Plant Cell, Tissue and Organ Culture (PCTOC) (2021) 144:397–407
https://doi.org/10.1007/s11240-020-01964-6

ORIGINAL ARTICLE

Highly efficient rapid micropropagation and assessment of genetic fidelity of regenerants by ISSR and SCoT markers of *Solanum khasianum* Clarke

Pavani Chirumamilla[1] · Chaitanya Gopu[1] · Phanikanth Jogam[1] · Shasthree Taduri[1]

Received: 28 March 2020 / Accepted: 27 October 2020 / Published online: 18 November 2020

Abstract

An efficient method of rapid micropropagation of *Solanum khasianum* Clarke was successfully established from the leaf, petiole, and nodal explants. The morphogenetic response of different concentrations of TDZ and BAP individually or in combination with auxins (IAA/IBA/2,4-D) was tested. Friable callus was obtained on different concentrations of BAP alone or in combination with IAA/IBA/2,4-D. Rapid multiple shoot induction was achieved from friable callus on MS medium supplemented with varying concentrations of TDZ and IBA. The leaf explants exhibited a high frequency of multiple shoots than petiole and nodal explants with an optimal percentage of response (92.73%), mean shoot number (53.5 ± 0.47), and shoot length (11.2 ± 0.53 cm) on MS medium augmented with TDZ (1.5 mg l^{-1}) and IBA (1.5 mg l^{-1}). Maximum rooting efficiency was achieved on MS medium with 1.5 mg l^{-1} IBA with 12.8 ± 0.36 mean number of roots. The in vitro rooted plants were acclimatized with a survival rate of 80%. The genetic fidelity of the regenerants assayed by the ISSR and the SCoT markers showed no genetic variation. The study examined the micropropagation responses of *S. khasianum* in the presence of various growth regulators and provided a simple and more suitable protocol adapted for the mass propagation of clones in this species.

Key message

We have established a highly efficient micropropagation system for large scale production in *Solanum khasianum*. Evaluation of clonal fidelity by using ISSR and SCoT markers detected no somaclonal variations. The present study helps to the enhancement of potential alkaloids (solasodine) with the help of biotechnological tools.

Keywords *Solanum khasianum* · Micropropagation · Genetic fidelity · ISSR · SCoT

Abbreviations

MS	Murashige and Skoogs medium
PGRs	Plant growth regulators
TDZ	Thidiazuron
BAP	Benzylaminopurine
IAA	Indole acetic acid
IBA	Indole butyric acid
2,4-D	2,4-Dichlorophenoxy acetic acid
KN	Kinetin
NAA	1- Naphthalene acetic acid
ANOVA	Analysis of variance
ISSR	Inter simple sequence repeats
SCoT	Start codon targeted
SPSS	Statistical package for the social sciences
NMR	Nuclear magnetic resonance
PCR	Polymerase chain reaction
EDTA	Ethylene diamine tetraacetic acid
CTAB	Cetyl trimethyl ammonium bromide
TAE	Tris-acetate-EDTA
DNA	Deoxyribonucleic acid

Communicated by Silvia Moreno.

✉ Shasthree Taduri
shastritaduri@gmail.com; tadurishastri@rediffmail.com

[1] Plant Cell Tissue and Organ Culture Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana State 506009, India

Introduction

Solanum khasianum Clarke (Solanaceae), commonly known as Kotahi Begena (Regional Research Laboratory, Jorhat), is a perennial shrub that grows up to a height of 2-3 m. The species has been reported to occur in Khasi, Jaintia, and Naga hills of Assam and Manipur. It also seems to grow widely from different parts of India like North-East, North-West, Southern, and Central India, and it spreads up to Burma and China (Singh and Kaushal 2007).

The members of Solanaceae have attained great significance as an alternative source of several alkaloids. The plant *S. khasianum* is known for its commercial and medicinal importance. It synthesizes a significant number of alkaloids like solamargine, khasianine, solanine, solasodine and solasonine from various plant parts (Srivastava et al. 2016; Kaunda and Zhang 2019). The important alkaloid solasodine, which has been identified in berries of *S. khasianum*, has proven to contain antiandrogenic, antibacterial and antifungal activity (Kim et al. 1996). The further pharmacological investigation of the berries has shown to possess anticancer (Rosangkima and Jagetia 2015), anthelmintic and anti-inflammatory properties (Jarald et al. 2008; Kaunda and Zhang 2019). Besides these properties, almost every part of the plant is used for treating several ailments like fever, smallpox, filaria (Ghosh et al. 1994), whooping cough, healing cuts and wounds (Chauhan 1999), trachoma, warts, back pain, male potency, snake bites, toothache, skin infections, bronchitis and rheumatism (Schmelzer et al. 2008).

Conventional plant breeding is time-consuming and very hard to achieve mass propagation. Micropropagation is the most promising method of plant tissue culture for the rapid multiplication and conservation of germplasm. Therefore, the correct choice of PGRs at varying concentrations and combinations is the prime requirement of micropropagation methods. In this regard, many plant regeneration protocols have been established in several species of Solanaceae such as *Solanum nigrum* (Padmapriya et al. 2011), *Solanum americanum* (O'Connor-Sanchez et al. 2010), *Lycopersicon esculentum* (Chaudhry et al. 2010), *Solanum melogena* (Ray et al. 2011) and *Solanum villosum* (Iftikhar et al. 2015).

Genetic fidelity screening is important to study the genetic variations in in vitro regenerated plants with the parent plant. The somaclonal variations may occur due to explant type, PGRs, and various subcultures during micropropagation (Lakshmanan et al. 2007). Different kinds of molecular markers such as SCoT and ISSR are available, which can screen total plant genome coding and noncoding sequences and discover the genetic variations (Collard and Mackill, 2009; Thakur et al. 2016). Moreover, genetic homogeneity studies must be analyzed in micropropagated plants, mainly where the industrial application is more. The present investigation has examined the micropropagation responses of *S. khasianum* in the presence of various plant growth regulators and here we are reporting first-time on rapid micropropagation and SCoT, ISSR based genetic fidelity studies in *S. khasianum*. Such a regeneration system would enable future studies on genetic transformation and enhancement of potential alkaloids (Solasodine) in *S. khasianum.*

Materials and methods

Collection of plant material and preparation of explants

The explants were collected from the 1 year old *Solanum khasianum* plants, maintained in the polyhouse, Biotechnology department, Kakatiya University, Warangal and cleansed thoroughly under running tap water for 10 min. The explants are now initially sterilized by treating with 1% bavistin (a fungicide) solution for 5 min, succeeded by rinsing with autoclaved distilled water for 3 times. Subsequent surface sterilization was achieved with 0.1% w/v $HgCl_2$ for 2 min, followed by sterile distilled water wash for 3 times to eliminate traces of chemical sterilant.

Culture media and conditions

The basal Murashige and Skoog's medium (1962) containing sucrose 3% is used for all the experiments in this study. The solidification of the medium was achieved by 0.8% agar before autoclaving. The medium's pH was adjusted to 5.7 by adding 1 N HCl/1 N NaOH and autoclaved at 121 °C for 15 min. The inoculated cultures were maintained at 25 ± 2 °C with 16 h photoperiod under white fluorescent light and 8 h dark.

Preparation of coconut milk

Coconut water was extracted from fresh coconut and then filtered using a 0.2 µM syringe filter. The filtrate was boiled at 50 °C for 2–3 min and filtered again. The resultant solution was stored at 0–4 °C for future use. The concentration of the coconut milk was made to 10–20% using sterile distilled water.

Callus induction

For callus induction, MS medium amended with BAP (0.5- 2.5 mg l^{-1}) alone or in combination with varying

concentrations of IAA/IBA/2,4-D were used to determine the morphogenic response of leaf, petiole, and nodal explants. The data pertaining to the texture and proliferation of callus, mean fresh weight (mg), and dry weight of callus (mg) was recorded after 4 weeks of culture.

Multiple shoot regeneration

For multiple shoot induction, a small piece of friable callus subcultured on MS medium amended with varying concentrations of TDZ alone or in combination with IAA/IBA was used to compare the regeneration capacity of different explants. Statistics concerning the % of regeneration, number of shoots regenerated from callus, and length were recorded after 8 weeks of culture.

In vitro rooting

The individual shoots were dissociated from the cluster of multiple shoots and shifted onto MS medium augmented with varying concentrations of IAA/IBA/NAA for in vitro rooting. At the end of the 4th week, the data regarding root number and root length were collected and recorded.

Hardening and acclimatization

The complete plantlets were taken away gently from the medium and cleaned under running tap water to eliminate the adhered media components and shifted to pots filled with sterile soil and sand in 2:1 proportion. After that, the pots were covered with polythene bags to retain the relative humidity and irrigated every alternate day with half-strength MS solution for 3 weeks. Later plants were acclimatized under greenhouse conditions.

DNA Isolation and genetic fidelity analysis by SCoT, ISSR markers

For genetic fidelity analysis, whole plant genomic DNA was extracted from 500 mg leaf samples of ten randomly selected in vitro propagated acclimatized plants and mother plant of *S. khasianum* by using the CTAB method (Doyle and Doyle 1987). The purity of isolated plant DNA was analyzed by agarose gel electrophoresis (0.8%). For analysis of genetic homogeneity, ten each type of SCoT and ISSR markers were used (Tables 4, 5). The total volume of 50 µl PCR reaction was carried out containing plant DNA (100 ng/µl), 10 p mole primer, and 2X master mix (GCC Biotech, India). The PCR technique was performed in a thermal cycler (Eppendorf, Germany) with the conditions of 95 °C initial denaturation for 5 min and followed by 35 cycles with 95 °C denaturation for 30 s and primer annealing for 45 s at 52 °C for SCoT, 50 °C for ISSR and extension for 2 min at 72 °C with a final extension at 72 °C for 10 min and the temperature was lowered down to 4 °C. The amplified PCR samples were separated by agarose gel electrophoresis (1%) using TAE (1X) buffer with 50 V for 2 h and 30 min. The gel was imaged using a gel documentation system (Bio-Rad, USA). The sizes of the amplified PCR bands were calculated by the 1 kb DNA marker (Thermo Scientific, USA). All the amplifications were repeated thrice with molecular markers (SCoT, ISSR) to check the banding pattern's consistency.

Statistical analysis

The entire experiments were conducted thrice with 10 replicates. The collected data was analyzed statistically by ANOVA (One-way). The significant difference between the sample means was detected by DMRT (Duncan's Multiple Range Test) at 5% level of significance ($p < 0.05$) using SPSS software.

Results and discussion

Callus induction and its proliferation

In the present investigation, the explants cultured on MS medium augmented with various BAP concentrations or combined with auxins like IAA/IBA/2,4-D achieved the only callus and failed to induce multiple shoots. Among all the explants, leaf explants promoted an optimal callus induction with a mean fresh and dry weight (220.1 ± 0.43 mg and 10.9 ± 0.43 mg) on MS medium amended with BAP (1.5 mg l^{-1}) and 2,4-D (1.5 mg l^{-1}) (Fig. 1b). Whereas the petiole explants exhibited a maximum fresh and dry weight (182.3 ± 0.47 mg and 9.2 ± 0.53 mg) on MS medium supplemented with BAP (1.0 mg l^{-1}) and 2,4-D (1.0 mg l^{-1}), followed by nodal explants (175.9 ± 0.37 mg and 8.9 ± 0.48 mg) on BAP (1.5 mg l^{-1}) and 2,4-D (1.5 mg l^{-1}) (Table 1). Similar findings were reported in *Citrullus colocynthis* (Savitha et al. 2010), *Trachyspermum ammi* (Fazeli-Nasab 2018), *Solanum trilobatum* (Pendli et al. 2019), and *Gynura procumbens* (Nurokhman et al. 2019), whereas in *Momordica cymbalaria,* MS medium augmented with 2,4-D alone induced maximum amount of callus (Chaitanya et al. 2020). The explants have shown various texture of callus on different PGR's tested. All the explants cultured on the MS medium supplemented with BAP and BAP + IAA developed green compact callus, whereas white nodular callus was developed on MS medium augmented with BAP + IBA and white friable callus on BAP + 2,4-D.

The other BAP combinations and with IAA/IBA tested showed less response than BAP and 2,4-D combination. MS medium augmented with BAP (1.5 mg l^{-1}) and IBA (1.5 mg l^{-1}) induced callus with a maximal mean fresh and

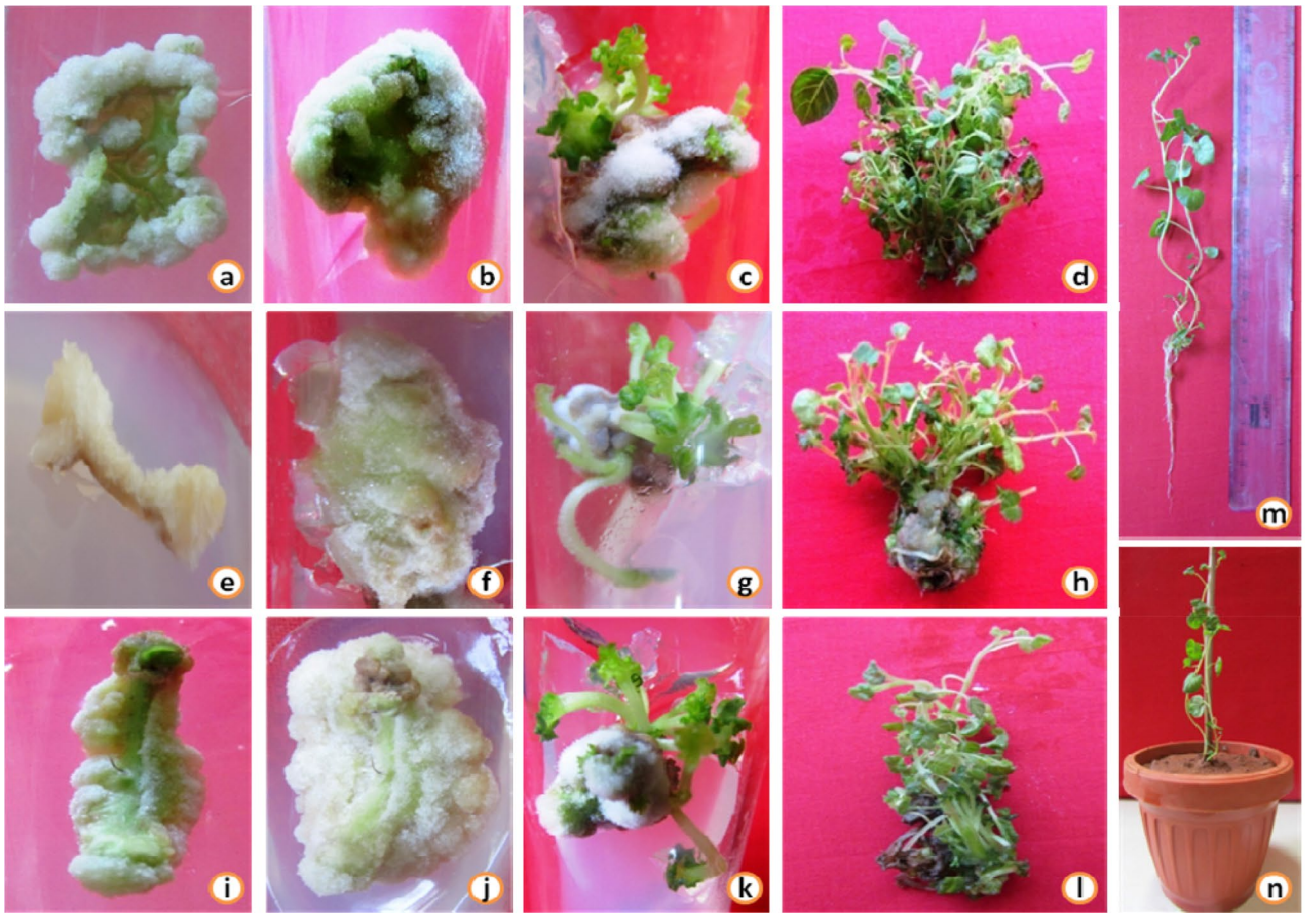

Fig. 1 Callus induction and multiple shoot regeneration with various explants and PGRs in *S. khasianum*. **a, e, i** Initiation of friable callus from leaf, petiole and nodal explants on BAP and auxin fortified medium, **b, f, j** induction of complete white friable callus from leaf, petiole and nodal explants, **c, d** initiation and regeneration of maximum frequency of multiple shoots from leaf explants after 8 weeks of culture on MS medium with TDZ (1.5 mg l^{-1}) and IBA (1.5 mg l^{-1}), **g, h** initiation and regeneration of maximum frequency of multiple shoots from petiole explants after 8 weeks of culture on TDZ (1.5 mg l^{-1}) and IBA (1.5 mg l^{-1}) medium, **k, l** initiation and regeneration of maximum frequency of multiple shoots from nodal explants after 8 weeks of culture on TDZ (1.5 mg l^{-1}) and IBA (1.5 mg l^{-1}) medium, **m** root induction on MS medium supplemented with IBA (1.5 mg l^{-1}) and measurement of shoot and root length, **n** hardening of plantlets

dry weight (219.4 ± 0.61 mg and 10.7 ± 0.42 mg) in petiole explants (Fig. 1f), followed by leaf explants (216.2 ± 0.80 mg and 10.6 ± 0.37 mg) and nodal explants (212.2 ± 0.41 mg and 10.5 ± 0.54 mg) (Fig. 1j; Table 1). But in contrast, BAP and IBA combination induced multiple shoots in *Cicer arietinum* (Sadhu et al. 2020).

On MS medium augmented with BAP (1.0 mg l^{-1}) and IAA (1.0 mg l^{-1}), optimal mean fresh and dry weight of callus (214.1 ± 0.43 mg and 10.5 ± 0.34 mg), (213.6 ± 0.60 mg and 10.5 ± 0.26 mg) and (192.2 ± 0.62 mg and 9.7 ± 0.36 mg) was observed in the petiole, leaf and nodal explants (Table 1). Shah et al. (2015) also reported high callus induction on BAP and IAA in *S. lycopersicum*. On MS medium amended with BAP (1.5 mg l^{-1}) induced an optimum fresh and dry weight of callus (214 ± 0.36 mg and 10.7 ± 0.30 mg) and (194.8 ± 0.80 mg and 9.8 ± 0.51 mg) in leaf and nodal explants, whereas the petiole explants exhibited optimal weight of callus (209.8 ± 0.41 mg and 10.4 ± 0.47 mg) at 1.0 mg l^{-1} BAP (Table 1). The supplementation of coconut milk (10% or 20%) to the MS medium either alone or in combination with BAP resulted in browning of callus or ceased tissue growth. A similar result of decreased growth parameters was observed on media supplemented with coconut milk (Baque et al. 2011; Souza et al. 2013). In contrast, MS medium fortified with BAP and Coconut milk induced green compact callus and later developed shoots in *S. trilobatum* (Alagumanian et al. 2004). The amount of callus produced on MS medium amended with BAP was comparatively lower when compared to BAP in combination with auxins IAA/IBA/2,4-D, which indicates the synergistic effect of BAP and auxins.

Table 1 Effect of BAP alone and in combination with IAA/IBA/2,4-D on callus induction from leaf, petiole, and node explants of *Solanum khasianum* Clarke

Plant growth regulators (in mg l^{-1})		Leaf explant		Petiole explant		Node explant	
		Mean fresh weight of callus (mg)	Mean dry weight of callus (mg)	Mean fresh weight of callus (mg)	Mean dry weight of callus (mg)	Mean fresh weight of callus (mg)	Mean dry weight of callus (mg)
BAP							
0.5		206.8 ± 0.78^{fg}	10.1 ± 0.27^{ab}	199.7 ± 0.68^{h}	10.0 ± 0.49^{abc}	185.9 ± 0.64^{g}	9.4 ± 0.56^{abcde}
1.0		212.5 ± 0.61^{d}	10.5 ± 0.34^{ab}	209.8 ± 0.41^{e}	10.4 ± 0.47^{ab}	188.1 ± 0.45^{f}	9.5 ± 0.34^{abcde}
1.5		214 ± 0.36^{cd}	10.7 ± 0.30^{ab}	207.9 ± 0.62^{f}	10.3 ± 0.44^{ab}	194.8 ± 0.80^{c}	9.8 ± 0.51^{abcd}
2.0		203.8 ± 0.53^{h}	10.0 ± 0.33^{ab}	204.1 ± 0.56^{g}	10.1 ± 0.31^{abc}	183.5 ± 0.50^{hi}	9.3 ± 0.42^{abcde}
2.5		193.6 ± 0.74^{k}	9.5 ± 0.22^{b}	194.6 ± 0.71^{i}	9.7 ± 0.30^{abc}	182.1 ± 0.76^{ij}	9.2 ± 0.32^{abcdef}
BAP	*IAA*						
0.5	0.5	208.3 ± 0.47^{f}	10.2 ± 0.32^{ab}	209.8 ± 0.41^{e}	10.3 ± 0.26^{ab}	189.3 ± 0.42^{ef}	9.5 ± 0.26^{abcde}
1.0	1.0	213.6 ± 0.60^{cd}	10.5 ± 0.26^{ab}	214.1 ± 0.43^{cd}	10.5 ± 0.34^{ab}	192.2 ± 0.62^{d}	9.7 ± 0.36^{abcd}
1.5	1.5	207.7 ± 0.63^{f}	10.1 ± 0.23^{ab}	211.1 ± 0.37^{e}	10.4 ± 0.42^{ab}	190.2 ± 0.59^{e}	9.6 ± 0.47^{abcd}
2.0	2.0	204.6 ± 0.54^{h}	9.9 ± 0.31^{ab}	207.1 ± 0.34^{f}	10.2 ± 0.38^{ab}	184.2 ± 0.84^{h}	9.3 ± 0.53^{abcde}
2.5	2.5	200.4 ± 0.76^{i}	9.6 ± 0.33^{b}	203.2 ± 0.44^{g}	10.1 ± 0.45^{abc}	181.0 ± 0.71^{j}	9.2 ± 0.38^{abcdef}
BAP	*IBA*						
0.5	0.5	210.6 ± 0.80^{e}	10.3 ± 0.30^{ab}	215.4 ± 0.65^{c}	10.5 ± 0.40^{ab}	206.9 ± 0.52^{b}	10.2 ± 0.55^{abc}
1.0	1.0	212.6 ± 0.73^{d}	10.5 ± 0.54^{ab}	217.2 ± 0.69^{b}	10.6 ± 0.42^{ab}	211.0 ± 0.64^{a}	10.4 ± 0.37^{ab}
1.5	1.5	216.2 ± 0.80^{b}	10.6 ± 0.37^{ab}	219.4 ± 0.61^{a}	10.7 ± 0.42^{a}	212.2 ± 0.41^{a}	10.5 ± 0.54^{a}
2.0	2.0	205 ± 0.73^{gh}	10.0 ± 0.39^{ab}	212.9 ± 0.10^{d}	10.4 ± 0.37^{ab}	210.7 ± 0.42^{a}	10.3 ± 0.42^{abc}
2.5	2.5	197.5 ± 0.80^{j}	9.6 ± 0.42^{b}	199.7 ± 0.42^{h}	10.0 ± 0.42^{abc}	205.5 ± 0.50^{b}	10.1 ± 0.52^{abc}
BAP	*2,4-D*						
0.5	0.5	215.2 ± 0.53^{bc}	10.5 ± 0.50^{ab}	176.4 ± 0.26^{k}	8.8 ± 0.38^{cd}	169.3 ± 0.57^{m}	8.5 ± 0.50^{def}
1.0	1.0	216.6 ± 0.45^{b}	10.6 ± 0.45^{ab}	182.3 ± 0.47^{j}	9.2 ± 0.53^{bcd}	173.5 ± 0.37^{l}	8.8 ± 0.57^{cdef}
1.5	1.5	220.1 ± 0.43^{a}	10.9 ± 0.43^{a}	166.3 ± 0.57^{l}	8.3 ± 0.47^{de}	175.9 ± 0.37^{k}	8.9 ± 0.48^{bcdef}
2.0	2.0	214.8 ± 0.57^{bc}	10.5 ± 0.47^{ab}	146.6 ± 0.70^{m}	7.4 ± 0.49^{e}	161.4 ± 0.52^{n}	8.0 ± 0.36^{ef}
2.5	2.5	200.1 ± 0.82^{i}	10.0 ± 0.25^{ab}	139.9 ± 0.60^{n}	7.1 ± 0.40^{e}	157.3 ± 0.57^{o}	7.8 ± 0.32^{f}

Each experiment was conducted thrice with 10 replicates and the data represent the mean ± SE. Means followed by the same letter are not significantly different (p = 0.05) as per Duncan's Multiple Range Test

Effect of PGR's on multiple shoot induction

The friable callus obtained on MS medium fortified with BAP (0.5- 2.5 mg l^{-1}) and auxins (0.5- 2.5 mg l^{-1}) was excised into small pieces and subcultured onto MS medium augmented with varying concentrations of TDZ alone or in combination with IAA/IBA showed a synergistic effect and successfully induced multiple shoots within 3 weeks (Fig. 1c, 1g, and 1k). Among the explants tested, leaf explants were predominant over petiole and nodal explants for multiple shoot induction. Among the different concentrations and combinations of TDZ tested, TDZ (1.5 mg l^{-1}) with IBA (1.5 mg l^{-1}) was considered the most efficient for multiple shoot induction in all the explants.

The leaf explants cultured on MS medium amended with TDZ (1.5 mg l^{-1}) and IBA (1.5 mg l^{-1}) showed the optimal percentage of response (92.73%) with maximal mean shoot number (53.5 ± 0.47) and shoot length (11.2 ± 0.53 cm) after 8 weeks of culture (Fig. 1d, 1m; Fig. 2; Table 2). Followed by petiole and nodal explants with an optimal percentage of response (85.76% and 78.50%) with mean shoot number (20.1 ± 0.49 and 15.1 ± 0.43) and shoot length (8.6 ± 0.26 cm and 8.4 ± 0.47 cm) on the same media (Fig. 1h,1l; Table 2). By raising or lowering the optimal concentration of TDZ alone or combined with IAA/IBA, showed the reduction of multiple shoot induction in all the explants. The combination of TDZ and IBA was also found to be effective in different species like *Ficus carica* (Kim et al. 2007), *Lythrum salicaria* (Turker et al. 2009), *Alocasia indica* (Rong et al. 2011), *Lycopersicon esculentum* (Vinoth et al. 2012), and *Malus niedzwetzkyana* (Jin et al. 2014; Kazemi et al. 2019).

The explants cultured on MS medium amended with different concentrations of TDZ and IAA achieved lower response than explants cultured on TDZ and IBA. Among the various concentrations of TDZ and IAA tested, TDZ (1.0 mg l^{-1}) and IAA (1.0 mg l^{-1}) were considered to be effective in leaf explants for induction of multiple

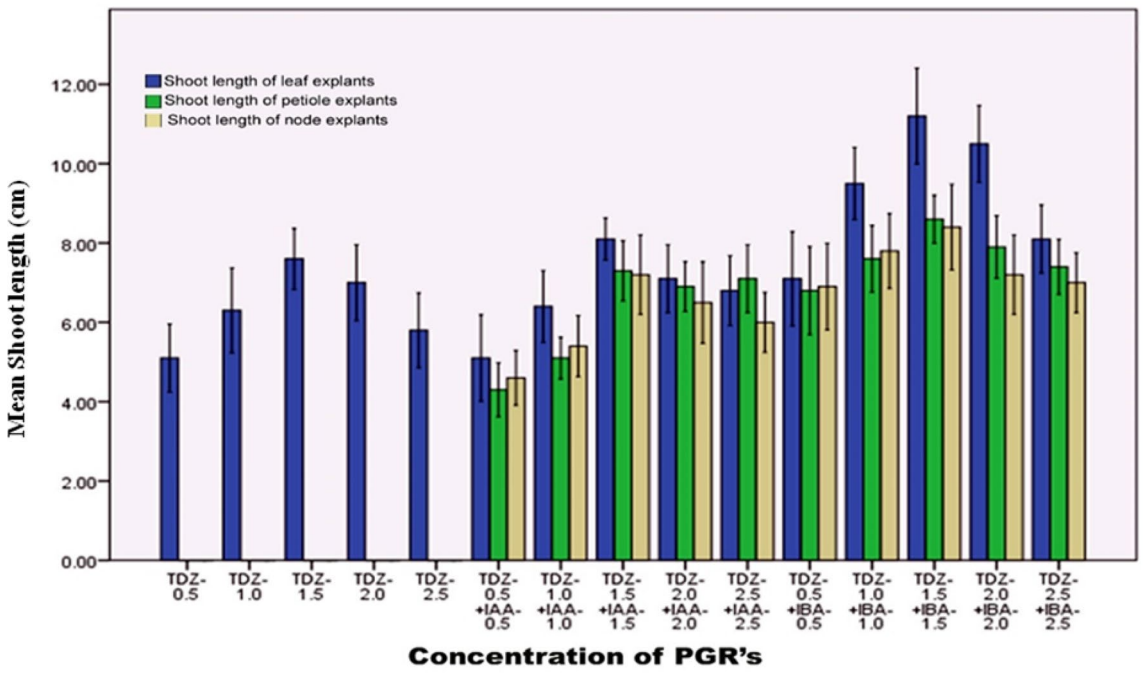

Fig. 2 Effect of TDZ alone and with IAA and IBA on shoot length of multiple shoots in leaf, petiole and nodal explants

Table 2 Effect of TDZ alone and in combination with IAA/IBA on multiple shoot induction from callus derived from leaf, petiole, and nodal explants of *Solanum khasianum* Clarke

Plant growth regulators (in mg l^{-1})		Leaf		Petiole		Node	
		% of Response	Mean no. of shoots/explant	% of Response	Mean no. of shoots/explant	% of Response	Mean no. of shoots/explant
TDZ							
0.5		65.36 ± 0.10^{j}	9.2 ± 0.58^{k}	0.0 ± 0.00^{i}	0.0 ± 0.00^{h}	0.0 ± 0.00^{f}	0.0 ± 0.00^{g}
1.0		69.46 ± 0.11^{i}	14.4 ± 0.84^{i}	0.0 ± 0.00^{i}	0.0 ± 0.00^{h}	0.0 ± 0.00^{f}	0.0 ± 0.00^{g}
1.5		73.33 ± 0.05^{ef}	23.2 ± 0.61^{e}	0.0 ± 0.00^{i}	0.0 ± 0.00^{h}	0.0 ± 0.00^{f}	0.0 ± 0.00^{g}
2.0		72.46 ± 0.14^{gh}	19.4 ± 0.81^{g}	0.0 ± 0.00^{i}	0.0 ± 0.00^{h}	0.0 ± 0.00^{f}	0.0 ± 0.00^{g}
2.5		61.06 ± 0.29	15.6 ± 0.69^{hi}	0.0 ± 0.00^{i}	0.0 ± 0.00^{h}	0.0 ± 0.00^{f}	0.0 ± 0.00^{g}
TDZ	*IAA*						
0.5	0.5	69.50 ± 0.14^{hi}	11.6 ± 0.77^{j}	64.33 ± 0.10^{g}	7.60 ± 0.40^{f}	62.33 ± 0.07^{e}	6.6 ± 0.37^{f}
1.0	1.0	87.43 ± 0.14^{b}	33.8 ± 0.56^{b}	73.66 ± 0.13^{de}	12.3 ± 0.37^{d}	69.23 ± 0.07^{cd}	9.3 ± 0.69^{e}
1.5	1.5	83.40 ± 0.13^{c}	25.9 ± 0.83^{d}	78.50 ± 0.12^{bc}	15.2 ± 0.56^{b}	74.96 ± 0.14^{ab}	13.1 ± 0.57^{b}
2.0	2.0	78.46 ± 0.14^{d}	21.8 ± 0.76^{ef}	71.33 ± 0.07^{f}	10.9 ± 0.53^{e}	70.86 ± 0.20^{bc}	10.5 ± 0.49^{d}
2.5	2.5	76.46 ± 0.14^{de}	17.0 ± 0.51^{h}	60.50 ± 0.10^{h}	5.9 ± 0.35^{g}	64.16 ± 0.26^{de}	7.0 ± 0.40^{f}
TDZ	*IBA*						
0.5	0.5	72.33 ± 0.13^{gh}	19.3 ± 0.91^{g}	70.66 ± 0.14^{f}	10.4 ± 0.37^{e}	70.63 ± 0.09^{cd}	10.1 ± 0.22^{de}
1.0	1.0	89.50 ± 0.11^{a}	31.1 ± 0.80^{c}	80.83 ± 0.05^{b}	16.2 ± 0.56^{b}	72.50 ± 0.09^{ab}	11.8 ± 0.66^{c}
1.5	1.5	92.73 ± 0.10^{a}	53.5 ± 0.47^{a}	85.76 ± 0.07^{a}	20.1 ± 0.49^{a}	78.50 ± 0.10^{a}	15.1 ± 0.43^{a}
2.0	2.0	86.00 ± 0.23^{bc}	26.9 ± 0.41^{d}	75.43 ± 0.21^{cd}	14.0 ± 0.88^{c}	71.50 ± 0.12^{bc}	11.0 ± 0.44^{cd}
2.5	2.5	81.16 ± 0.28^{c}	20.7 ± 0.37^{fg}	64.53 ± 0.11^{g}	7.3 ± 0.53^{f}	62.20 ± 0.03^{e}	6.7 ± 0.54^{f}

Each experiment was conducted thrice with 10 replicates and the data represent the mean ± SE. Means followed by the same letter are not significantly different (p = 0.05) as per Duncan's Multiple Range Test

shoots, whereas, in petiole and nodal explants, TDZ (1.5 mg l^{-1}) and IAA (1.5 mg l^{-1}) showed to the best combination. Therefore the optimal percentage of response (87.43 ± 0.14, 78.50 ± 0.12, and 74.96 ± 0.14) and mean shoot number (33.8 ± 0.56, 15.2 ± 0.56 and 13.1 ± 0.57) was obtained in leaf, petiole, and nodal explants on TDZ and IAA (Table 2). The similar result were reported in *Capsicum annuum (*Kasula et al. 2008), *Woodfordia fruticosa* (Bulle et al. 2012), and *Citrullus colocynthis* (Dasari et al. 2015), where TDZ + IAA showed maximum regeneration of multiple shoots.

The MS medium augmented with TDZ alone failed to regenerate multiple shoots in petiole and node explants, whereas only leaf explants developed multiple shoots. The highest percentage of response (73.33 ± 0.05) and number of multiple shoots (23.2 ± 0.61) was achieved on TDZ (1.5 mg l^{-1}) (Table 2). TDZ alone was also found to be effective in *Solanum aethiopicum* and *Solanum macrocarpon* (Gisbert et al. 2006), *Solanum melongena* (Mallaya et al. 2013), *Vigna subterranea* (Silue et al. 2016), *Bienertia sinuspersici* (Northmore et al. 2016), *Solanum nigrum* (Afrasiab et al. 2017) and *Solanum erianthum* (Sarkar and Banerjee 2020).

Thidiazuron, substituted phenyl urea, is a potent cytokinin for the regeneration of multiple shoots in several plants (Sajid and Aftab 2009; Gharari et al. 2019; Matand et al. 2020; Savitikadi et al. 2020). TDZ is also showed to have better efficiency than other cytokinins like BA or Kinetin, even at low concentrations (Bhattacharyya et al. 2016).

Rooting and acclimatization

The in vitro developed shoots were rooted efficiently on the MS medium amended with auxins like IAA, IBA, and NAA (0.5 mg l^{-1} to 2.5 mg l^{-1}). The rooting efficiency varied significantly among the three auxins tested. The cultured shoots induced root formation after 2 weeks of culture. Among all the auxins tested, IBA was superior to IAA and NAA. The optimum number of roots (12.8 ± 0.36) per shoot was observed on MS medium amended with IBA (1.5 mg l^{-1}), subsequently followed by NAA (1.0 mg l^{-1}) with 7.9 ± 0.52 mean number of roots, whereas negligible rooting response (1.9 ± 0.32) was achieved on MS medium fortified with IAA (1.0 mg l^{-1}) (Table 3). Kannan et al. (2006) also reported similar results in *Solanum nigrum* with 0.5 mg/L IBA. IBA also induced in vitro rooting in different plants (Hussain et al. 2018; Vemula et al. 2019; Jogam et al. 2020). The high frequency of in vitro rooting with IBA is due to its fine structure, stability, and easy translocation to tissues (Hussain et al. 2018). The well-rooted complete plantlets were acclimatized with a high survival rate of 80% (Fig. 1n).

Table 3 Effect of various auxins on the induction of rooting in *S. khasianum* Clarke

Plant growth regulators (in mg l^{-1})			Mean No. of Roots/shootlet + SE	Mean Root Length (cm) + SE
NAA	*IBA*	*IAA*		
0.5			5.3 ± 0.64^{f}	7.4 ± 0.25^{bc}
1.0			7.9 ± 0.52^{d}	8.1 ± 0.29^{ab}
1.5			4.9 ± 0.41^{f}	8.7 ± 0.28^{a}
2.0			3.7 ± 0.40^{g}	7.8 ± 0.39^{ab}
2.5			3.5 ± 0.31^{g}	6.3 ± 0.31^{de}
	0.5		8.9 ± 0.35^{cd}	6.2 ± 0.31^{de}
	1.0		11.2 ± 0.39^{b}	6.5 ± 0.32^{cd}
	1.5		12.8 ± 0.36^{a}	5.9 ± 0.29^{def}
	2.0		9.2 ± 0.44^{c}	5.7 ± 0.34^{defg}
	2.5		6.6 ± 0.51^{e}	5.4 ± 0.29^{efg}
		0.5	1.3 ± 0.24^{hi}	4.7 ± 0.34^{gh}
		1.0	1.9 ± 0.32^{h}	5.1 ± 0.35^{fg}
		1.5	1.2 ± 0.29^{hi}	3.9 ± 0.26^{hi}
		2.0	1.0 ± 0.24^{hi}	3.7 ± 0.40^{i}
		2.5	0.6 ± 0.15^{i}	3.2 ± 0.23^{i}

Means followed by the same letter are not significantly different (p = 0.05) using Duncan's Multiple Range Test

SCoT and ISSR molecular markers based on genetic fidelity studies

Genetic variations are common in in vitro raised plantlets. So, molecular marker-based PCR studies are preferable to study the variations among micropropagated plants with the mother plant. In the present study, SCoT and ISSR molecular markers are used to check the somaclonal variations. The ten SCoT primers amplified 50 scorable amplicons with the approximate range from 400 to 3000 bp size (Table 4; Fig. 3a). The average of 5 PCR bands per one SCoT primer was amplified. The ten ISSR primers amplified 40 scorable amplicons with an approximate range of 300 to 3100 bp size (Table 5; Fig. 3b). The average of 4 PCR bands per one ISSR primer was amplified. All the molecular markers amplified PCR products were found to be monomorphic.

SCoT and ISSR are most advanced and have high nucleotide length compared with RAPD markers (Goyal et al. 2015). These markers have a high annealing temperature to result in better reproducibility without nonspecific amplification (Bornet and Branchard 2011). Screening of genetic homogeneity among micropropagated plants by two molecular markers is always preferable to reconfirm the genetic variations (Rohela et al. 2019). ISSR and SCoT markers are constant, low cost, reproducible, examining plants' total genome randomly (Rohela et al. 2020). Similar Genetic fidelity studies of in vitro regenerants using SCoT molecular marker was reported in *Pisum sativum* (Ajithan et al. 2019),

Table 4 List of SCoT primers used and their response on the assessment of genetic fidelity studies in *S. khasianum* Clarke

S. No	Primer code	Primer sequence (5′–3′)	Number of bands amplified	Length of PCR bands (bp)
1	S1	CAACAATGGCTACCACCC	8	500–3000
2	S2	GCAACAATGGCTACCACC	4	1000–2500
3	S3	CAACAATGGCTACCACCT	4	1000–2000
4	S4	ACCATGGCTACCACCGCC	3	1100–1900
5	S5	CAACAATGGCTACCACCA	7	600–3000
6	S6	CAACAATGGCTACCACGA	3	500–2000
7	S7	ACGACATGGCGACCAACG	6	400–2500
8	S8	ACCATGGCTACCACCGAC	3	600–1400
9	S9	AAGCAATGGCTACCACCA	7	600–2500
10	S10	CATGGCTACCACCGGCCC	5	500–3000

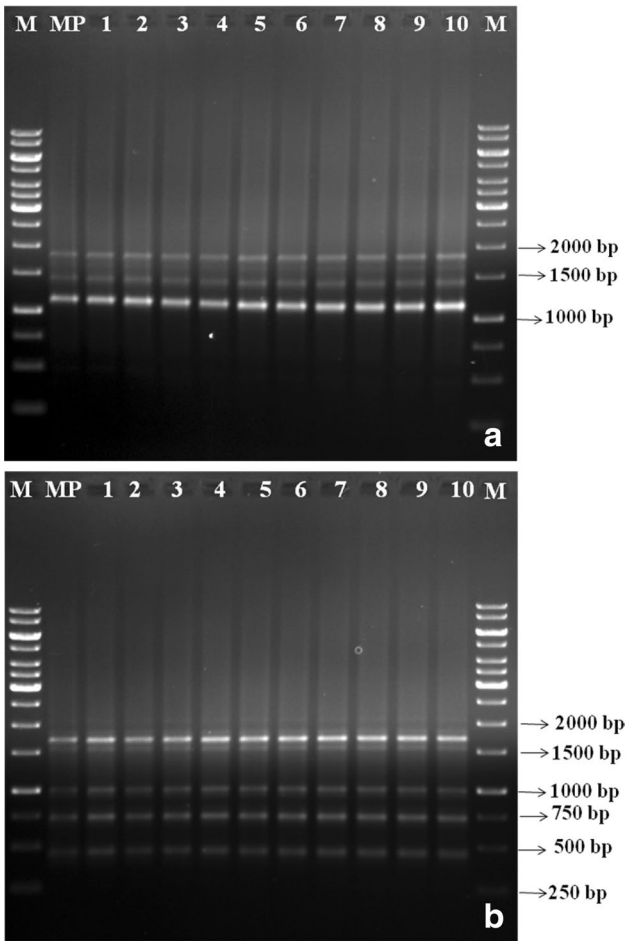

Fig. 3 **a** SCoT markers based genetic fidelity analysis of *S. khasianum* amplified with S4 primer. Lane M: 1 kb marker, Lane MP: PCR amplification of mother plant DNA, Lane 1–10: PCR amplification of in vitro regenerated plants DNA. **b** ISSR markers based genetic fidelity analysis of *S. khasianum* amplified with I8 primers. Lane M: 1 kb marker, Lane MP: PCR amplification of mother plant DNA, Lane 1–10: PCR amplification of in vitro regenerated plants DNA

Helicteres isora (Muthukumar et al. 2020), and *Cicer arietinum* (Sadhu et al. 2020) and ISSR molecular marker-based genetic fidelity among micropropagated plants were reported in *Eleusine coracana* (Babu et al. 2018), *Morus spp* (Rohela et al. 2018), and *Flemingia macrophylla* (Sirikonda et al. 2020).

Conclusion

The micropropagation technique is the best-employed method for the regeneration of *S. khasianum*. Among all the three explants (leaf, petiole, and node) used, leaf explants showed maximum multiple shoot induction (53.5 ± 0.47) on TDZ (1.5 mg l^{-1}) with IBA (1.5 mg l^{-1}) from friable callus. The variations in the regeneration frequencies are due to the type of explants and their physiological conditions. The regenerated plantlets showed no somaclonal variations when assessed by ISSR and SCoT markers. In the present investigation, we have developed an efficient micropropagation method for mass propagation in *S. khasianum* plants without any genetic variability with biotechnological tools.

Table 5 List of ISSR molecular markers used and their response on the assessment of genetic fidelity studies in *S. khasianum* Clarke

S. No	Primer code	Primer Sequence (5′–3′)	Number of bands amplified	Length of PCR bands (bp)
1	I1	GAGAGAGAGAGAGAGAYG	3	500–1000
2	I2	AGAGAGAGAGAGAGAGG	6	300–1300
3	I3	CACACACACACACACARC	3	500–1500
4	I4	GAGAGAGAGAGAGAGAC	4	500–1100
5	I5	ACACACACACACACACT	5	500–1500
6	I6	CTCCTCCTCCTCCTCCTC	5	1200–3100
7	I7	AGAGAGAGAGAGAGAGTC	3	650–1100
8	I8	GAGAGAGAGAGAGAGAYT	5	450–1750
9	I9	TGTGTGTGTGTGTGTGRA	2	1500–2000
10	I10	AGAGAGAGAGAGAGAGYT	4	500–1400

Acknowledgement The authors duly acknowledge the Department of Biotechnology, Kakatiya University, Warangal for providing all the facilities for carrying out the research. We also thank Prof. N. Ramaswamy (Rtd), Department of Biotechnology and Dr. P. Venkataiah, Department of Microbiology, for their valuable suggestions.

Author contributions PC conducted the experimental work, analyzed the data and designed the manuscript. CG helped in experimental work, designing tables and plates. PJ did genetic fidelity studies. ST extended overall guidance and finalized the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest in this study.

References

Afrasiab H, Rashid N, Akram M (2017) An efficient method for direct shoot regeneration from leaf explants of *Solanum nigrum* induced by Thidiazuron. Int J Agric Biol 19(2):348–354

Ajithan C, Vasudevan V, Sathish D, Sathish S, Krishnan V, Manickavasagam M (2019) The influential role of polyamines on the in vitro regeneration of pea (*Pisum sativum* L.) and genetic fidelity assessment by SCoT and RAPD markers. Plant Cell Tissue Organ Cult 139(3):547–561

Alagumanian S, Perumal VS, Balachandar R, Rameshkannan K, Rao MV (2004) Plant regeneration from leaf and stem explants of *Solanum trilobatum* L. Curr Sci 86(11):1478–1480

Babu GA, Vinoth A, Ravindhran R (2018) Direct shoot regeneration and genetic fidelity analysis in finger millet using ISSR markers Plant Cell. Tissue and Organ Cult 132(1):157–164

Baque MA, Shin YK, Lee EJ, Paek KY (2011) Effect of light quality, sucrose and coconut water concentration on the microporpagation of Calanthe hybrids (''Bukduseong''' Hyesung' and' Chunkwang'' Hyesung'). Aust J Crop Sci 5(10):1247

Bhattacharyya P, Kumaria S, Job N, Tandon P (2016) En-masse production of elite clones of *Dendrobium crepidatum*: a threatened, medicinal orchid used in Traditional Chinese Medicine (TCM). J Appl Res Med Aromat Plants 3:168–176

Bornet B, Branchard M (2011) Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol Biol Rep 19(3):209–215

Bulle M, Kota S, Rathakatla D, Aileni M, Kokkirala VR, Gadidasu KK, Abbagani S (2012) An efficient in vitro leaf-based regeneration and evaluation of genetic fidelity using ISSR markers in *Woodfordia fruticosa* (L.) Kurz. J Herbs Spices Med Plants 18(2):178–90

Chaitanya G, Suvarchala V, Ramakrishna D, Sunitha D, Shasthree T (2020) In vitro plant regeneration of *Momordica cymbalaria* Fenzl. and assessment of genetic fidelity using ISSR primers. Res J Biotechnol 15(2):103–110

Chauhan NS (1999) Medicinal and aromatic plants of Himachal Pradesh. Indus publishing company, New Delhi, pp 381–383

Chaudhry Z, Abbas S, Yasmin A, Rashid H, Ahmed H, Anjum MA (2010) Tissue culture studies in tomato (*Lycopersicon esculentum*) var. Moneymaker Pak J Bot 42(1):155–163

Collard BCY, Mackill DJ (2009) Conserved DNA-derived polymorphism (CDDP): a simple and novel method for generating DNA markers in plants. Plant Mol Biol Rep 27:558–562

Dasari R, Narra M, Ellendula R, Kota S, Taduri S (2015) Efficient in vitro propagation system via multiple shoot induction and assessment of clonal fidelity of regenerants in *Citrullus colocynthis* (L.) Schard. Plant Cell Biotechnol Mol Biol 16(3&4):108–118

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Fazeli-Nasab B (2018) The effect of explant, BAP and 2, 4-D on callus induction of *Trachyspermum ammi*. Potravinarstvo 12(1):578–586

Gharari Z, Bagheri K, Sharafi A, Danafar H (2019) Thidiazuron induced efficient in vitro organogenesis and regeneration of *Scutellaria bornmuelleri*: an important medicinal plant. Vitro Cell Dev Biol Plant 55(2):133–138

Ghosh M, Sinhababu SP, Sukul NC, Sahu NP, Mahato SB (1994) Antifilarial effect of solamargine isolated from *Solanum khasianum*. Int J Pharmacogn 32(2):184–190

Gisbert C, Prohens J, Nuez F (2006) Efficient regeneration in two potential new crops for subtropical climates, the scarlet (*Solanum aethiopicum*) and gboma (*Solanum macrocarpon*) eggplants. N Z J Crop Hortic 34:55–62

Goyal AK, Pradhan S, Basistha BC, Sen A (2015) Micropropagation and assessment of genetic fidelity of *Dendrocalamus strictus* (Roxb.) nees using RAPD and ISSR markers. 3 Biotech 5(4):473–482

Hussain SA, Ahmad N, Anis M (2018) Synergetic effect of TDZ and BA on minimizing the post-exposure effects on axillary shoot proliferation and assessment of genetic fidelity in *Rauvolfia tetraphylla* (L.). Rend Lincei Sci Fis Nat 29:109–115

Iftikhar A, Qureshi R, Munir M, Shabbir G, Hussain M, Khan MA (2015) In vitro micropropagation of *Solanum Villosum*—a potential alternative food plant. Pak J Bot 47(4):1495–1500

Jarald EE, Edwin S, Saini V, Deb L, Gupta VB, Wate SP, Busari KP (2008) Anti-inflammatory and anthelmintic activities of *Solanum khasianum* Clarke. Nat Prod Res 22(3):269–274

Jin W, Wang Y, Wang H (2014) Adventitious shoot regeneration from leaves of apple rootstock 'Pingyitiancha'(*Malus hupehensis* var. pinyiensis) and genetic fidelity of regenerated plantlets using SSR markers. Can J Plant Sci 94(8):1345–54

Jogam P, Sandhya D, Shekhawat MS, Alok A, Manokari M, Abbagani S, Allini VR (2020) Genetic stability analysis using DNA barcoding and molecular markers and foliar micro-morphological analysis of in vitro regenerated and in vivo grown plants of *Artemisia vulgaris* L. Ind Crops Prod 151:112476

Kannan TM, Nagarajan SM, Kulothungan S (2006) Micropropagation of *Solanum nigrum* L.-a medicinal herb. Plant Arch 6:97–99

Kasula K, Prasad S, Umate P, Gadidasu K, Abbagani S (2008) Efficient TDZ and IAA-assisted plant regeneration from cotyledon and leaf explants of *Capsicum annuum* L.—one-step protocol for shoot bud differentiation and elongation. Int J Plant Dev Biol 2:114–117

Kaunda JS, Zhang YJ (2019) The Genus Solanum: an ethnopharmacological, phytochemical and biological properties review. Nat Products Bioprospect 9:77–137

Kazemi N, Kermani MJ, Habashi AA (2019) Sodium nitroprusside stimulates micropropagation and TDZ induces adventitious shoots regeneration in red flesh apple *Malus niedzwetzkyana* Koehne Dieck ex. J Hort Res 27(2):23–30

Kim KM, Kim MY, Yun PY, Chandrasekhar T, Lee HY, Song PS (2007) Production of multiple shoots and plant regeneration from leaf segments of fig tree (*Ficus carica* L.). J Plant Biol 50(4):440–6

Kim YC, Che QM, Gunatilaka AL, Kingston DG (1996) Bioactive steroidal alkaloids from *Solanum umbelliferum*. J Nat Prod 59(3):283–285

Lakshmanan V, Reddampalli Venkataramareddy S, Neelwarne B (2007) Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. Electron J Biotechnol 10(1):106–113

Mallaya NP, Ravishankar GA (2013) In vitro propagation and genetic fidelity study of plant regenerated from inverted hypocotyl explants of eggplant (*Solanum melongena* L.) cv Arka Shirish. 3 Biotech 3(1):45–52

Matand K, Shoemake M, Li C (2020) High frequency in vitro regeneration of adventitious shoots in daylilies (Hemerocallis sp) stem tissue using thidiazuron. BMC Plant Biol 20(1):31

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Muthukumar M, Muthukrishnan S, Kumar TS, Rao MV (2020) Direct regeneration, microshoot recovery and assessment of genetic fidelity in *Helicteres isora* L., a medicinally important tree. Biocatal Agric Biotechnol 23:101415

Northmore JA, Sigurdson D, Schoor S, Rustum A, Chuong SD (2016) Thidiazuron induces high-frequency indirect shoot organogenesis of *Bienertia sinuspersici*: a single-cell C 4 species. Plant Cell Tissue Organ Cult 126(1):141–151

Nurokhman A, Faizah H, Sugiharto ES, Manuhara YS (2019) Effect of plant growth regulator and explant types on in vitro callus induction of *Gynura procumbens* (Lour.) Merr. Res J Biotechnol 14:9

O'Connor-Sánchez A, Domínguez-May ÁV, Keb-Llanes MA, González-Estrada TA, Peña-Ramírez YJ (2010) Efficient plant regeneration from leaf explants of *Solanum americanum*. Afr J Biotechnol 9(36):5830–5835

Padmapriya H, Karthikeyan AV, Jahir Hussain G, Karthi C, Velayutham P (2011) An efficient protocol for in vitro propagation of *Solanum nigrum* L. from nodal explants. J Agric Technol 7(4):1063–1073

Pendli S, Rohela GK, Jogam P, Bylla P, Korra R, Thammidala C (2019) High frequency in vitro plantlet regeneration in *Solanum trilobatum* L. an important ethno-medicinal plant and confirmation of genetic fidelity of R 1 plantlets by using ISSR and RAPD markers. Vegetos 32(4):508–520

Ray BP, Hassan L, Nasiruddin KM (2011) In vitro regeneration of brinjal (*Solanum melongena* L.). Bangladesh J Agr Res 36(3):397–406

Rohela GK, Jogam P, Shabnam AA, Shukla P, Abbagani S, Ghosh MK (2018) In vitro regeneration and assessment of genetic fidelity of acclimated plantlets by using ISSR markers in PPR-1 (Morus sp.): an economically important plant. Sci Hortic 241:313–321

Rohela GK, Jogam P, Bylla P, Reuben C (2019) Indirect regeneration and assessment of genetic fidelity of acclimated plantlets by SCoT, ISSR, and RAPD markers in *Rauwolfia tetraphylla* L.: an endangered medicinal plant. BioMed Res Int 2019

Rohela GK, Jogam P, Mir MY, Shabnam AA, Shukla P, Abbagani S, Kamili AN (2020) Indirect regeneration and genetic fidelity analysis of acclimated plantlets through SCoT and ISSR markers in *Morus alba* L. cv. Chinese white. Biotechnol Rep e00417

Rong CH, Qingling L, Changsan Z (2011) Study on application of TDZ in tissue culture of *Alocasia indica*. J Anhui Agric Sci 39(36):22224–22225

Rosangkima G, Jagetia GC (2015) In-vitro anticancer screening of medicinal plants of Mizoram State, India, against dalton's lymphoma, MCF-7 and HELA cells. Int J Recent Sci Res 6:5648–5653

Sadhu S, Jogam P, Thampu RK, Abbagani S, Penna S, Peddaboina V (2020) High efficiency plant regeneration and genetic fidelity of regenerants by SCoT and ISSR markers in chickpea (*Cicer arietinum* L.). Plant Cell Tissue Organ Cult 2:1–3

Sajid ZA, Aftab F (2009) Effect of thidiazuron (TDZ) on in vitro micropropagation of *Solanum tuberosum* L. cvs. Desiree and Cardinal. Pak J Bot 41:1811–1815

Sarkar J, Banerjee N (2020) Influence of different cytokinins on micropropagation of an important medicinal plant, *Solanum erianthum* D. Don, and assessment of the genetic fidelity of the regenerants. Vitro Cell Dev Biol Plant 56:480–490

Savitha R, Shasthree T, Mallaiah B (2010) High frequency of plantlet regeneration and multiple shoot induction from leaf and stem explant of *Citrullus colosynthis* (L.) Schrad, an endangered medicinal cucurbit. Int J Pharma Bio Sci V1(2):1–8

Savitikadi P, Jogam P, Rohela GK, Ellendula R, Sandhya D, Allini VR, Abbagani S (2020) Direct regeneration and genetic fidelity analysis of regenerated plants of *Andrographis echioides* (L.)—an important medicinal plant. Ind Crops Prod 155:112766

Schmelzer GH, Gurib Fakim A (2008) Medicinal plants 1. In: Plant resources of Tropical Africa, vol 11, Netherlands, pp 518–520

Shah SH, Ali S, Jan SA, Din J, Ali GM (2015) Callus induction, in vitro shoot regeneration and hairy root formation by the assessment of various plant growth regulators in tomato (*Solanum lycopersicum* Mill.). J Anim Plant Sci 25(2):528–538

Silué N, Koné T, Soumahoro AB, Koné M (2016) In vitro shoot tip multiplication of bambara groundnut [*Vigna subterranea* (L.) Verdc.]. Plant Cell Tissue Organ Cult 127(3):603–611

Singh KN, Kaushal R (2007) Comprehensive notes on commercial utilization, characteristics and status of steroid yielding plants in India. Ethnobot Leaflets 11:45–51

Sirikonda A, Jogam P, Ellendula R, Kudikala H, Mood K, Allini VR (2020) In vitro micropropagation and genetic fidelity assesment in *Flemingia macrophylla* (Willd.) Merr: an ethnomedicinal plant. Vegetos. https://doi.org/10.1007/s42535-020-00106-9

Souza RA, Braga FT, Setotaw TA, Vieira Neto J, Azevedo PH, Azevedo VH, Cançado GM (2013) Effect of coconut water on growth of olive embryos cultured in vitro. Ciência Rural 43(2):290–296

Srivastava M, Sharma S, Misra P (2016) Elicitation based enhancement of secondary metabolites in Rauwolfia serpentina and *Solanum khasianum* hairy root cultures. Pharmacogn Mag Suppl 3:S315–S320

Thakur J, Dwivedi MD, Sourabh P, Uniyal PL, Pandey AK (2016) Genetic homogeneity revealed using SCoT, ISSR and RAPD markers in micropropagated *Pittosporum eriocarpum* Royle—an endemic and endangered medicinal plant. PLoS ONE 9(3)

Turker AU, Yucesan B, Gurel E (2009) An efficient in vitro regeneration system for *Lythrum salicaria*. Biol Plant 53(4):750

Vemula S, Koppula T, Jogam P, Mohammed M (2019) In vitro high frequency multiplication and assessment of genetic fidelity of *Corallocarpus epigaeus*: an endangered medicinal plant. Vegetos 33:63–73

Vinoth S, Gurusaravanan P, Jayabalan N (2012) Effect of seaweed extracts and plant growth regulators on high-frequency in vitro mass propagation of *Lycopersicon esculentum* L (tomato) through double cotyledonary nodal explant. J Appl Phycol 24(5):1329–1337