 15 days before being transferred to a light environment. Records of the visual observations were collected. Explants that were preconditioned in the dark tended to survive longer and have less bacterial contamination. Mitsukuri *et al.*, (2009) [15] showed that dark preconditioning reduces browning and bacterial contamination in *Habenaria radiata*.

3.3 *In vitro* Establishment of shoot tip

The highest establishment of culture for the Nendran variety (88.60%) was seen in medium comprising 5 mgL⁻¹ BAP, while for Nanjangud Rasabale variety highest establishment (86.58%) was seen in MS medium supplemented with 6 mgL⁻¹ BAP. The lowest establishment of culture were recorded in medium containing 2 mgL⁻¹ BAP (0.46% and 0.84%) for Nendran and Nanjangud Rasabale, respectively. Similar results were reported by Venkatachalam *et al.*, (2006) [24], which shows highest establishment in MS medium supplemented with 5 mgL⁻¹ BAP in banana.

3.4 *In vitro* shoot regeneration

The Nendran variety showed the highest shoot induction frequency in MS medium containing 6 mgL⁻¹ BAP (78.28%) in 20 days time period. While, MS medium containing 5 mgL⁻¹ BAP (81.55%) had the highest frequency of shoot induction in 18 days for Nanjangud Rasabale. Koli *et al.*, (2014), observed 99% shooting on media MS + 4 mgL⁻¹ BAP + 2 mgL⁻¹ IAA. Jaisy and Ghai (2011) [10], who recorded 4 weeks

to shoot production, Goswami *et al.*, (2013) [9]-recorded 21-34 days required to shoot induction.

On kinetin medium, the highest shoot multiplication ratio was observed in MS medium containing 6 mgL⁻¹ Kin (63.83%) for Nendran variety. Whereas, for Nanjangud Rasabale highest shoot multiplication was shown in MS medium containing 6 mgL⁻¹ Kin (63.52%).

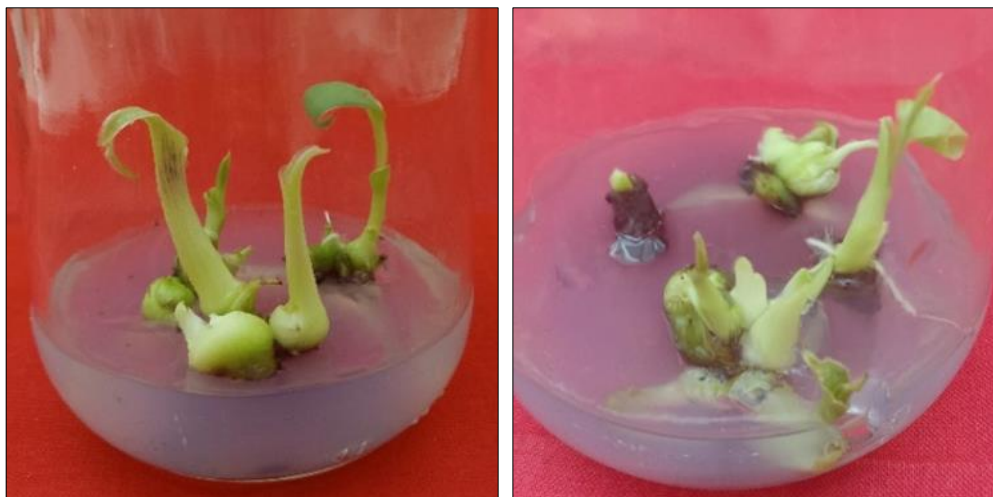


Fig 3: A. Shoot induction of Nendran on MS + 6 mgL⁻¹ BAP B. Shoot induction of Nanjangud Rasabale on MS + 5 mgL⁻¹ BAP

3.5 Subculturing for multiple shoot regeneration

For the multiplication Nendran variety, explants were subcultured every 30 days on new medium supplemented with 6 mgL⁻¹ BAP and 6 mgL⁻¹ kinetin and for Nanjangud Rasabale variety 5 mgL⁻¹ BAP and 6 mgL⁻¹ kinetin for multiplication. Shashikumar *et al.*, (2015) [23] showed that subculturing at 15 days interval resulted in increase in rate of multiplication of shoots. Maximum shoot multiplication rate was observed up to 4th subculture with 1:3.30 multiplication rate and 2.2 cm shoot height in Nendran. And in Nanjangud Rasabale maximum multiplication rate was observed up to 3rd subculture with 1: 3.40 multiplication rate and 2.7cm shoot height. On kinetin medium Nendran shows maximum shoot multiplication rate in 3rd subculture with 1:3.20 ratio and 1.9 cm shoot height, while Nanjangud Rasabale shows maximum shoot multiplication rate in 3rd subculture with the ratio 1:3.30 and 2.5 cm shoot height.

3.6 *In vitro* Root Regeneration

Medium fortified with MS + 2 mgL⁻¹ IBA recorded maximum (91.48%) frequency of rooting in 13 days for the Nendran variety of banana. Kelta *et al.*, (2018), showed MS media supplemented with 1.5 mgL⁻¹ IBA produced 60% root induction. However, in Nanjangud Rasabale variety, maximum frequency (80.29%) of rooting in 15 days was observed in medium which was fortified with MS + 2.0 mgL⁻¹ NAA. Lohidas *et al.*, (2015) [14] described, when the MS medium supplemented with 2.5 mg/l of IBA a good performance of rooting was observed in banana. Dagnev *et al.*, (2012) [6] observed plantlets rooted *in vitro* with MS supplemented with 2.12 mgL⁻¹ NAA was best for rooting. Lowest root induction (42.44% and 36.26%) in 19 days of time period was observed in medium supplemented with MS + 0.5 mgL⁻¹ NAA in both Nendran and Nanjangud Rasabale, respectively.



Fig 4: A. Root induction of Nendran on MS + 2 mgL⁻¹ IBA B. Root induction of Nanjangud Rasabale on MS + 2 mgL⁻¹ NAA

3.7 Root development

For Nendran, the rooting medium containing MS + 2.5 mgL⁻¹ IBA was found significantly superior over all rooting media with an average of 9.58 roots per shooted plant. Kumari and Misra (2016) [13] observed that 5 roots/explant on 0.5 mgL⁻¹ IAA + 0.5 mgL⁻¹ IBA. While, for Nanjangud Rasabale, The rooting medium S11 containing MS + 2.5 mgL⁻¹ NAA was found significantly superior over all rooting media with an average of 9.11 roots per shooted plant. Similarly, Rahman *et al.*, (2002) [20] observed plantlets rooted *in vitro* with MS supplemented with 2.5 mgL⁻¹ NAA (6 roots/plant).

3.8 Hardening

In a greenhouse, the highest per centage of plantlets that survived was 89.57% was found in potting mixture comprising Red Soil + FYM + Sand (1:1:1) mixture for Nendran variety. For Nanjangud Rasabale variety, the highest percent of plantlets survival 88.55% was found in potting mixture comprising Red soil + FYM + Sand (1:1:1). Sindha *et al.*, (2011) [22] were transferred the rooted plantlets into a poly bag containing fine sterilized sand, sterilized soil and a farmyard manure (1:1:1) mixture. After 4 weeks of hardening these plantlets survival in their native habitat was observed. Therefore, among the numerous combinations utilized in the current study for hardening: soil, FYM and sand mix in the ratio of 1:1:1 demonstrated to be the most successful. Nandhakumar *et al.*, (2017) [17] was done acclimatization by transferring sufficiently grown plants into poly bags containing sterilized pot mixture 1:1:1 ratio of Sand, Red soil and FYM observed maximum survival.

4. Conclusion

From the present investigation it could be concluded that, for *in vitro* regeneration of variety Nendran banana the treatment in the sequential order of 1% Savlon for 10 minutes + 0.5% Tween 20 for 10 minutes. followed by 0.1% Carbendazim + 200 mgL⁻¹ streptomycin solution for 30 minutes, and 200 mgL⁻¹ Cefotaxime + 200 mgL⁻¹ streptomycin solution for 30 minutes, then Ethanol 70% for 1 minute, Sodium Hypochlorite 10% for 10 minutes and Mercuric Chloride 0.1% for 10 minutes produces 78.65% aseptic culture. While, for variety Nanjangud Rasabale 57 banana the treatment in the sequential order of 1% Savlon for 10 minutes + 0.5% Tween 20 for 10 minutes followed by 0.1% carbendazim + 200 mgL⁻¹ streptomycin solution for 30 minutes, and 200 mgL⁻¹ Cefotaxime + 200 mgL⁻¹ streptomycin solution for 30 minutes, then Ethanol 70% for 1 minute, Sodium Hypochlorite 10% for 10 minutes and Mercuric Chloride 0.15% for 5 minutes produces 69.48% aseptic culture The MS medium supplemented with 5 mgL⁻¹ BAP for establishment, 6 mgL⁻¹ BAP for proliferation, 2 mgL⁻¹ IBA for root regeneration was found superior in Nendran variety of banana. While, The Ms medium supplemented with 6 mgL⁻¹ BAP for establishment, 5 mgL⁻¹ BAP for proliferation, 2 mgL⁻¹ NAA for root regeneration was found superior in Nanjangud Rasabale. The plantlets hardened in the potting mixture (1:1:1) of Red soil, FYM and Sand with survivability 89.57% and 88.55% for Cv. Nendran and Nanjangud Rasabale, respectively.

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