***In vitro* propagation of banana (*Musa* spp.) *cv.* Neypoovan through male bud culture**

ABSTRACT

Neypoovan is an important banana cultivar widely cultivated in South India. The demand for planting material of this cultivar is huge and tissue-cultured plantlets are not available owing to its inherent problems in the initial establishment of the culture and slow rate of multiplication by using shoot tips as explant. The present study investigated on direct regeneration and mass multiplication of the banana cultivar Neypoovan (AB) through *in vitro* male bud culture. The experiment which was conducted at the College of Agriculture, Vellayani explored both flower buds and floral hands as explants. The establishment media evaluated involved plain MS as control and 15 hormone treatments involving different levels of BA (2, 4 and 6 mgL^{-1}), TDZ (0.2, 0.4 and 0.6 mgL^{-1}), kinetin (0.5, 1.0 and 2.0 mgL^{-1}), combination of BA (2, 4 and 6 mgL^{-1}) with kinetin (1.0 mgL^{-1}) and combination of BA (2, 4 and 6 mgL^{-1}) with NAA (1.0 mgL^{-1}). Floral hands in 4 mgL^{-1} BA + 1mgL^{-1} NAA achieved higher culture establishment percentage (100) and lesser number of days for establishment (26.78 days) when compared to whole buds. Standardization of shoot multiplication involved three levels of BA *viz.*, 2, 4 and 6 mgL^{-1} in standard MS solid media. The treatment with 4 mgL^{-1} BA registered the least number of days to shoot initiation (96.45 days) and significantly higher number of shoots per culture (9.73). The study involved different rooting *viz.*, MS, MS + 250 mg charcoal, MS + 1mgL^{-1} NAA and MS + 0.5mgL^{-1} NAA. Days to root initiation was least in full MS (5.25 days) which also recorded the highest root length (10.23 cm). The present investigation highlighted the possibility of using floral hands as a promising explants type for direct regeneration of banana cv. Neypoovan.

Keywords: Banana, Neypoovan, floral meristem, *in vitro* propagation, plant growth regulators, Benzyl Adenine

1. INTRODUCTION

Banana (*Musa* spp.) is a perennial monocotyledonous herb belonging to the genus *Musa* of the Musaceae family. It is cultivated in warm, humid tropical, and subtropical regions of the world (D'hont *et al*., 2012). They are one of the significant tropical fruits (Giuggioli *et al*., 2024) and the fourth most important food crop after rice, wheat, and maize (Voora *et al.,* 2020). Ripe banana consists of about 70% water, with 23.43% carbohydrates, 1.03% protein, 0.48% fat, and various minerals and vitamins, providing 67 -137 calories per 100 grams (Aurore *et al.,* 2009). Although bananas have considerable commercial value, the crop faces significant threats from various pests and diseases, largely due to propagation through low-quality suckers. However, in recent years, *in vitro* propagation techniques have been introduced, allowing for large-scale multiplication of both traditional banana varieties and new hybrids. This method helps ensure the availability of quality planting materials for both farmers and researchers (Kavitha *et al*., 2020).

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Neypoovan (Syn. Njalipoovan, Elakkibale, Safed Velchi; AB genome) is one of the most favoured cultivars of the South Indian state of Kerala. The cultivar is known for its special taste, aroma, and long-keeping quality of its fruits (Udaya, 2024). The demand for planting material for this cultivar is huge and tissue-culture plantlets are not available owing to its inherent problems in the initial establishment of the culture in the medium and slow multiplication rate (Meenakshi *et al.*, 2014). The apical meristem of the rhizome is the most commonly used explant for banana micropropagation. Moreover, *in vitro* propagation using shoot tips presents challenges, particularly with surface sterilization and significant loss of explants due to contamination from endogenous bacteria (Titov *et al.*, 2006; Uma *et al.*, 2024).

In contrast, immature male floral hands offer a promising alternative as explants due to their lower contamination rate and reduced phenolic exudation (Kavitha *et al.*, 2018). They also contribute to the development of cultivars with desirable yield traits, as they are selected *in situ* after the bunching (Resmi and Nair, 2007). Micropropagation using male floral meristems has demonstrated significantly less somaclonal variation compared to soil-grown suckers and a single flower can yield hundreds of successful clones (Vincent and Anushma, 2018).

The feasibility of utilizing male immature flower buds as explants for the rapid propagation of bananas has been studied in cultivars like Berangan (Harirah and Khalid, 2006), Sannachenkadali and Red Banana (Resmi and Nair, 2007), Sabari (Sultan *et al.*, 2011), Virupakshi, Sirumalai (Mahadev *et al.*, 2011), Poovan (Nair *et al.*, 2018 ; Sivakumar and Visalakshi, 2021), Kadali (Lakshmi *et al.,* 2019) and Rajapuri Bale (Habibi and Guranna, 2023). However, only a few studies have been reported about the micropropagation technique of banana cv. Neypoovan (AB). The development of a protocol suitable for each variety through direct regeneration from male buds is very much in need. Hence, the present study aims at direct regeneration and mass multiplication of the banana cultivar Neypoovan through *in vitro* male bud culture.

2. MATERIALS AND METHODS

2.1 Explant collection and surface sterilization: The present investigation was carried out at the Department of Fruit Science, College of Agriculture, Vellayani, Kerala Agricultural University from 2023 to 2024. The study involved the use of floral meristem as explant and for which immature male flower buds were collected from healthy field-grown Neypoovan banana plants at 2-3 weeks after the bunch emergence (Fig. 1a&b). The bracts were then carefully removed (Fig. 1c) and reduced to a size of 4-5 cm before being surface sterilized in laminar air flow by wiping with 70% ethyl alcohol (Kavitha *et al.*, 2018: Shukla *et al.*, 2020). Development of *in vitro* cultures involved two types of explants *viz.*, flower buds and floral hands (Fig. 2a-d). Flower buds were reduced to 1–2 cm, inoculated as whole bud, dissected longitudinally into two and four parts while the buds of the same size were dissected to isolate young male flowers positioned 14–22 from the meristematic dome under laminar airflow for culture initiation.

2.2 Development of *in vitro* cultures of Neypoovan: The explants were inoculated (Fig. 3 a&b) in plain MS (Murashige and Skoog, 1962) solid basal medium (T_0) and MS media supplemented with 15

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treatments involving plant growth regulators (T_1 to T_{15}). The hormone treatments involved three levels of BA (2, 4 and 6 mg l^{-1}), TDZ (0.2, 0.4 and 0.6 mg l^{-1}), kinetin (0.5, 1.0 and 2.0 mgL^{-1}), combination of BA (2, 4 and 6 mgL^{-1}) with kinetin (1.0 mg L^{-1}) and combination of BA (2, 4 and 6 mgL^{-1}) with NAA (1.0 mgL^{-1}). All 16 treatments were imposed on male buds and floral hands separately for establishment (Table 1). Observations on the morphological characters of the explant, percentage of cultures established, and days for culture establishment were made after 4 weeks of culturing.

2.3 Standardization of shoot multiplication: The proliferating white meristem clumps from the best treatment after establishment were selected and used for shoot multiplication. Standardization of shoot multiplication involved three levels of BA *viz*., 2, 4, and 6 mgL^{-1} in standard MS solid media. The explants were transferred to fresh media at three-week intervals until the formation of shoot buds. The multiple shoots induced were separated into clumps of 2-3 shoot clumps and sub-cultured at 30 days intervals. Observations on days to shoot initiation, number of shoots per culture at 3 weeks, length of shoots at 3 weeks and number of leaves were recorded.

2.4 Root induction and hardening: The proliferated multiplied shoots were transferred into the following treatments viz., full MS, full MS + 250 mg charcoal, full MS + 1 mgL^{-1} NAA, and full MS + 0.5 mgL^{-1} NAA to optimize the media for *in vitro* rooting. Observations on number of roots, root length (cm), and days to root initiation were recorded four weeks after culturing. The regenerated plants with fully developed leaves and roots were washed in running tap water to remove the media particles and primarily hardened in portrays filled with coir pith compost. Hardened plantlets were maintained in the greenhouse at a temperature of 27-28°C and a relative humidity of 60% to 80%. Observations were taken on plant survival percentage along with morphological parameters like plant height, number of leaves, leaf length and leaf width at two weeks after hardening.

2.5 Statistical analysis: The experiment was laid out in a Completely Randomized Design (CRD). The data was subjected to statistical analysis by analysis of variance (ANOVA) using GRAPES (General R-based Analysis Platform Empowered by Statistics) software (Gopinath *et al.,* 2020).

3. RESULTS AND DISCUSSION

3.1 Development of *in vitro* cultures of Neypoovan

3.1.1 Morphological characters of explant

Morphological responses of flower buds and floral hands revealed distinct variations in their morphogenic potential, reflecting their suitability for *in vitro* culture establishment. Whole flower buds and those split into two halves showed browning at the tips, followed by internal necrosis within three weeks of inoculation, leading to culture failure. In contrast, buds dissected into four longitudinal sections recorded green coloration within 7-10 days of inoculation, indicating viable morphogenic activity (Fig 4b&d). These observations align with previous findings by Ganapathi *et al.* (1999), Harirah and Khalid (2006), Nair *et al.* (2018) and Lusiyanto et al. (2021) who reported that smaller flower bud sections improved culture establishment.

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Floral hands however displayed faster and more robust morphological responses compared to flower buds. Green coloration developed within 5–7 days, along with the formation of white bud like structures (WBLS) by the third week (Fig 4a&c). This was more prominent in media containing 4 mgL^{-1} BA and 1 mgL^{-1} NAA which was consistent with the observations made by Lakshmi *et al.* (2019) in cultivar Kadali. The basal regions of floral hands exhibited higher morphogenic activity, supporting the findings of Darvari *et al.* (2010) and Lusiyanto *et al.* (2021), who emphasized the critical role of meristematic activity at the flower base in successful culture initiation.

3.1.2 Percentage of culture established

The effects of explant and hormones on percentage of cultures established are given in the Tables 2a & 2b. Floral hands, exhibited a higher percentage of successful culture initiation (72.22%) compared to flower buds (53.12%). This could be due to the fact that smaller male inflorescences or floral clusters are reported to give better culture initiation in various banana genotypes (Darvari *et al.*, 2010; Mukunthakumar *et al.*, 2011). Interaction effects between explants and treatments revealed that the highest percentage of culture establishment was observed in floral hands receiving BA + NAA combination viz., T_{13}, T_{14} and T_{15} which was on par with T_1, T_2, T_3 and T_{12}. In general, floral hands exhibited significantly higher establishment than flower buds. Among flower buds, the highest percentage of culture establishment was recorded in T_3 which was on par with T_{12}. In both floral hands and flower bud, kinetin treatments viz., T_7, T_8 and T_9 registered the lowest percentage of culture establishment. The faster response observed with floral hands can be attributed to their higher meristematic activity, as seen in the early formation of WBLS and cauliflower like bodies (CLBs). The superior response of floral hands is also consistent with studies by Sultan *et al.* (2011) and Zuhry and Razmy (2019), who found that smaller floral parts or dissected buds exhibited better tissue culture responses.

3.1.3 Days for culture establishment

The influence of explant and hormones on days for culture establishment are given in Table 3. Floral hands in general recorded the least number of days to establish (26.79 to 34.22 days) when compared to male flower bud (31.33 to 40.66 days). This result was consistent with Sultan *et al.* (2011), who observed varying culture establishment times based on the specific bracts used in male flower buds. The floral hands in 4 mgL^{-1} BA + 1 mg L^{-1} NAA was the best recording 26.78 days for culture establishment and was on par with 6 mgL^{-1} BA + 1 mg L^{-1} NAA (27.39 days). In male flower bud, T_3 observed the least number of days for culture establishment (31.00 days). Flower bud in plain MS (control) took the highest number of days for culture initiation (40.66 days). The findings of this study is in contrast with earlier research by **Ganapathi *et al.* (1999**) and Harirah and Khalid (2006), which emphasized the use of male flower buds for banana tissue culture.

The higher establishment rates and shorter time to initiation observed with floral hands suggests that they are more efficient and reliable as an explant for *in vitro* culture initiation, particularly for cv. Neypoovan. These findings have practical implications for improving large-scale micropropagation protocols, as floral hands offer a more efficient and cost-effective alternative to

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flower buds. Considering the performance of floral hands in media containing 4 mgL^{-1} BA and 1 mg L^{-1} NAA (T_{14}), were selected as the best treatment for culture establishment of Neypoovan, and thus used for further studies on shoot multiplication.

3.2 Standardization of shoot multiplication

In the shoot multiplication stage, cytokinins like 6-benzyl adenine (BA) stimulate axillary meristem proliferation, promoting shoot organogenesis. Following Wong (1986), Prabhath and De Silva, (2014) and Nugrahani *et al.* (2024), BA was included in the shoot multiplication media, enabling floral hand cultures to proliferate via non-dominant lateral meristems. Effect of different levels of BA on shoot initiation are presented in Table 4.

3.2.1 Days to shoot initiation

The floral hand cultures in different BA concentrations displayed varied responses after establishment. Cultures in 4 mgL^{-1} BA (T_2) showed distinct green nodule-like structures within 21-25 days, which developed into tiny shoots at 105-110 days (Fig 5 a-d). However, cultures in 2 mgL^{-1} (T_1) and 6 mgL^{-1} BA (T_3) exhibited signs of shoot initiation but failed to form proper shoots, eventually turning brown. Among the treatments, 4 mgL^{-1} BA (T_2) was the most effective, producing the shortest period for average shoot initiation (96.45 days), significantly better than 2 mgL^{-1} (108.60 days) and 6 mgL^{-1} BA (107.40 days). This indicates that an intermediate BA concentration effectively overcomes apical dominance and enhances meristematic activity. Similar findings have been reported by Mukunthakumar *et al.* (2011), emphasizing the role of moderate cytokinin levels in promoting shoot multiplication in banana tissue culture.

3.2.2 Number and length of shoots per culture

BA at 4 mgL^{-1} produced an average of 6.93 shoots per culture with a shoot length of 0.83 cm, significantly outperforming lower (4.80 shoots) and higher (5.00 shoots) BA concentrations. Excessive cytokinin concentrations can cause physiological stress, limiting shoot proliferation, as observed by Punyarani *et al.* (2017) and Nandariyah *et al.* (2021).

Further subculturing in 4 mgL^{-1} BA for 60 days resulted in shoot elongation, leaf formation, and multiple shoots (Fig 5 e-f). Effect of T_2 (4 mgL^{-1} BA) on shoot proliferation at 60 days after shoot initiation are given in Table 5. Cultures produced 9.73 shoots per culture with a shoot length of 3.7 cm, along with 3.16 leaves per shoot and 11.13 leaves per culture. This supports the role of BA in enhancing both shoot initiation and subsequent growth by creating an optimal hormonal balance conducive to cell division and elongation. These results align with findings by Nair *et al.* (2018), who reported that BA concentrations between 4–6 mgL^{-1} promote shoot primordia development and elongation. Similarly, Resmi and Nair (2007) highlighted the superior performance of intermediate BA concentrations in promoting robust shoot and leaf development in *Musa* spp.

Overall, 4 mgL^{-1} BA emerged as the optimal concentration for shoot initiation, multiplication, and leaf development in banana cv. Neypoovan from male bud explants. This treatment consistently produced the best outcomes, providing a basis for standardizing BA use in banana micropropagation while

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highlighting the need for cultivar-specific optimization. While BA proved most effective in this study, other cytokinins like kinetin and TDZ have shown efficacy in specific banana cultivars. For example, Smitha *et al.* (2014) found TDZ effective in cv. Matti, while Hrahsel *et al.* (2014) reported kinetin's superiority in cv. Vaibalhla. This suggested that shoot multiplication can vary with the type of cytokinin used, cultivar as well as culture conditions.

3.3 Standardization of rooting media

Rooting and acclimatization are critical stages in the micropropagation of bananas, directly influencing the establishment and survival of tissue-cultured plants in *ex-vitro* environments. Influence of different media on *in vitro* rooting are presented in Table 6.

3.3.1 Days to root initiation

In this study, T_1 (Full MS medium) exhibited the shortest root initiation period (5.25 days), highlighting its efficiency in inducing early root development (Fig 6a). Conversely, the addition of activated charcoal (T_2) delayed root initiation significantly (14 days), potentially due to the adsorption of essential nutrients and hormones like auxins, as noted in prior studies by Yeoman (1991), Mahadev *et al.* (2011) and Rakshi *et al.* (2022).

Resmi and Nair (2007) also observed rapid root induction within 10–15 days in basal MS medium in banana cv. Sannachenkadali. Interestingly, NAA-supplemented treatments showed moderate performance, with T_3 (1 mgL^{-1} NAA) and T_4 (0.5 mgL^{-1} NAA) initiating roots in 7.50 and 8.25 days respectively. The slightly delayed root initiation in the presence of higher NAA concentrations suggests an intricate balance between auxin concentration and root induction as observed by Mante and Tepper (1983) and Helal (2020).

3.3.2 Number of roots

The highest root proliferation was observed in T_4 (0.5 mgL^{-1} NAA), averaging 9.25 roots per plantlet, confirming that lower auxin concentrations are more conducive to root formation. This observation aligns with studies by Nair *et al.* (2018), where 1.0 mgL^{-1} IBA produced the highest rhizogenic activity. However, high auxin levels in T_3 (1 mgL^{-1} NAA) led to fewer roots (5.75 roots), supporting findings by Cronauer and Krikorian (1985), who noted that excess auxin could prioritize root elongation over root number. T_1 (Full MS) produced 4.75 roots per plantlet and was significantly on par with T_3 (1 mgL^{-1} NAA) confirming that higher levels of NAA does not exert much influence on root proliferation. The least number of roots (3.00) were recorded in T_2 (Full MS + charcoal) indicating that charcoal also does not have any influence in rooting of shoots. The varying effects of activated charcoal observed here are also consistent with findings by Mukunthakumar *et al.* (2010), who highlighted its dual role in improving rooting conditions and occasionally inhibiting hormone availability.

3.3.3 Root length (cm)

While more number of roots were observed in T_4 (0.5 mgL^{-1} NAA), root elongation was most pronounced in full MS (T_1) registering an average root length of 10.23 cm. This superior root

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elongation reflects the findings by Hrahsel *et al.* (2014), who reported an average root length of 20.23 cm in *Musa acuminata* using basal MS medium. Conversely, the shorter roots in T_2 highlight the inhibitory effect of activated charcoal on nutrient and hormone availability, consistent with Mahadev *et al.* (2011). The shorter root lengths in NAA treatments *viz.*, T_3 and T_4 indicate that auxin supplementation supports root proliferation but may not always enhance elongation.

The results of this study align closely with existing literature on banana tissue culture. Mahadev *et al.* (2011) and Hrahsel *et al.* (2014) demonstrated the efficacy of basal MS medium for rooting, while Banerjee and de Langhe (1985) and Sultan *et al.* (2011) emphasized the importance of auxins like NAA and IBA for root induction. The present study identifies the treatment T_1 (Full MS medium) as best for quicker root initiation and better root elongation in Neypoovan. The rooted plantlets were subjected to primary hardening and recorded 90 % survival measuring an average plant height of 9.75 cm (Fig 6b).

4. CONCLUSION

- The study evaluated the *in vitro* regeneration of 'Neypoovan' banana using male immature flower buds and floral hands as explants. Among the explants, floral hands proved superior due to their higher culture establishment rate and faster establishment time compared to the flower bud. The treatment combination of 4 mgL^{-1} BA + 1 mgL^{-1} NAA was the most effective for initiating cauliflower-like bodies (CLBs), facilitating subsequent shoot induction and multiplication. During shoot multiplication, 4 mgL^{-1} BA produced the highest number of shoots, longer shoots and better leaf development. For rooting, full MS media significantly enhanced root initiation, producing the longest roots within 5 days. The hardened plantlets exhibited 90% survival rate at 2 weeks after planting. The present study demonstrated that in addition to commonly used vegetative shoot tips as explants, flower buds in the form of floral hands offers an opportunity for mass multiplication of Neypoovan banana especially when suckers are in shortage. Each male bud can provide atleast 9-10 explants, yielding 2-3 meristematic clumps per explant producing an average of 10 shoots per culture which can be mass multiplied through subsequent subculturing. The standardized technology on male bud culture with floral hands as explants is a viable strategy to meet the large-scale demand for planting materials of Neypoovan. This could be further scaled up through developing bioreactor-based systems for cost-effective, high-volume production of plantlets.

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Fig. 1 (a) Banana cultivar Neypoovan (b) Male buds collected at 2-3 weeks after bunch emergence (c) Preparation of explants by removal of bracts

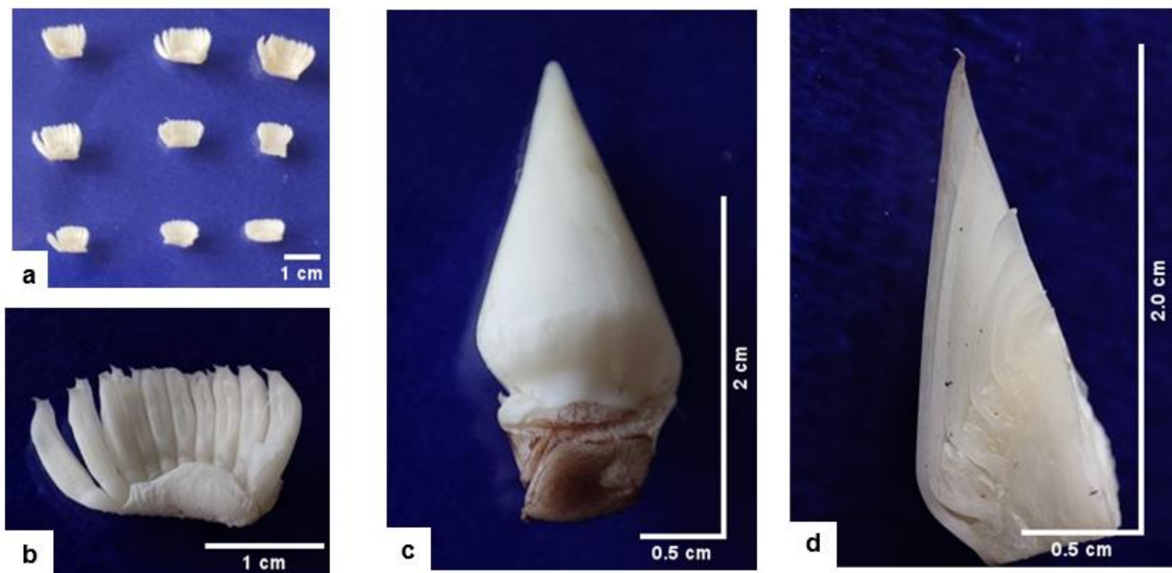

Fig. 2 Preparation of explant (a) floral hands obtained from single flower bud (b) single floral hand (c) whole flower bud (d) whole flower bud longitudinally dissected into four sections

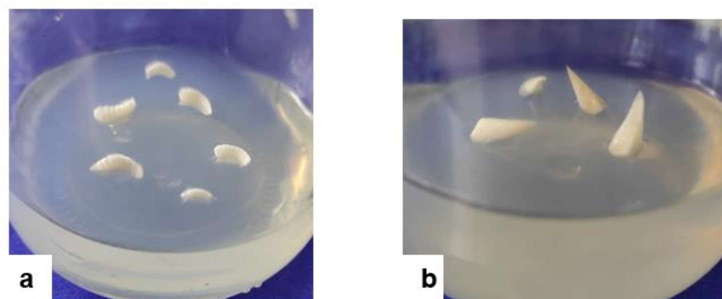

Fig. 3 Inoculation of explant into treatment media (a) floral hands (b) flower bud

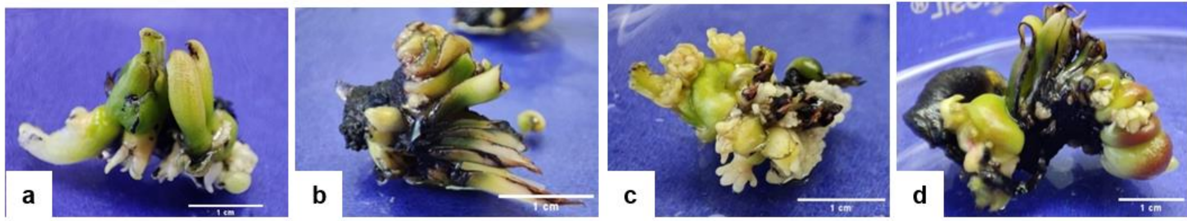

Fig. 4 Response of floral hands and male bud in establishment media (a) Floral hands in 4mgL^{-1} BA + 1mgL^{-1} NAA 25-30 days after inoculation (b) Flower buds in 6mgL^{-1} BA 30-35 days after inoculation (c) Floral hands in 4mgL^{-1} BA + 1mgL^{-1} NAA 55-60 days after inoculation (d) Floral buds in 6mgL^{-1} BA 55-60 days after inoculation

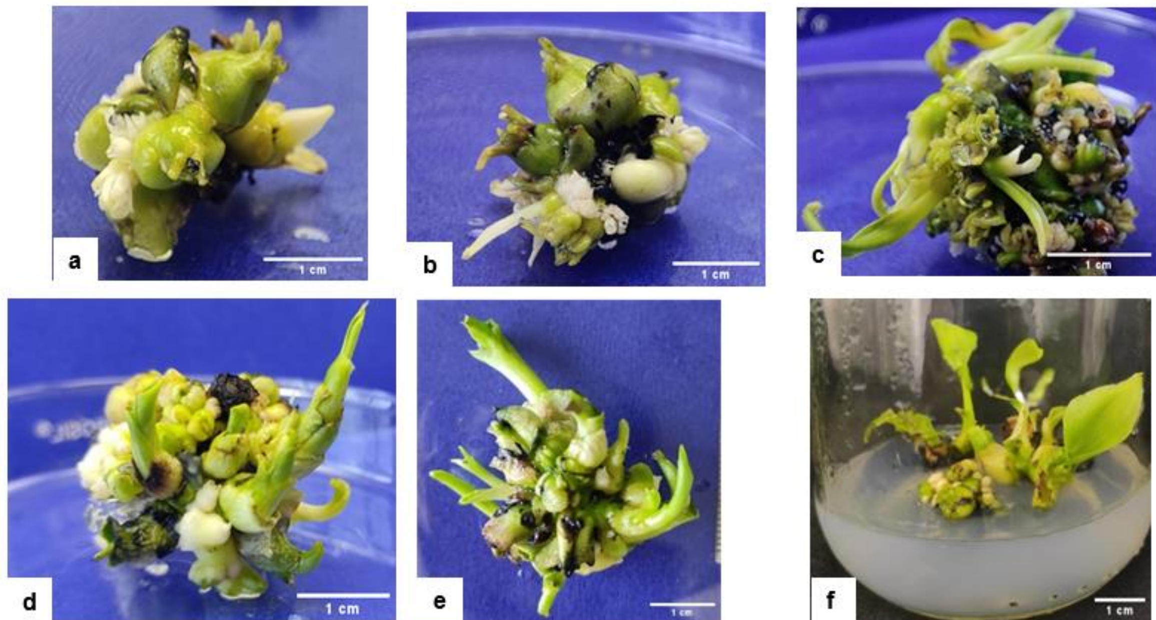

Fig. 5 Response of floral hand cultures in shoot multiplication media (a) Proliferation of white floral meristems in 4 mgl^{-1} BA at 21-25 days (b) at 45-50 days (c) 75- 80 days (d) Shoot initiation in 4 mgl^{-1} BA at 105-110 days (e) Shoot multiplication and elongation in 4 mgl^{-1} BA at 30 days after shoot initiation (f) Formation of leaves at 60 days after shoot initiation.

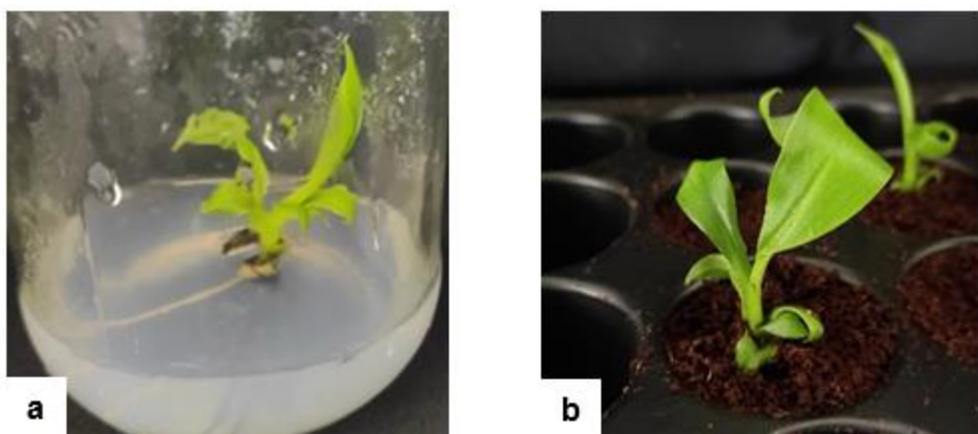

Fig. 6 In vitro rooting and hardening of plantlets(a) *in vitro* rooting in full MS (b) hardening

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Table 1. Treatment combinations for culture establishment

Treatments	
T_0	MS (control)
T_1	MS + 2 mgl^{-1} BA
T_2	MS + 4 mgl^{-1} BA
T_3	MS + 6 mgl^{-1} BA
T_4	MS + 0.2 mgl^{-1} TDZ
T_5	MS + 0.4 mgl^{-1} TDZ
T_6	MS + 0.6 mgl^{-1} TDZ
T_7	MS + 0.5 mgl^{-1} Kinetin
T_8	MS + 1 mgl^{-1} Kinetin
T_9	MS + 2 mgl^{-1} Kinetin
T_{10}	MS + 2 mgl^{-1} BA + 1 mgl^{-1} Kinetin
T_{11}	MS + 4 mgl^{-1} BA + 1 mgl^{-1} Kinetin
T_{12}	MS + 6 mgl^{-1} BA + 1 mgl^{-1} Kinetin
T_{13}	MS + 2 mgl^{-1} BA + 1 mgl^{-1} NAA
T_{14}	MS + 4 mgl^{-1} BA + 1 mgl^{-1} NAA
T_{15}	MS + 6 mgl^{-1} BA + 1 mgl^{-1} NAA

Table 2a. Effect of explant on the percentage of cultures established

Explant	Percentage of cultures established
Flower bud	$53.12 (0.82)^b$
Floral hands	$72.22 (1.04)^a$
SEm±	0.02
CD(0.05)	0.06

*Transformed values are given in brackets

Table 2b. Effect of explant and hormone treatments in percentage of cultures established

Treatments		Percentage of cultures established	
		Floral hands	Flower buds
T_0	MS (control)	50.00(0.80)^{fgh}	33.33(0.61)^{hi}
T_1	MS + 2 mgL^{-1} BA	88.89(1.22)^{abc}	58.33 (0.87)^{efg}
T_2	MS + 4 mgL^{-1} BA	94.44(1.29)^{ab}	66.67(0.96)^{defg}
T_3	MS + 6 mgL^{-1} BA	94.44(1.29)^{ab}	75.00 (1.05)^{cde}
T_4	MS + 0.2 mgL^{-1}TDZ	66.67(0.96)^{defg}	58.33 (0.87)^{efg}
T_5	MS + 0.4 mgL^{-1} TDZ	72.22(1.02)^{cdef}	58.33 (0.87)^{efg}
T_6	MS + 0.6 mgL^{-1} TDZ	66.67(0.95)^{defg}	50.00(0.78)^{gh}
T_7	MS + 0.5 mgL^{-1} Kinetin	27.78(0.55)^{i}	25.00(0.52)^{i}
T_8	MS + 1 mgL^{-1} Kinetin	22.22(0.49)^{i}	25.00(0.52)^{i}
T_9	MS + 2 mgL^{-1} Kinetin	33.33(0.61)^{hi}	25.00(0.52)^{i}
T_{10}	MS + 2 mgL^{-1} BA + 1 mgL^{-1} Kinetin	77.78(1.08)^{bcde}	58.33 (0.87)^{efg}
T_{11}	MS + 4 mgL^{-1} BA + 1 mgL^{-1} Kinetin	77.78(1.08)^{bcde}	58.33 (0.87)^{efg}
T_{12}	MS + 6 mgL^{-1} BA + 1 mgL^{-1} Kinetin	83.33(1.15)^{abcd}	75.00 (1.05)^{cde}
T_{13}	MS + 2 mgL^{-1} BA + 1 mgL^{-1} NAA	100.00(1.36)^{a}	58.33(0.87)^{efg}
T_{14}	MS + 4 mgL^{-1} BA + 1 mgL^{-1} NAA	100.00(1.36)^{a}	58.33(0.87)^{efg}
T_{15}	MS + 6 mgL^{-1} BA + 1 mgL^{-1} NAA	100.00(1.36)^{a}	66.67 (0.96)^{defg}
SE (m)		0.08	
CD (0.05)		0.23	

*Transformed values are given in brackets

Table 3. Effect of explant and hormone treatments in the days for culture establishment

Treatment		Days for culture establishment	
		Floral hands	Flower buds
T_0	MS (control)	34.22^{d}	40.66^{a}
T_1	MS + 2 mgL^{-1} BA	29.12^{h}	33.00^{e}
T_2	MS + 4 mgL^{-1} BA	27.78^{jk}	31.67^{f}
T_3	MS + 6 mgL^{-1} BA	27.99^{ijk}	31.00^{cd}
T_4	MS + 0.2 mgL^{-1} TDZ	29.37^{h}	35.00^{d}
T_5	MS + 0.4 mgL^{-1} TDZ	28.93^{hi}	34.67^{cd}
T_6	MS + 0.6 mgL^{-1} TDZ	29.16^{h}	35.67^{d}
T_7	MS + 0.5 mgL^{-1} Kinetin	31.00^{fg}	38.33^{b}
T_8	MS + 1 mgL^{-1} Kinetin	30.50^{g}	38.33^{b}
T_9	MS + 2 mgL^{-1} Kinetin	30.67^{g}	37.67^{b}
T_{10}	MS + 2 mgL^{-1} BA + 1 mgL^{-1} Kinetin	28.72^{ij}	37.67^{b}
T_{11}	MS + 4 mgL^{-1} BA + 1 mgL^{-1} Kinetin	27.97^{ijk}	34.33^{d}
T_{12}	MS + 6 mgL^{-1} BA + 1 mgL^{-1} Kinetin	28.60^{ij}	31.67^{f}
T_{13}	MS + 2 mgL^{-1} BA + 1 mgL^{-1} NAA	28.44^{ij}	34.67^{d}
T_{14}	MS + 4 mgL^{-1} BA + 1 mgL^{-1} NAA	26.78^{l}	32.67^{e}
T_{15}	MS + 6 mgL^{-1} BA + 1 mgL^{-1} NAA	27.39^{kl}	31.33^{fg}
SEm±		0.35	
CD (0.05)		0.99	

Table 4. Effect of different levels of BA on shoot initiation

Treatment	Days to shoot initiation	Number of shoots per culture	Length of shoots (in cm

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			(at 110 days)	at 110 days)
T_1	MS + 2 mgL^{-1} BA	108.60^a	4.80^b	0.47^c
T_2	MS + 4 mgL^{-1} BA	96.45^b	6.93^a	0.83^a
T_3	MS + 6 mgL^{-1} BA	107.40^a	5.00^b	0.61^b
SEm±		1.34	0.24	0.04
CD(0.05)		4.14	0.75	0.11

Table 5. Effect of T_2 (4 mgL^{-1} BA) on shoot proliferation at 60 days after shoot initiation

Parameter	Observation
Number of shoots per culture	9.73 ± 1.43
Length of shoots (cm)	3.7 ± 0.42
Number of leaves per culture	11.13 ± 1.50
Number of leaves per shoot	3.16 ± 0.44

Table 6. Influence of different media on *in vitro* rooting

Treatments		Days to root initiation	Number of roots	Length of roots (cm)
T_1	Full MS	5.25^c	4.75^b	10.23a
T_2	Full MS + 250 mg charcoal	14.00^a	3.00^c	8.95b
T_3	Full MS + 1mgL^{-1} NAA	7.50^b	5.75^b	3.64d
T_4	Full MS + 0.5 mgL^{-1} NAA	8.25^b	9.25^a	5.49c
SEm±		0.37	0.46	0.41
CD(0.05)		1.13	1.28	1.27

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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