
Micropropagation and genetic fidelity analysis using SCoT marker in *Syzygium cumini* (L.) Skeels

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Abstract This study optimized in vitro regeneration of *Syzygium cumini* (L.) Skeels and validated clonal fidelity using Start Codon Targeted (SCoT) markers. Cytokinins significantly affected the plant height, shoot number and leaf number. The tallest shoots were obtained with 3 mg/L of kinetin (2.96 ± 0.21 cm), while the greatest shoot proliferation and leaf production (9.09 ± 1.02 shoots; 24.25 ± 2.53 leaves per explant) were obtained with 2 mg/L of BAP. Auxins significantly affected all growth characteristics. The maximum shoot height (4.25 ± 0.42 cm) was obtained at 2 mg/L of IAA and the best rooting (3.09 ± 0.21 roots; 3.21 ± 0.51 cm root length) was also observed with this concentration of IAA. NAA (2 mg/L) inhibited morphogenesis and stimulated callus. The genetic fidelity of 20 regenerated plantlets was assessed by 20 SCoT primers producing 162 scorable bands (8.1 bands/primer) in the range of 250–2500 bp. No polymorphism was detected in comparison to the donor plant, confirming true-to-type regeneration. Optimum BAP (2 mg/L) was used for shoot and leaf proliferation, kinetin (3 mg/L) for shoot elongation and IAA (2 mg/L) for root induction and elongation. The SCoT profiles validated the protocol for large scale propagation and conservation of *S. cumini*.

Keywords: *Syzygium cumini*, Micropropagation, Plant growth regulators, SCoT, Genetic fidelity.

Introduction

Syzygium cumini (L.) Skeels, commonly called jamun, black plum, or Indian blackberry, is a tropical evergreen tree belonging to the family Myrtaceae. It is widely distributed in South and Southeast Asia, including India, Sri Lanka, Malaysia and Thailand and has been introduced to several tropical regions of the world. The species has tremendous ecological, nutritional and medicinal importance. Its fruits are rich in anthocyanins, flavonoids and phenolic compounds contributing to its high antioxidant capacity as well as health benefits such as antidiabetic, hepatoprotective, cardioprotective and anticancer activity (Bangar *et al.*, 2025). Moreover, seeds, leaves and bark of *S. cumini* have been

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widely used in traditional systems of medicine particularly Ayurveda for the treatment of diabetes mellitus, gastrointestinal disorders and inflammation. Besides its pharmacological importance, *S. cumini* is an economically important multipurpose tree providing timber, fodder and shade (Kaur *et al.*, 2024).

Its wide utility is impeded by problems in large scale propagation and conservation of *S. cumini*. Traditional propagation techniques such as seed germination and vegetative cuttings are limited by several factors (Gupa *et al.*, 2024). The high recalcitrancy of *S. cumini* seeds leads to rapid loss of viability, limiting long-term storage and germplasm conservation (Jain and Babbar, 2000). Vegetative propagation of the species is possible, but the efficiency is low and the success rate is dependent on the season. Further, natural populations of *S. cumini* are threatened owing to anthropogenic pressures, urbanization, and overexploitation for medicinal purposes, highlighting the need for effective strategies for its multiplication, conservation and sustainable utilization (Rasheed *et al.*, 2024).

Plant tissue culture technology, especially micropropagation, is a reliable means of rapid multiplication of large numbers of uniform plants from elite genotypes. This technique enables the mass production of uniform plantlets within a short period of time regardless of the season. It is also useful in conservation of genetic material of rare, threatened and medicinal plants (Wijerathna-Yapa *et al.*, 2022). Studies on micropropagation of Myrtaceae species are numerous. However, studies in *S. cumini* are still scarce and sometimes contradictory (Meena *et al.*, 2023). It is important to establish a uniform and efficient micropropagation system to provide reliable planting material for both conservation and commercial purposes.

But one of the major concerns with in vitro propagation is somaclonal variation. This change may lead to genetic change at the chromosomal level or at the level of the DNA sequence. Such variation can be useful for generating novel traits but can compromise the genetic fidelity of regenerated plantlets when uniformity is desired. Therefore, it is important to assess the clonal fidelity of micropropagated plants to ensure their true-to-type nature in relation to the donor mother plant (Mehbub *et al.*, 2022; Siringam and Vanijajiva, 2023).

Molecular markers are useful tools to assess the genetic stability of plants propagated through tissue culture. They are more reliable than morphological or biochemical markers, as they are not influenced by the environment. These markers can identify differences at the DNA level. Start Codon Targeted (SCoT) polymorphism has been of interest as a gene targeted marker technique among various marker systems. SCoT markers are reproducible, cheap and highly informative for studying genetic diversity, identification of cultivars, and clonal fidelity assessment based on the short-conserved regions flanking the ATG

translation start codon. Compared to other random markers like RAPD (Random Amplified Polymorphic DNA) (Hromadová *et al.*, 2023) or ISSR (Inter Simple Sequence Repeat) (Igwe *et al.*, 2022), SCoT markers are more reproducible and are directly associated with functional genes, thus providing a reliable approach to monitor the genetic stability (Rai, 2023). In recent years, SCoT markers have been applied successfully in fidelity analysis of tissue culture derived plants in many species (Badhepuri *et al.*, 2024; Sirangi *et al.*, 2024). The studies validate the utility of these in confirming genetic homogeneity among regenerated plants thereby augmenting their credibility for large scale propagation and field deployment (Mustafa, 2024). However, the literature on the application of SCoT markers in *S. cumini* is rare. This needs to be resolved before micropropagated plantlets can be reliably used in conservation and commercial programmes.

The objectives were to establish an efficient micropropagation protocol from elite genotypes of *S. cumini* and to evaluate the genetic fidelity of the in vitro regenerated plantlets using SCoT markers.

Materials and methods

Plant material and preparation of explants

Young and healthy shoots of *S. cumini* were collected from about 10 mature mother plants growing in Chaibadan district, Lopburi province, Thailand (15.1454° N, 101.0693° E). Representative voucher specimens were prepared and deposited at Chaibadanpiphat College, Phranakhon Rajabhat University as a permanent reference collection for future taxonomic and molecular studies.

The shoots collected from the field were washed thoroughly under running tap water to remove surface debris for initiation of tissue culture. Nodal segments (3.0 ± 0.5 cm) were cut and washed in sterile distilled water for 15 minutes. Surface sterilization was done by dipping the explants in 70% (v/v) ethanol for 1 min followed by agitation in 10% (v/v) sodium hypochlorite solution with two drops of Tween-20 at 200 rpm for 10 min. A second sterilization step was performed using 5% (v/v) sodium hypochlorite for 5 min with the same agitation. Then the explants were washed 5 times with sterilized distilled water to remove any sterilizing agents.

The sterilized nodal segments were cut to a final length of 1.5 ± 0.5 cm and placed on Murashige and Skoog (MS) basal medium containing 30 g/L sucrose and solidified with 4.0 g/L gellan gum (Kelcogel®, CP Kelco, USA). The pH of the medium was adjusted to 5.6 ± 0.2 before autoclaving. Cultures were grown at a constant temperature of 25 ± 1 °C, under a 16-h photoperiod of cool-white fluorescent light and an 8-h dark period. Explants were kept under these conditions for 8 weeks to assess their in vitro responses.

In vitro shoot multiplication

The surface-sterilized nodal segments of *S. cumini* were trimmed to a length of 1.5 ± 0.5 cm and cultured on Murashige and Skoog (MS) basal medium supplemented with 30 g/L sucrose and solidified with 4.0 g/L gellan gum (Kelcogel®, CP Kelco, USA). The medium was further supplemented with different concentrations of 6-benzylaminopurine (BAP) or kinetin (Kn) (0.5, 1.0, and 2.0 mg/L), while a hormone-free medium served as the control. The pH was adjusted to 5.6 ± 0.2 before autoclaving. Cultures were maintained at 25 ± 1 °C under a 16-h photoperiod (cool-white fluorescent light) and an 8-h dark cycle for 8 weeks. At the end of the incubation period, morphological parameters including mean shoot length, number of shoots, and number of leaves per explant were recorded (Siringam and Vanijajiva, 2023).

In vitro rooting

The in vitro-derived shoot explants of *S. cumini* were excised to a uniform length of 1.5 ± 0.5 cm and transferred to MS medium supplemented with 30 g/L sucrose and solidified with 4.0 g/L gellan gum (Kelcogel®, CP Kelco, USA). The medium was further amended with different concentrations of α -naphthaleneacetic acid (NAA) or indole-3-butyric acid (IBA) (0.5, 1.0, and 2.0 mg/L), while a hormone-free medium served as the control. The pH of the medium was adjusted to 5.6 ± 0.2 prior to autoclaving. Cultures were incubated at 25 ± 1 °C under a 16-h photoperiod followed by an 8-h dark cycle for 8 weeks. At the end of the culture period, rooting responses were evaluated based on the mean number of roots per explant and root length (Siringam and Vanijajiva, 2023).

Genetic fidelity analysis using SCoT markers

To assess the genetic fidelity of the regenerated *S. cumini* plantlets, genomic DNA was extracted from 500 mg of fresh young leaves obtained from the mother plant and 20 randomly selected acclimatized plantlets using a modified cetyl trimethyl ammonium bromide (CTAB) protocol (Siringam *et al.*, 2024). DNA purity was evaluated using a Nanodrop Spectrophotometer (Thermo Scientific Nanodrop 1000, USA) by measuring the absorbance ratio at 260/280 nm, with values between 1.7 and 1.8 considered optimal. DNA integrity was further verified by electrophoresis on 0.8% agarose gel in $1\times$ TAE buffer, and the samples were stored at -20 °C until used for PCR amplification.

Genetic stability was evaluated using the Start Codon Targeted (SCoT) marker system (Collard and Mackill, 2009). A total of 20 SCoT primers were initially screened, of which all primers produced clear and reproducible amplification patterns (Table 1). PCR amplification was performed in a Thermohybrid Px2 thermocycler (Roche Molecular Systems, Inc., USA). Each 20 µL reaction contained 50 ng of template DNA, 1× PCR buffer (20 mM Tris-HCl, pH 8.4; 50 mM KCl), 2.0 mM MgCl₂, 0.24 mM of each dNTP, 0.5 U of Taq DNA polymerase (Promega, USA), and 0.8 µM of a single primer.

Table 1. SCOT primers used in this study

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
SCoT1	CAACAATGGCTACCACCA	SCoT11	AAGCAATGGCTACCACCA
SCoT2	CAACAATGGCTACCACCC	SCoT12	ACGACATGGCGACCAACG
SCoT3	CAACAATGGCTACCACCG	SCoT13	ACGACATGGCGACCATCG
SCoT4	CAACAATGGCTACCACCT	SCoT14	ACGACATGGCGACCACGC
SCoT5	CAACAATGGCTACCACGA	SCoT15	ACGACATGGCGACCGCGA
SCoT6	CAACAATGGCTACCACGC	SCoT16	ACCATGGCTACCACCGAC
SCoT7	CAACAATGGCTACCACGG	SCoT17	ACCATGGCTACCACCGAG
SCoT8	CAACAATGGCTACCACGT	SCoT18	ACCATGGCTACCACCGCC
SCoT9	CAACAATGGCTACCAGCA	SCoT19	ACCATGGCTACCACCGGC
SCoT10	CAACAATGGCTACCAGCC	SCoT20	ACCATGGCTACCACCGCG

The thermal cycling program consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50–60 °C (depending on the primer) for 1 min, and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min. Amplification reproducibility was verified by repeating each PCR reaction twice.

PCR products were separated on 1.8% (w/v) agarose gel in 1×TAE buffer at 120 V for 90 min. Gels were stained with ethidium bromide (10 mg mL⁻¹) and visualized with a Syngene Bio-Imaging System (GeneGenius, UK). The molecular weight marker used was 100 bp DNA ladder (Promega, USA). Scorable bands were recorded for further analysis and reproducibility was confirmed by repeated amplification.

Statistical analysis

The experiment was conducted using a completely randomized design (CRD) with five replicates per treatment. Data was analyzed by one-way analysis of variance (ANOVA), and mean separations were performed using Duncan's multiple range test (DMRT) at a significance level of $P \leq 0.05$. Statistical analyses were carried out using IBM SPSS Statistics software (IBM Corp., Armonk, NY, USA) (Siringam and Vanijajiva, 2023).

Results

Effects of cytokinins on growth and development of S. cumini

The effects of cytokinin supplementation on the in vitro growth of *S. cumini* were significant ($P \leq 0.05$) for plant height, shoot number, and leaf number (Table 2). Explants cultured on MS medium supplemented with kinetin at 3 mg/L produced the tallest shoots (2.96 ± 0.21 cm). However, values did not differ significantly from those obtained with kinetin at 2 mg/L (2.40 ± 0.23 cm) or BAP at 1 mg/L (2.34 ± 0.13 cm). In contrast, the lowest shoot height (1.13 ± 0.14 cm) was observed on MS medium with 3 mg/L BAP, which was not statistically different from 1 mg/L kinetin (1.54 ± 0.27 cm) (Table 2; Figure 1).

Table 2. Plant Height (cm), Number of Shoots (shoots), and Number of Leaves (leaves) of *S. cumini* Tissue Cultured on MS Medium (1962) Supplemented with BAP and Kinetin at Concentrations of 0, 1, 2, and 3 mg/L for 12 weeks

Treatment	Plant Height (cm)	Number of Shoots (shoots)	Number of Leaves (leaves)
0 mg/L (Control)	1.78 ± 0.29 bc ^{1/}	1.08 ± 0.08 c	4.67 ± 0.49 b
BAP 1 mg/L	2.34 ± 0.13 ab	4.75 ± 0.55 b	22.42 ± 1.33 a
BAP 2 mg/L	1.81 ± 0.10 bc	9.09 ± 1.02 a	24.25 ± 2.53 a
BAP 3 mg/L	1.13 ± 0.14 d	7.25 ± 2.08 ab	18.92 ± 4.12 a
Kinetin 1 mg/L	1.54 ± 0.27 cd	1.00 ± 0.00 c	4.25 ± 0.60 b
Kinetin 2 mg/L	2.40 ± 0.23 ab	1.42 ± 0.21 c	6.92 ± 0.50 b
Kinetin 3 mg/L	2.96 ± 0.21 a	1.75 ± 0.16 c	8.67 ± 0.85 b
F-test	*	*	*
C.V. (%)	20.79	48.23	30.35

^{ns} No significant difference.

* Significant difference at the 0.05 significance level ($p \leq 0.05$).

^{1/} The values are presented as mean $\pm$ standard deviation ($n = 5$) and means followed by the same letter in a column are not significantly different at the 0.05 significance level ($p \leq 0.05$) when compared using Duncan's Multiple Range Test (DMRT).

Shoot proliferation was most pronounced on MS medium with 2 mg/L BAP, producing 9.09 ± 1.02 shoots per explant, though this was not significantly different from 3 mg/L BAP (7.25 ± 2.08 shoots). The fewest shoots were observed with 1 mg/L kinetin (1.00 ± 0.00), comparable to the control (1.08 ± 0.08) and kinetin treatments at 2 and 3 mg/L (1.42 ± 0.21 and 1.75 ± 0.16 , respectively).

Leaf production was highest on MS medium containing 2 mg/L BAP (24.25 ± 2.53 leaves per explant), with no significant difference compared to BAP at 1 mg/L (22.42 ± 1.33) and 3 mg/L (18.92 ± 4.12). The lowest leaf number was obtained with 1 mg/L kinetin (4.25 ± 0.60), which did not differ significantly

from the control (4.67 ± 0.49) or kinetin treatments at 2 and 3 mg/L (6.92 ± 0.50 and 8.67 ± 0.85 , respectively) (Table 2; Figure 1).

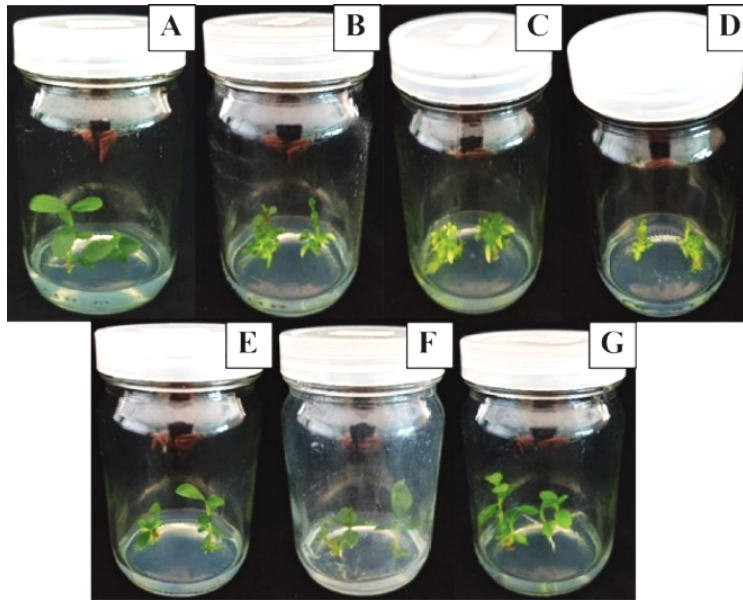


Figure 1. Growth and development of shoot tissue of *S. cumini* cultured on MS medium: (A) Control MS medium, (B) BAP concentration 1 mg/L, (C) BAP concentration 2 mg/L, (D) BAP concentration 3 mg/L, (E) Kinetin concentration 1 mg/L, (F) Kinetin concentration 2 mg/L, (G) Kinetin concentration 3 mg/L, cultured for 12 weeks

Effects of auxin on growth and development of S. cumini

Significant differences ($P \leq 0.05$) were observed in plant height, shoot number, leaf number, root number, and root length among explants cultured with different auxin treatments. The maximum shoot height was obtained on MS medium supplemented with 2.0 mg/L IAA (4.25 ± 0.42 cm), although this did not differ significantly from treatments with 0.5 mg/L IBA (3.87 ± 0.39 cm), 0.5 mg/L IAA (3.33 ± 0.27 cm), and 1.0 mg/L IAA (3.46 ± 0.39 cm). Explants cultured on 2.0 mg/L NAA failed to develop shoots and instead produced callus, while explants with 0.5 and 1.0 mg/L NAA showed stunted growth (0.67 ± 0.36 cm and 0.54 ± 0.36 cm, respectively) (Table 3; Figure 2).

Table 3. Height (cm), Number of Shoots (shoots), and Number of Leaves (leaves) of *S. cumini* Cultured on MS Medium (1962) Supplemented with NAA, IBA, and IAA at Concentrations of 0.0, 0.5, 1.0, and 2.0 mg/L for 12 Weeks

Treatment	Plant Height (cm)	Number of Shoots (shoots)	Number of Leaves (leaves)
0 mg/L (Control)	1.75±0.20 c ^{1/}	1.00±0.00 abc	4.92±0.39 b
NAA 0.5 mg/L	0.67±0.36 de	0.33±0.14 d	1.34±0.77 de
NAA 1.0 mg/L	0.54±0.36 de	0.17±0.10 de	1.34±0.77 de
NAA 2.0 mg/L	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e
IBA 0.5 mg/L	3.87±0.39 ab	0.84±0.10 bc	6.17±0.69 ab
IBA 1.0 mg/L	1.21±0.41 cd	0.33±0.14 d	2.25±0.76 cd
IBA 2.0 mg/L	2.79±0.46 b	0.75±0.08 c	4.17±0.78 bc
IAA 0.5 mg/L	3.33±0.27 ab	1.08±0.08 ab	5.92±0.72 ab
IAA 1.0 mg/L	3.46±0.39 ab	1.17±0.10 a	7.75±0.44 a
IAA 2.0 mg/L	4.25±0.42 a	0.92±0.08 abc	7.42±0.95 a
F-test	*	*	*
C.V. (%)	32.05	27.96	32.91

ns No significant difference.

* Significant difference at the 0.05 significance level ($p \leq 0.05$).

^{1/} The values are presented as mean $\pm$ standard deviation ($n = 5$) and means followed by the same letter in a column are not significantly different at the 0.05 significance level ($p \leq 0.05$) when compared using Duncan's Multiple Range Test (DMRT).

Shoot proliferation was generally low across all auxin treatments. The highest number of shoots was recorded on MS medium supplemented with 1.0 mg/L IAA (1.17 ± 0.10 shoots per explant), which was not significantly different from the control or from 0.5 and 2.0 mg/L IAA treatments (1.00 ± 0.00 , 1.08 ± 0.08 , and 0.92 ± 0.08 , respectively). NAA treatments strongly suppressed shoot formation, with no shoots produced at 2.0 mg/L and only 0.17 ± 0.10 shoots at 1.0 mg/L. (Table 3; Figure 2).

Leaf production was highest in explants cultured with 1.0 mg/L IAA (7.75 ± 0.44 leaves per explant), but not significantly different from 0.5 mg/L IAA (5.92 ± 0.72), 2.0 mg/L IAA (7.42 ± 0.95), and 0.5 mg/L IBA (6.17 ± 0.69). NAA treatments again suppressed organogenesis; explants with 2.0 mg/L NAA failed to form new leaves, while those with 0.5–1.0 mg/L NAA produced only 1.34 ± 0.77 leaves per explant. (Table 3; Figure 2).

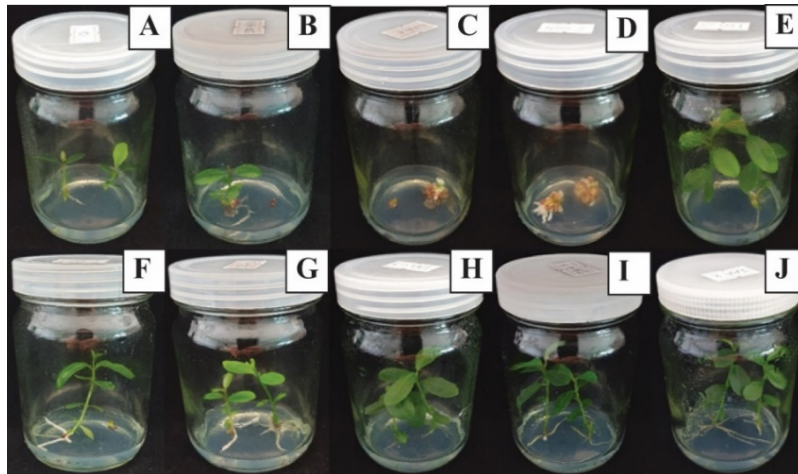


Figure 2. The growth and development of the apical tissue of *S. cumini* cultured on MS control medium (A) NAA concentration 0.5 mg/L (B) NAA concentration 1.0 mg/L (C) NAA concentration 2.0 mg/L (D) IBA concentration 0.5 mg/L (E) IBA concentration 1.0 mg/L (F) IBA concentration 2.0 mg/L (G) IAA concentration 0.5 mg/L (H) IAA concentration 1.0 mg/L (I) IAA concentration 2.0 mg/L (J) after 12 weeks of culture

Root induction and development in response to auxins

Explants of *S. cumini* cultured on MS medium supplemented with 2.0 mg/L IAA produced the highest mean number of roots (3.09 ± 0.21 roots per explant). However, this was not significantly different from explants grown with 2.0 mg/L NAA (1.75 ± 0.80), 0.5 mg/L IBA (2.75 ± 0.50), 2.0 mg/L IBA (2.42 ± 0.48), 0.5 mg/L IAA (2.25 ± 0.25), or 1.0 mg/L IAA (1.92 ± 0.16). Root length was also greatest on medium containing 2.0 mg/L IAA (3.21 ± 0.51 cm), but did not differ significantly from 0.5 mg/L IBA (2.29 ± 0.40 cm). In contrast, explants cultured with 2.0 mg/L NAA developed the shortest roots (0.34 ± 0.15 cm), which were statistically comparable to those of the control (0.88 ± 0.14 cm), as well as 0.5 mg/L NAA (0.79 ± 0.28 cm), 1.0 mg/L NAA (0.79 ± 0.52 cm), and 1.0 mg/L IBA (1.17 ± 0.40 cm) (Table 4; Figure 3).

Table 4. The number of roots (roots) and root length (centimeters) of *S. cumini* tissue cultured on MS (1962) medium supplemented with NAA, IBA, and IAA at concentrations of 0.0, 0.5, 1.0, and 2.0 mg/L for 12 weeks

Treatment	number of roots (roots)	Root length (centimeters)
0 mg/L (Control)	0.58±0.16 c ^{1/}	0.88±0.14 de
NAA 0.5 mg/L	0.67±0.27 c	0.79±0.28 de
NAA 1.0 mg/L	1.25±0.55 bc	0.79±0.52 de
NAA 2.0 mg/L	1.75±0.80 abc	0.34±0.15 e
IBA 0.5 mg/L	2.75±0.50 a	2.29±0.40 ab
IBA 1.0 mg/L	1.17±0.57 bc	1.17±0.40 cde
IBA 2.0 mg/L	2.42±0.48 ab	1.75±0.25 bcd
IAA 0.5 mg/L	2.25±0.25 ab	1.67±0.12 bcd
IAA 1.0 mg/L	1.92±0.16 abc	2.09±0.29 bc
IAA 2.0 mg/L	3.09±0.21 a	3.21±0.51 a
F-test	*	*
C.V. (%)	49.78	45.12

ns No significant difference.

* Significant difference at the 0.05 significance level ($p \leq 0.05$).

^{1/} The values are presented as mean $\pm$ standard deviation ($n = 5$) and means followed by the same letter in a column are not significantly different at the 0.05 significance level ($p \leq 0.05$) when compared using Duncan's Multiple Range Test (DMRT).

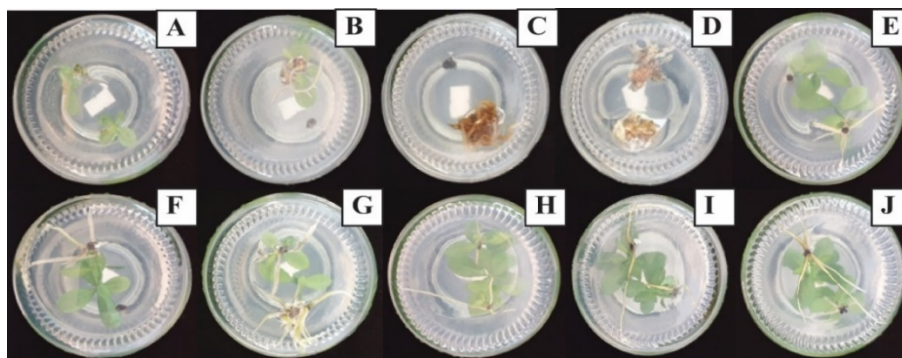


Figure 3. The growth and development of the root tissue of *S. cumini* cultured on MS control medium (A) NAA concentration 0.5 mg/L (B) NAA concentration 1.0 mg/L (C) NAA concentration 2.0 mg/L (D) IBA concentration 0.5 mg/L (E) IBA concentration 1.0 mg/L (F) IBA concentration 2.0 mg/L (G) IAA concentration 0.5 mg/L (H) IAA concentration 1.0 mg/L (I) IAA concentration 2.0 mg/L after (J) 12 weeks of culture

Genetic fidelity analysis using SCoT markers

Micropropagation is an essential tool in plant biotechnology for large-scale propagation of select genotypes. However, its commercial use is constrained by

somaclonal variation among regenerated plantlets (Rohela *et al.*, 2020). Regular monitoring of clonal fidelity ensures that in vitro-derived progeny remain genetically identical to their donor plants. Plantlets derived from organized meristems, which are generally less prone to genomic alterations (Borsai *et al.*, 2020), can have their stability efficiently confirmed by molecular marker analysis.

The genetic fidelity of twenty tissue culture regenerated *S. cumini* plantlets, selected randomly were assessed with the mother plant using Start Codon Targeted (SCoT) markers. Reproducible amplification products were obtained from 20 SCoT primers screened. Amplicon sizes ranged from 250 bp to 2500 bp, representing different genomic regions targeted by primers. A total of 162 different bands were amplified by all primers with an average of 8.1 bands per primer (Table 2). The number of bands per primer varied from five (SCoT6, SCoT18) to twelve (SCoT11, SCoT17). SCoT11 and SCoT17 were the most informative primers producing 12 scorable fragments each, while the least number of bands was obtained with SCoT6 and SCoT18 (Table 5).

Table 5. Types of Primers, Number of DNA Bands, and DNA Sizes

Primer	Number of DNA Bands	DNA Size (bp)
SCoT1	9	400, 450, 600, 650, 750, 800, 1000, 1400, 1500
SCoT2	10	350, 500, 700, 750, 850, 900, 1000, 1300, 1400, 1500
SCoT3	9	400, 450, 600, 650, 750, 850, 1000, 1400, 1500
SCoT4	6	400, 450, 590, 600, 800, 900
SCoT5	6	550, 650, 700, 1000, 1100, 2200
SCoT6	5	500, 600, 670, 800, 1000
SCoT7	9	400, 450, 600, 650, 750, 850, 1000, 1400, 1500
SCoT8	6	510, 680, 790, 900, 1300, 2300
SCoT9	9	400, 510, 680, 750, 800, 950, 1400, 1600, 1900
SCoT10	6	510, 680, 790, 900, 1300, 2300
SCoT11	12	380, 410, 480, 550, 600, 670, 800, 850, 1000, 1200, 1500, 2500
SCoT12	11	280, 310, 380, 420, 510, 580, 650, 790, 900, 1000, 1200, 1500,
SCoT13	10	250, 300, 440, 510, 600, 780, 900, 1000, 1300, 1500
SCoT14	9	400, 510, 680, 750, 800, 950, 1400, 1600, 1900
SCoT15	6	510, 680, 790, 900, 1300, 2300
SCoT16	9	310, 400, 480, 500, 600, 650, 800, 1000, 1300
SCoT17	12	280, 300, 380, 500, 590, 650, 700, 880, 1000, 1200, 1400, 1700
SCoT18	5	510, 600, 700, 1000, 1200
SCoT19	7	500, 590, 800, 1000, 1200, 1700, 2000
SCoT20	6	400, 500, 750, 800, 900, 1500
total	162	
mean	8.1	

The reproducible and clear amplification patterns showed the efficiency of SCoT markers for genetic analysis in *S. cumini*. No polymorphism was observed between the mother plant and the in vitro regenerated plantlets indicating no somaclonal variation and genetic fidelity of the regenerated material. The result

shows the application of the developed micropropagation protocol for the production of true-to-type *S. cumini* plants.

Discussion

The micropropagation response of *S. cumini* was influenced to a great extent by the plant growth regulators. 6-benzylaminopurine (BAP) and kinetin stimulate shoot development among cytokinins. The maximum number of shoots and leaves was obtained using BAP at concentrations of 1 to 3 mg / L and the maximum elongation of shoots using kinetin at 2 to 3 mg / L. These results are consistent with previous studies in other plant species. The overall evidence supports the role of BAP in stimulating axillary bud proliferation and leaf formation while kinetin mainly promotes shoot elongation (Naaz *et al.*, 2019; Siringam and Vanijajiva, 2023).

Auxin supplementation also played a crucial role in morphogenesis. Among the tested auxins, indole-3-acetic acid (IAA) at 0.5–2.0 mg/L was the most effective for plant height, shoot number, leaf number, root induction, and root elongation (Ohbayashi *et al.*, 2022). However, high concentrations of naphthaleneacetic acid (NAA; 2.0 mg/L) inhibited growth and caused callus formation, indicating toxicity at higher concentrations. These results agree with results obtained in several plant species in which IBA and IAA were more effective than NAA in stimulating root development. The better performance of IBA and IAA may be due to their stability and metabolic conversion pathways. In contrast, the accumulation of NAA can cause induction of ethylene and thus growth inhibition (Jain and Babbar, 2003).

Morphogenic outcomes are determined by the balance between cytokinins and auxins, as demonstrated in previous studies (Sosnowski *et al.*, 2023). The optimum concentrations and ratios of these vary with species, explant and culture conditions and this stresses the need for optimization of protocols for each target plant.

One of the major problems in micropropagation is somaclonal variation which may either occur spontaneously during culture or be induced by the composition of the medium, growth regulators or stress conditions. Such variations can be useful in generating diversity but are not desirable for clonal fidelity. To address this concern, the current study employed Start Codon Targeted (SCoT) markers to evaluate the genetic stability of twenty regenerated plantlets of *S. cumini* (Vrundha and Thomas, 2023). Crucially, all 20 primers generated distinct, reproducible amplification profiles with fragments between 250 and 2500 bp. A total of 162 scorable bands were generated across primers (mean 8.1 bands/ primer) with SCoT11 and SCoT17 giving the highest polymorphic information content. Importantly, no polymorphism was observed

between the regenerated plantlets and the mother plant indicating that the present micropropagation protocol produced true-to-type clones (Rathore *et al.*, 2014).

The present results validate the reliability of SCoT markers in evaluating the fidelity of *S. cumini* and corroborate the findings of other species (Badhepuri *et al.*, 2024; Sirangi *et al.*, 2024). SCoT markers are a potent tool to ensure the genetic integrity of micropropagated plants due to their reproducibility, cost-effectiveness and targeting ability of gene-associated regions (Gautam *et al.*, 2025).

Overall, BAP was best for increasing shoot number, kinetin was best for increasing shoot length and IAA was best for supporting both shoots and roots in *S. cumini*. High levels of NAA slowed growth indicating the importance of titrating the auxin levels. The SCoT marker analysis confirmed the genetic fidelity of the in vitro regenerated plantlets and found them to be true to type and the protocol is reliable for large scale propagation and conservation of *S. cumini*. The method provides a reliable means of supporting sustainable use, conservation and potential commercialisation of this important species.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

- Badhepuri, M. K., Beeravelli, P. R., Arolla, R. G., Jogam, P., Rohela, G. K. and Singisala, N. R. (2024). Micropropagation and genetic fidelity analysis using SCoT and ISSR markers in *Muehlenbeckia platyclada* (F. Muell.) Meisn. *Plant Cell, Tissue and Organ Culture*, 157:51.
- Bangar, N. S., Dixit, A., Apte, M. M. and Tupe, R. S. (2025). *Syzygium cumini* (L.) skeels mitigate diabetic nephropathy by regulating Nrf2 pathway and mitochondrial dysfunction: In vitro and in vivo studies. *Journal of Ethnopharmacology*, 336:118684.

- Borsari, B., Villegas-Mirón, P., Laayouni, H., Segarra-Casas, A., Bertranpetit, J., Guigó, R. and Acosta, S. (2020). Intronic enhancers regulate the expression of genes involved in tissue-specific functions and homeostasis. *bioRxiv*, 2020-08.
- Collard, B. C. and Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant molecular biology reporter*, 27:86-93.
- Gautam, N., Faroda, P. and Gupta, A. K. (2025). Encapsulation, short-term storage and SCoT based genetic homogeneity assessment of *Moringa Concanensis* Nimmo.: A multipurpose wild drumstick. *South African Journal of Botany*, 184:995-1000.
- Gupa, M. A., Garba, M., Sa'idu, M., Adamu, A. and Muhammad, A. H. (2024). Seed Germination of *Syzygium cumini* (Java Plum) Under Different Desiccation Period in Borno State, Nigeria. *African Journal of Agricultural Science and Food Research*, 15:9-16.
- Hromadová, Z., Gálová, Z., Mikolášová, L., Balážová, Ž., Vivodík, M. and Chňapek, M. (2023). Efficiency of RAPD and SCoT markers in the genetic diversity assessment of the common bean. *Plants*, 12:2763.
- Igwe, D. O., Iheahuru, O. C., Osano, A. A., Acquah, G. and Ude, G. N. (2022). Assessment of genetic diversity of *Musa* species accessions with variable genomes using ISSR and SCoT markers. *Genetic Resources and Crop Evolution*, 69:49-70.
- Jain, N. and Babbar, S. B. (2000). Recurrent production of plants of black plum, *Syzygium cuminii* (L.) Skeels, a myrtaceous fruit tree, from in vitro cultured seedling explants. *Plant Cell Reports*, 19:519-524.
- Jain, N. and Babbar, S. B. (2003). Regeneration of juvenile plants of black plum, *Syzygium cuminii* Skeels, from nodal exp of mature trees. *Plant Cell, Tissue and Organ Culture*, 73:257-263.
- Kaur, D., Yousuf, B. and Qadri, O. S. (2024). *Syzygium cumini* anthocyanins: recent advances in biological activities, extraction, stability, characterisation and utilisation in food systems. *Food Production, Processing and Nutrition*, 6:34.
- Meena, M. K., Bhatnagar, P., Bharadwaj, M., Shukla, U. and Sharma, Y. K. (2023). Effect of foliar application of potassium silicate on growth attributes of Jamun (*Syzygium cumini* (L.) Skeels.) Cv. Goma Priyanka. *International Journal of Environment and Climate Change*, 13:1559-1569.

- Mehbub, H., Akter, A., Akter, M. A., Mandal, M. S. H., Hoque, M. A., Tuleja, M. and Mehraj, H. (2022). Tissue culture in ornamentals: Cultivation factors, propagation techniques, and its application. *Plants*, 11:3208.
- Mustafa, Z. (2024). Screening Turkish pea (*Pisum sativum*) germplasm with start codon targeted (SCoT) polymorphism marker for evaluation of genetic diversity. *Crop and Pasture Science*, 75. <https://doi.org/10.1071/CP24149>
- Naaz, A., Hussain, S. A., Naz, R., Anis, M. and Alatar, A. A. (2019). Successful plant regeneration system via de novo organogenesis in *Syzygium cumini* (L.) Skeels: an important medicinal tree. *Agroforestry Systems*, 93:1285-1295.
- Ohbayashi, I., Sakamoto, Y., Kuwae, H., Kasahara, H. and Sugiyama, M. (2022). Enhancement of shoot regeneration by treatment with inhibitors of auxin biosynthesis and transport during callus induction in tissue culture of *Arabidopsis thaliana*. *Plant Biotechnology*, 39:43-50.
- Rathore, N. S., Rai, M. K., Phulwaria, M., Rathore, N. and Shekhawat, N. S. (2014). Genetic stability in micropropagated Cleome gynandra revealed by SCoT analysis. *Acta physiologiae plantarum*, 36:555-559.
- Rai, M. K. (2023). Start codon targeted (SCoT) polymorphism marker in plant genome analysis: current status and prospects. *Planta*, 257:34.
- Rasheed, H. U., Shoukat, U., Arshad, U., Afzal, S., Khan, M. S., Aleem, S. ... and Arshad, M. A. (2024). Adaptation and Agricultural Significance of *Syzygium cumini* L. *Saline Environments: A Global Perspective on Jamun Cultivation and Salt Stress Resilience*. Haya: The Saudi Journal of Life Sciences, 9:172-187.
- Rohela, G. K., Jogam, P., Mir, M. Y., Shabnam, A. A., Shukla, P., Abbagani, S. and Kamili, A. N. (2020). Indirect regeneration and genetic fidelity analysis of acclimated plantlets through SCoT and ISSR markers in *Morus alba* L. cv. Chinese white. *Biotechnology Reports*, 25:e00417.
- Sirangi, S., Sandhya, D., Rohela, G. K., Shekhawat, M. S., Ajmeera, R. and Raju, V. S. (2024). Leaf explant based direct and indirect regeneration and SCoT marker assisted genetic fidelity analysis of endemic taxon *Corynandra chelidonii* var. pallae. *Vegetos*, 37:2588-2599.
- Siringam, T. and Vanijajiva, O. (2023). The effect of plant growth regulators on micropropagation of *Melientha suavis* Pierre. and assessment of genetic fidelity of

regenerants based on iPBS and SRAP markers. *Biodiversitas Journal of Biological Diversity*, 24:4628-4634.

Siringam, T., Vanijajiva, O. and Leebonoi, W. (2024). Analysis of genetic polymorphisms in wild dioecious vegetable populations of *Melientha suavis* Pierre (Opiliaceae) using start codon targeted (SCoT) markers. *International Journal of Agricultural Technology*, 20:1575-1590.

Sosnowski, J., Truba, M. and Vasileva, V. (2023). The impact of auxin and cytokinin on the growth and development of selected crops. *Agriculture*, 13:724.

Vrundha, C. K. and Thomas, T. D. (2023). Indirect shoot regeneration from root explants, assessment of clonal fidelity of regenerated plants using SCoT primers and antioxidant analysis in *Thottea siliquosa* (Lamk.) Ding Hou. *Plant Cell, Tissue and Organ Culture*, 155:255-266.

Wijerathna-Yapa, A., Ramtekey, V., Ranawaka, B. and Basnet, B. R. (2022). Applications of in vitro tissue culture technologies in breeding and genetic improvement of wheat. *Plants*, 11:2273.

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