Journal of Phytology 2026, 18: 67-73
https://doi.org/10.25081/jp.2026.v18.9970

Research Article

Genetic fidelity assessment of micropropagated *Lycium truncatum* Y.C.Wang, the Mongolian rare shrub, using RAPD and ISSR markers

Selenge Munkhtsetseg[1], Altanzul Bazarvaani[1], Khaliunaa Tuvshinjargal[2], Oyunbileg Yungeree[1], Altanzul Khorolragchaa[1], Kalaiselvi Senthil[3]*, Khongorzul Odgerel[1]*

[1]*Laboratory of Plant Biotechnology, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia*
[2]*Laboratory of Molecular Biology, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia*
[3]*Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641043, Tamil Nadu, India*

(Received: February 23, 2026; Revised: May 25, 2026; Accepted: June 09, 2026; Published: June 27, 2026)

*Corresponding author: Kalaiselvi Senthil, E-mail: kalaiselvi_bc@avinuty.ac.in; Khongorzul Odgerel, E-mail: khongorzul@mas.ac.mn

Abstract

Lycium truncatum Y.C.Wang is a medicinal and environmentally valuable shrub belonging to the family of Solanaceae. Due to desertification, prolonged drought, overgrazing, and mining activities, it has been listed as a rare shrub in the Mongolian Red Book and the Checklist of Native Vascular Plants of Mongolia. To establish an initial report on the genetic fidelity assessment of micropropagated *L. truncatum*, two-DNA-based techniques, Random amplified polymorphic DNA (RAPD) and Inter-simple sequence repeats (ISSR) marker analysis were performed. The plantlets were micropropagated on DKW medium supplemented with 2.0 mg/L kinetin (Kin) and 0.1 mg/L naphthaleneacetic acid (NAA), and acclimatised in the culture room (*ex vitro*) after hardening off in a peat moss (2:1) mixture. A total of 10 RAPD and 10 ISSR primers were used in the present study; 14 primers produced clear and scorable amplicons. RAPD analysis generated a total of 37 bands, of which 30 (81.1%) were monomorphic. The percentage of polymorphism was low, with 18.9% polymorphism among all samples. The similarity coefficient was between 0.90 and 0.91 between the hardened plants and between the wild type. ISSR analysis revealed a total of 52 bands, of which 31 (59.6%) were monomorphic. The percentage of polymorphism was 40.4% for all samples. The similarity coefficient ranged from 0.77 to 0.83, while the mean similarity between the two primer sets was 0.84 to 0.87. The result could provide the theoretical basis for a large-scale micropropagation protocol of *L. truncatum*.

Keywords: *Lycium truncatum*, Genetic fidelity, RAPD-PCR, ISSR-PCR, Dice's similarity coefficient

Introduction

The genus *Lycium* belongs to the Solanaceae family and comprises more than 97 species and six varieties, which are distributed in South America (32 species), North America (24 species), South Africa (24 species) and temperate Europe and Asia (12 species). *Lycium* species are shrubs or small trees that occur in the arid and semi-arid regions of the temperate zones (Grubov, 2001). The species belonging to this genus often have thorns on their stems and leaves (Yao *et al.,* 2018). *Lycium truncatum* is widespread in Central Asia, Mongolia and China. The plant is a 1-1.5 m tall, erect shrub with a spiny stem, small fruits that are bright orange-red in colour and orange seeds (Oyuntsetseg *et al.,* 2022). The fruit of *L. truncatum*, similar to the other *Lycium* species, is rich in nutrients such as β-carotene, organic acids and tannins, but has the lowest vitamin C content (Yao *et al.,* 2018). Despite their nutritional value, the fruits of *Lycium* spp. are known to have a variety of uses in human life, such as eye-healing and diuretic effects (Zhurba *et al.,* 2021). The root bark of *L. truncatum* is used as a medicinal ingredient in China (Grubov, 2001).

Due to desertification, persistent drought, the destruction of pastureland by livestock, and mining, *L. truncatum* is considered a rare woody shrub, which is listed in the Red Book of Mongolia and in the Checklist of Native Vascular Plants of Mongolia (Shiirevdamba, 2013; Baasanmunkh *et al.,* 2022).

Although *L. truncatum* can be propagated by seeds in its natural habitat, it does not produce them every year, and collecting the seeds is both time-consuming and labour-intensive (Oyutolgoi, 2022). Therefore, *in vitro* micropropagation is essential to ensure a continuous and reliable supply of explant material throughout the year. For decades, plant micropropagation has been used as a technique to preserve and promote the economic value of many agricultural and rare species (Mamdouh *et al.,* 2021). For this reason, we have developed an effective micropropagation protocol for the rare Mongolian shrub *L. truncatum*, and the results of this study can be effectively used in restoration after land degradation and mining activities (Erdenetuul *et al.,* 2024).

In plant tissue culture, variability can occur spontaneously and be the result of transient changes or permanent genetic alterations in cells or tissues (Ali *et al.,* 2019). Explant types play a crucial role in the induction of somaclonal variation in *in vitro* propagated plants. The involvement of the callus phase in plant regeneration is more susceptible to genetic variation than plants developing through organised meristematic tissues such as axillary buds and shoot apices (Kumar, 2023).

Therefore, it is crucial to determine the genetic uniformity of micropropagated plants, as the main objective of tissue culture is to produce uniform genetic material for further use.

Therefore, it is crucial to determine the genetic uniformity of micropropagated plants, as the main objective of tissue culture is to produce uniform genetic material for further use. For this purpose, various methods have been reported for assessing clonal fidelity, including morphological, biochemical, cytological, and molecular approaches. Among these, molecular methods are regarded as the most widely used and reliable methods, as they enable the detection of genetic variations at the DNA level. Particularly, hybridization-based restriction fragment length polymorphism (RFLP) or methylation-sensitive amplified polymorphism (MSAP) and PCR-based arbitrary markers, including random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and sequence-characterized amplified region (SCAR) were widely used in plant genetic studies. Nowadays, molecular marker research has increasingly shifted toward co-dominant simple sequence repeat (SSR) markers, sequence-based markers such as single-nucleotide polymorphisms (SNPs), and gene-targeted arbitrary markers, including start codon targeted (SCoT) polymorphism markers (Bohar *et al.,* 2020; Rai, 2023). Since the DNA markers can be useful to determine the individual genotypic differences within the same or different species (Amiteye, 2021), RAPD and ISSR-PCR are widely utilized due to their simplicity, cost-effectiveness, rapid and reproducible performances, as it does not require prior knowledge of the plant genome to detect genetic variations (Serrote *et al.,* 2020; Mamdouh *et al.,* 2021).

In the molecular phylogenetic study, Zhang (2024) sequenced and aligned the CPGs (chloroplast genomes) of a total of 15 *Lycium* species, distributed in China, and revealed a 155,638 bp length of *L. truncatum* (Genbank accession number: PP239386) genomic sequence with a typical quadripartite structure, for the first time (Zhang *et al.,* 2024). Despite this, information about the molecular genetics of *L. truncatum* is scarce. Previously, genetic fidelity assessments using MSAP, RAPD, and ISSR markers were done for *L. barbarum* and *L. chinense* Miller (Liu *et al.,* 2020a, b), *L. schweinfurthii* (Mamdouh *et al.,* 2021), and *L. ruthenicum* (Gao *et al.,* 2021). To the best of our knowledge, no studies have yet reported the assessment of genetic fidelity among micropropagated plants in comparison with the mother plant in *L. truncatum*.

The aim of this study was to analyse the genetic fidelity of the micropropagated plants and compare them with the mother plant *L. truncatum* using RAPD and ISSR markers to detect the occurrence of somaclonal variations.

Material and methods

Plant material and in vitro micropropagation

The seeds and mother plants of *Lycium truncatum* were collected in the province of Umnugobi in Mongolia (42°48’50.93” N, 107°0’11.48” E). The seeds were used to obtain seedlings for explant preparation *in vitro*, which were maintained at the Laboratory of Plant Biotechnology, Institute of Biology, Mongolian Academy of Sciences. Sterilisation of seeds and plant regeneration *via* tissue culture were performed in a previous study (Erdenetuul *et al.,* 2024). Plantlets were cultured for three generations on DKW (Driver & Kuniyuki, 1984) medium supplemented with kinetin (Kin) and naphthaleneacetic acid (NAA). All media components used for these experiments were purchased from PhytoTechnology Laboratories® (Shawnee Mission, KS, US).

Ex vitro acclimatization

A total of 90 micropropagated plantlets were removed from the nutrient-agar medium, washed thoroughly to remove any traces of DKW medium, and hardened off in pots filled with a soil: peat moss (2:1) mixture (Khulert Khors LLC, Ulaanbaatar, Mongolia). Plantlets were then acclimatised in the culture room (*ex vitro*) under the following conditions: 24±2 °C temperature, 35±5% relative humidity, and 16/8 h light/dark photoperiod for three months. Each pot was covered with polyethylene bags to maintain high humidity and watered regularly, 2 times a week. Ten days after transplanting, small holes have been made in the polyethylene covering to reduce humidity gradually. The survival rates were measured three months after *ex vitro* acclimatization. After a successful acclimatization period, the plants were used for morphological evaluation and genetic fidelity analyses.

DNA extraction

Genomic DNA was isolated using 100 mg of fresh leaves from mother plants and *ex vitro* plantlets of *L. truncatum* according to the TaKaRa MiniBEST Plant Genomic DNA extraction kit (Takara Bio Inc., Shiga, Japan), and concentrations were checked using the NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Delaware, US). The DNA samples adjusted to a final concentration of 15 ng/µL and were stored at -20 °C for RAPD and ISSR analysis.

RAPD amplification

The RAPD analysis was performed according to a protocol adapted from Williams *et al.* (1990). Seven primers, selected from an initial set of ten primers, were used for the analysis and are listed in Table 1. PCR was performed in a reaction mixture totalling 25 µL containing 1 µL template DNA, 10 µM primers, 200 µM dNTPs, 1 unit of Taq polymerase and 1X Taq buffer (Thermo Fisher Scientific, Delaware, US). Amplification was performed using a thermal cycler (Takara Bio Inc., Shiga, Japan) under the following conditions: initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, annealing temperature 30-35 °C for 30 s, extension of 1 min at 72 °C and final extension for 7 min at 72 °C. The amplification fragments were separated with a 1.5% (w/v) agarose gel using 1x TAE buffer at 100 V for 30 min. The gel was stained with ethidium bromide and visualised using a gel documentation system, the Gel Doc EZ Imager (Bio-Rad, California, US).

ISSR amplification

The ISSR analysis was performed according to a protocol derived from Zietkiewicz *et al.* (1994). Seven primers, selected from an initial set of ten primers, were used for the analysis and are listed in Table 2. PCR was performed in a reaction mixture totalling 25 µL containing 1 µL template DNA, 10 µM primers, 200 µM dNTPs, 1 unit of Taq polymerase, and 1X Taq buffer (Thermo Fisher Scientific, Delaware, US). Amplification was performed using a thermal cycler (Takara Bio Inc., Shiga, Japan) for touchdown PCR of the ISSR marker: initial denaturation at 94 °C for 5 min, 22 cycles of denaturation at 94 °C for 30 s, annealing at 65-44 °C for 30 s (1 °C per cycle), and extension at 72 °C for 1 min 30 s. Then a second step with 13 cycles of denaturation at 94 °C for 30 s, annealing at 43 °C for 30 s, and extension at 72 °C for 1 min 30 s. All PCRs were followed by a final extension step at 72 °C for 7 min. The amplification fragments were separated with 1.5 % (w/v) agarose gel using 1x TAE buffer at 100 V for 30 min. The gel was stained with ethidium bromide and viewed in a gel documentation system, the Gel Doc EZ Imager (Bio-Rad, California, US).

Data analysis

Genetic fidelity analyses were performed on three technical replicates with three biological replicates from hardened plants per marker. The bands were scored as present (1) and absent (0) and a binary matrix was computed; only clear and distinct bands were included in the analysis. Genetic distance and similarity were determined by Dice's coefficient of similarity. Dice's coefficient of similarity presented as a percentage was calculated for pairwise comparisons between individual samples according to the formula:

$$Dice(WT,S) = \frac{2 \times n_{ij}}{n_i + n_j}$$

where:

n_{ij} is common band number between samples *WT (Wild type)* and *S (Samples, 1-3)*

n_{ij} band number for samples

Results

Ex vitro acclimatization

In vitro plant regeneration protocol of *L.truncatum* was published in a previous study (Erdenetuul *et al.,* 2024), and a total of 90 *in vitro* plantlets were transferred to a soil mixture (soil: peat moss 2:1). After three months of plant acclimatization in soil (*ex vitro)* conditions, the survival rate was evaluated. The acclimatization leads to high plantlet survival, which 91.7% were successfully acclimatized. Hardened plantlets were visually inspected for any differences, and phenotypic characteristics, such as leaves and stems, were similar to those of the parent plants (Figure 1). The stems are flexible with a light green color, leaves solitary on long shoots, clustered on short shoots, leaf blade linear-lanceolate or lanceolate, 3.0-4.5 cm × 5-10 mm, base cuneate, decurrent, apex acute, mid vein evident (Figure 1).

RAPD analysis

Of the 10 primers analysed, only 7 primers produced clearly and detectably amplified DNA. A total of 37 recognisable bands between 700-3000 bp were amplified with seven RAPD primers (Table 1 & Figure 2). The Dice similarity coefficient ranged from 0.90 to 0.91. The percentage of 100% monomorphism was observed in OPA-01, 07, 09, and polymorphism ranged from a minimum value of 20% in OPA-08 to a maximum value of 33.3% in OPA-06, 10, with an average of 15.9%. Of the 37 DNA fragments, thirty monomorphic fragments were recorded.

Table 1: A list of primers and their nucleotide sequences, range of band products, number of monomorphic and polymorphic bands produced by RAPD primers

Primer code	Nucleotide sequence 5' to 3'	Size range (bp)	Total bands (bp)	Monomorphic bands	Polymorphic bands
OPA-01	CAGGCCCTTC	750-2000	5	5	0
OPA-05	AGGGGTCTTG	1000-2000	4	3	1
OPA-06	GGTCCCTGAC	700-3000	6	4	2
OPA-07	GAAACGGGTG	700-2700	5	5	0
OPA-08	GTGACGTAGG	1250-2700	5	4	1
OPA-09	GGGTAACGCC	800-1250	3	3	0
OPA-10	GTGATCGCAG	850-2700	9	6	3
Total			37	30	7

Table 2: A list of primers and their nucleotide sequences, range of band products, and number of monomorphic and polymorphic bands produced by ISSR primers

Primer code	Nucleotide sequence 5' to 3'	Size range (bp)	Total bands (bp)	Monomorphic bands	Polymorphic bands
UBC-811	$(GA)_8C$	750-1700	7	5	2
UBC-815	$(CT)_8G$	1200-4000	6	6	0
UBC-823	$(TC)_8C$	900-4700	12	5	7
UBC-835	$(AG)_8TC$	350-1750	11	4	7
UBC-836	$(AG)_8TA$	500-4000	7	5	2
UBC-841	$(GA)_8TC$	500-1400	4	4	0
UBC-845	$(CT)_8GG$	1000-3000	5	2	3
Total			52	31	21

Figure 1: *Ex vitro* acclimatization of micropropagated *L. truncatum.* a) Potted plantlets in soil mixture (soil: peat moss 2:1) after 1 month of growth in culture room, b) Hardened plants after 3 months of growth in culture room and, c) The mother plant grown in natural habitat, Umnugobi, Mongolia

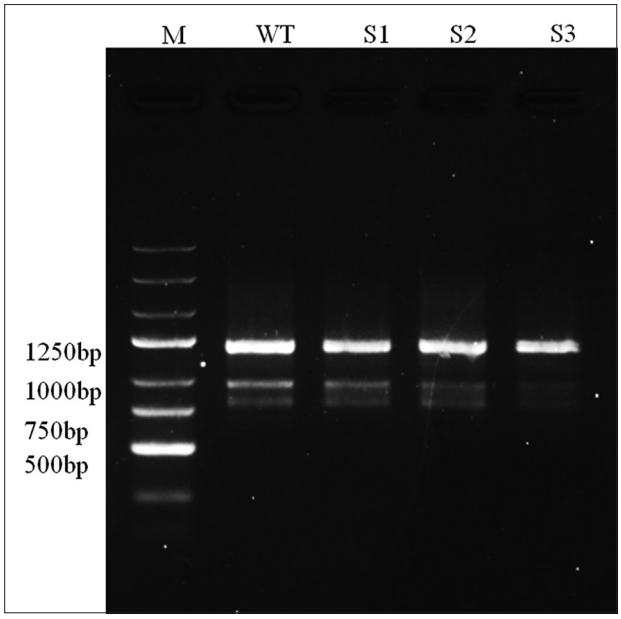

Figure 2: PCR amplification profiles obtained with RAPD primer OPA-09 showing monomorphism among the *ex vitro* and wild-type of *L. truncatum*. M- Marker, WT- Wild type; S1-S3-Samples (Plantlets)

ISSR analysis

Of the 10 primers analysed, only 7 primers produced clearly and detectably amplified DNA. A total of 52 recognisable bands between 350-4700 bp were amplified with seven ISSR primers (Table 2 & Figure 3). The Dice similarity coefficient ranged from 0.77 to 0.83. The percentage of 100% monomorphism was observed in UBC-815, 841, and polymorphism ranged from a minimum value of 28.6% in UBC-811, 836 to a maximum value of 63.6% in UBC-835, with an average of 47.8%. Thirty-one monomorphic fragments out of 52 DNA fragments were recorded.

The results were evaluated using Dice's similarity coefficient, which ranges from 0 (completely different)

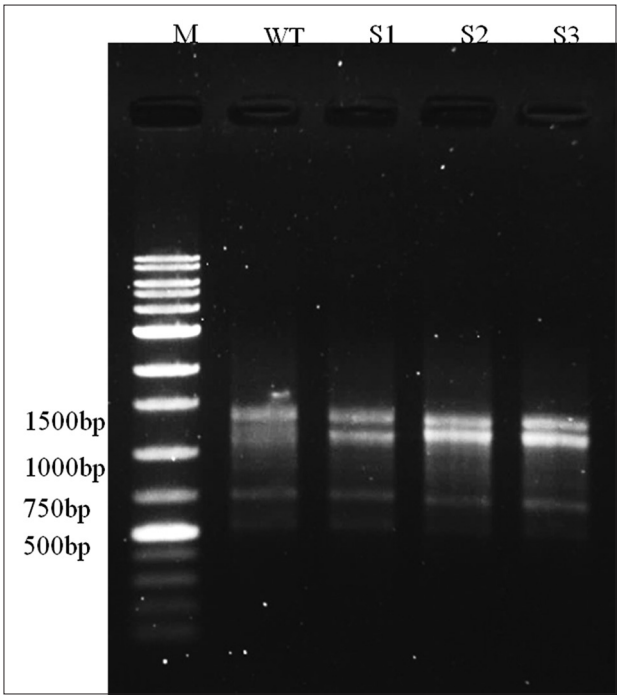

Figure 3: PCR amplification profiles obtained with ISSR primer UBC-841 showing monomorphism among *ex vitro* and wild type of *L. truncatum*. M-Marker, WT-Wild type; S1-S3-Samples (Plantlets)

Table 3: Genetic similarity among samples based on Dice's similarity coefficient derived from DNA fragment patterns generated through RAPD and ISSR marker analyses

	WT	S1	S2	S3
WT				
S1	0.87			
S2	0.84	0.96		
S3	0.84	0.94	0.97	

to 1 (identical). Compared with the wild-type plants, the micropropagated generation showed a similarity coefficient ranging from 0.84 to 0.87, representing the mean value obtained from two primers. In contrast, the micropropagated plantlets (S1–S3) exhibited higher similarity coefficients within the group, ranging from 0.94 to 0.97, which also represent the mean values obtained from two primers (Table 3).

Discussion

Tissue culture of plants is an efficient method of clonal propagation; however, the resulting regenerants often exhibit a number of somaclonal variations. These somaclonal variations are mainly caused by newly created mutations resulting from the tissue culture process. The triggers for mutations in tissue culture have been attributed to numerous stress factors, including wounding, exposure to sterilants during sterilisation, imbalances of media components such as high concentrations of plant growth regulators (PGRs), light conditions, and the disturbed relationship between high humidity and transpiration (Krishna *et al.,* 2016). In addition, the *in vitro* cultures are exposed to a high level of oxidative stress. This can lead to structural changes in chromatin due to chromosome breaks and rearrangements, DNA methylation, point mutations, histone modifications,

etc., resulting in potential (Alsafran *et al.,* 2022). For these reasons, it is necessary to check the genetic uniformity of micropropagated plantlets (Mamdouh *et al.,* 2021).

In plant tissue culture and micropropagation, acclimatization or hardening of *in vitro* propagated plantlets is considered the most critical step. In this study, plantlets with similar sizes of roots and shoots (n=90) were harvested from the culture media, transferred to the soil for three months for acclimatization, and 91.7% of the plantlets survived. Up to 95% of survival rates were previously observed in *Lycium* species after acclimatization (Gao *et al.,* 2021; Mamdouh *et al.,* 2021; Erdenetuul *et al.,* 2024), and optimal conditions in a culture room, composted soil, and less contamination may lead to high plantlet survival.

L. truncatum is an excellent economic shrub with physiological characteristics that confer drought and salt tolerance, making it highly suitable for desertification control and ecological rehabilitation (Dimitrova *et al.,* 2017), while also possessing significant nutritional and medicinal properties (Grubov, 2001; Yao *et al.,* 2018).

Although numerous studies have investigated the functional properties of its fruits, limited attention has been given to somaclonal variation. Molecular markers have been used as a powerful tool to detect somaclonal variations, such as ISSR, RFLP, SCoT, SSR, SNPs, and RAPD. They are widely used due to their simplicity, cost-effectiveness, and proven reliability in evaluating genetic fidelity and diversity without the need for prior sequence information (Bohar *et al.,* 2020; Alsafran *et al.,* 2022; Rai, 2023). In addition, the parallel use of different markers provides better opportunities for genetic variation between different clones (Mamdouh *et al.,* 2021).

Genetic similarity or polymorphism of *ex vitro* plants using SDS-PAGE, RAPD and ISSR markers has been found in many studies, e.g. in three species of the genus *Mesembryanthemum* (Soliman *et al.,* 2014), *Swertia chirayita* (Sharma *et al.,* 2016), *Orthosiphon stamineus* (Ali *et al.,* 2019), *Hippophae rhamnoides* (Sandag *et al.,* 2021), *Acorus calamus* (Parasher *et al.,* 2024), *Scutellaria tuvensis* (Muraseva & Guseva, 2021), *Lagerstroemia speciosa* (Wu *et al.,* 2024), *Stachys byzantine* (Hatzilazarou *et al.,* 2025), *Nothapodytes foetida* (Attaluri & Dharavath, 2025), *Eleusine coracana* (Maharajan *et al.,* 2025) and new varieties of turmeric, ginger, coriander, and fenugreek (Giridhari *et al.,* 2020), and *Lycium* (Mei *et al.,* 2017; Liu *et al.,* 2020a). In the present study, two PCR-based techniques, such as RAPD and ISSR marker analyses, were used to test the genetic fidelity between the *ex vitro* or hardened plantlets and the wild type of *L. truncatum*. After RAPD analysis, the *ex vitro* plantlets showed a higher similarity coefficient with the wild-type plants, ranging between 0.90 and 0.91. Previously, Liu (2020a) characterized similarities between different *Lycium* varieties based on ISSR analysis and RAMP-PCR markers. The similarity coefficient index between *Lycium* species from different geographical areas was found to be 0.36-0.98 by both analyses, which concluded that RAMP-PCR and ISSR were both mutually consistent and the measured polymorphism was related to the different origins of the plant samples. RAMP-PCR is an improved RAPD technique, where the ramp time is prolonged at the stage from annealing to extension, the resolution and production are greatly increased compared to regular RAPD (Mei *et al.,* 2017; Liu *et al.,* 2020a).

In the present ISSR analysis, *ex vitro* plantlets showed a similarity coefficient of 0.77-0.83 (40.4% polymorphism) with the wild-type plants, while RAPD marker analysis showed 0.9-0.91 (18.9% polymorphism). The reason ISSR markers showed a higher degree of polymorphism and lower genetic fidelity compared to RAPD markers could be related to the abundant nature of ISSR microsatellites caused by slippage during DNA replication (Muthusamy *et al.,* 2008; Ali *et al.,* 2019). ISSR primers are usually 16-25 bp long and consist of short 2-4 nucleotide repeats anchored at the 3' or 5' end, with 1 to 4 degenerate bases extended into the flanking sequences (Muraseva & Guseva, 2021). Therefore, ISSR fingerprints are considered to detect more polymorphic loci than RAPD fingerprints (Devarumath *et al.,* 2002). We used touchdown PCR to increase the specificity, sensitivity, and PCR product yield. Touchdown PCR offers a simple and reliable approach to improve reaction stringency and eliminates the need to optimise amplifiers, salt concentrations, melting or annealing temperatures (Korbie & Mattick, 2008; Green & Sambrook, 2018; Muraseva & Guseva, 2021).

Moreover, it is known that different growth conditions reduce the tendency of the plants to be genetically stable. For instance, 6-benzyladenine (BAP) and NAA were found to cause DNA mutations on *L. schweinfurthii* and Vitis species (Alizadeh & Singh, 2009; Mamdouh *et al.,* 2021). The observed polymorphism in the present study may be associated with both *in vitro* and *ex vitro* conditions, particularly the PGRs used in the culture medium and the cultivation conditions.

On the whole, this is an initial report on the genetic fidelity assessment of micropropagated *L. truncatum* using RAPD and ISSR, which may provide a theoretical basis for the mass multiplication, germplasm conservation, and further genetic transformation assays of Mongolian medicinal and economically important plants.

Conclusion

In this study, the genetic fidelity of micropropagated plantlets of *L. truncatum* was evaluated in comparison with the wild type using RAPD and ISSR marker analyses. Both marker systems revealed monomorphic banding patterns, confirming the genetic uniformity of the regenerated plantlets and their genetic stability relative to the donor plant. These findings demonstrate that the established micropropagation protocol is suitable for large-scale *in vitro* propagation of *L. truncatum* without genetic variation. Furthermore, the assessment of genetic fidelity provides a reliable basis for the conservation, mass propagation, and future biotechnological applications of this rare medicinal shrub.

Acknowledgement

The present study was funded by the "Priority plant propagation study" project by the Oyu Tolgoi LLC [Contract No. CW2193872, PO-3106462122]. We would like to thank Tseesuren Oyun-Erdene, Advisor, Environment and Land, Oyu Tolgoi LLC, Ulaanbaatar, Mongolia, for providing the wild-type sample, and Ulziisaikhan Tumendemberel, Laboratory of Genetics, Institute of Biology, Mongolian Academy of Sciences, for his insightful comments throughout the study.

Author contributions

Selenge Munkhtsetseg and Altanzul Bazarvaani performed all the experiments, and Selenge Munkhtsetseg prepared the original draft of the manuscript. Khaliunaa Tuvshinjargal was involved in designing the experiments. Khongorzul Odgerel, Oyunbileg Yungeree and Altanzul Khorolragchaa contributed to the suggestion and conception of the study. Khongorzul Odgerel and Kalaiselvi Senthil revised the manuscript. All authors have read and approved the final manuscript.

References

Ali, H., Musa, I. F., Abu Bakar, N. A., Karsani, S. A., & Yaacob, J. S. (2019). In vitro regeneration and ISSR-based genetic fidelity analysis of *Orthosiphon stamineus* Benth. *Agronomy, 9*(12), 778. https://doi.org/10.3390/agronomy9120778

Alsafran, M., Wickramanayake, K., Usman, K., & Ahmed, T. (2022). Efficient shoot regeneration of medicinal plant *Haplophyllum tuberculatum* by direct and indirect organogenesis and genetic fidelity assessment using Inter Simple Sequence Repeats markers. *Frontiers in Plant Science, 13*, 995825. https://doi.org/10.3389/fpls.2022.995825

Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon, 7*(10), e08093. https://doi.org/10.1016/j.heliyon.2021.e08093

Attaluri, S., & Dharavath, R. (2025). The synergistic effect of plant growth regulators and polyamines on regeneration of Nothapodytes foetida, and assessment of genetic fidelity using ISSR and SCoT markers. *Plant Cell, Tissue and Organ Culture (PCTOC), 160*, 42. https://doi.org/10.1007/s11240-025-02964-0

Baasanmunkh, S., Urgamal, M., Oyuntsetseg, B., Sukhorukov, A. P., Tsegmed, Z., Son, D. C., Erst, A., Oyundelger, K., Kechaykin, A. A., Norris, J., Kosachev, P., Ma, J.-S., Chang, K. S., & Choi, H. J. (2022). Flora of Mongolia: annotated checklist of native vascular plants. *PhytoKeys, 192*, 63-169. https://doi.org/10.3897/phytokeys.192.79702

Bohar, R., Chitkineni, A., & Varshney, R. K. (2020). Genetic molecular markers to accelerate genetic gains in crops. *BioTechniques, 69*(3), 158-160. https://doi.org/10.2144/btn-2020-0066

Devarumath, R., Nandy, S., Rani, V. Marimuthu, S., Muraleedhara, N., & Raina, S. (2002). RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* ssp. *assamica* (Assam-India type). *Plant Cell Reports, 21*, 166-173. https://doi.org/10.1007/s00299-002-0496-2

Dimitrova, V., Ivanova, K., Petrov, P., Anev, S., Tzvetkova, N., Georgieva, T., & Markovska, Y. (2017). Influence of salt stress on leaf morphology and photosynthetic gas exchange in two *lycium* species. *Proceedings of the Bulgarian Academy of Sciences, 70*(3), 367-374.

Driver, J. A., & Kuniyuki, A. H. (1984). In vitro propagation of Paradox walnut rootstock. *HortScience*, 19(4), 507-509. https://doi.org/10.21273/HORTSCI.19.4.507

Erdenetuul, D., Selenge, M., Bolortuya, U., Uyanga, B., Altantsetseg, B., Senthil, K., & Oyunbileg, Y. (2024). Micropropagation protocol for Mongolian rare shrub Lycium truncatum Y.C. Wang. *Journal of Phytology, 16*, 176-182. https://doi.org/10.25081/jp.2024.v16.9212

Gao, Y., Wang, Q.-M., An, Q., Cui, J., Zhou, Y., Qi, X., Zhang, L., & Li, L. (2021). A novel micropropagation of *Lycium ruthenicum* and epigenetic fidelity assessment of three types of micropropagated plants *in vitro* and *ex vitro*. *PloS One, 16*(2), e0247666. https://doi.org/10.1371/journal.pone.0247666

Giridhari, A., Vijesh Kumar, I. P., & Sheeja, T. (2020). Discrimination of a selected set of turmeric, ginger, fenugreek and coriander varieties using ISSR markers. *Journal of Plantation Crops, 48*(3), 161-172. https://doi.org/10.25081/jpc.2020.v48.i3.6622

Green, M. R., & Sambrook, J. (2018). Touchdown Polymerase Chain Reaction (PCR). *Cold Spring Harbor Protocols, 2018*(5), 436-456. https://doi.org/10.1101/pdb.prot095133

Grubov, V. I. (2001). *Key to the Vascular Plants of Mongolia*. Science Publishers, Inc.,

Hatzilazarou, S., Kantere, C., Kotoula, A.-A., Economou, A., Bertsouklis, K., Darras, A., & Kostas, S. (2025). *In Vitro* propagation and genetic stability assessment using the ISSR markers of *Stachys byzantina* K. Koch, a promising ornamental species. *Horticulturae, 11*(5), 530. https://doi.org/10.3390/horticulturae11050530

Korbie, D. J., & Mattick, J. S. (2008). Touchdown PCR for increased specificity and sensitivity in PCR amplification. *Nature Protocols, 3*, 1452-1456. https://doi.org/10.1038/nprot.2008.133

Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M., & Sadh, R. K. (2016). Somaclonal variations and their applications in horticultural crops improvement. *3 Biotech, 6*, 54. https://doi.org/10.1007/s13205-016-0389-7

Kumar, N. (2023). *Biotechnology and Crop Improvement Tissue Culture and Transgenic Approaches*. (1st ed.). CRC Press.

Liu, X., Cheng, J., Mei, Z., Wei, C., Khan, M. A., Peng, J., & Fu, J. (2020b). SCAR marker for identification and discrimination of specific medicinal *Lycium chinense* Miller from *Lycium* species from ramp-PCR RAPD fragments. *3 Biotech, 10*, 334. https://doi.org/10.1007/s13205-020-02325-y

Liu, X., Du, J., Khan, M. A., Cheng, J., Wei, C., Mei, Z., Chen, H., He, T., Fu, J. (2020a). Analysis of genetic diversity and similarities between different Lycium varieties based on ISSR analysis and RAMP-PCR markers. *World Academy of Sciences Journal, 2*(2), 83-90. https://doi.org/10.3892/wasj.2020.39

Maharajan, T., Duraipandiyan, V., & Krishna, T. P. A. (2025). Direct shoot regeneration from the finger millet's *in vitro*-derived shoot apex and genetic fidelity study with ISSR markers. *Biotech, 14*(2), 29. https://doi.org/10.3390/biotech14020029

Mamdouh, D., Mahgoub, H. A. M., Gabr, A. M. M., Ewais, E. A., & Smetanska, I. (2021). Genetic stability, phenolic, flavonoid, ferulic acid contents, and antioxidant activity of micropropagated *Lycium schweinfurthii* plants. *Plants, 10*(10), 2089. https://doi.org/10.3390/plants10102089

Mei, Z., Zhang, X., Liu, X., Imani, S., & Fu, J. (2017). Genetic analysis of *Canarium album* in different areas of China by improved RAPD and ISSR. *Comptes Rendus*

Biologies, 340(11-12), 558-564. https://doi.org/10.1016/j.crvi.2017.09.006

Muraseva, D. S., & Guseva, A. A. (2021). ISSR primer screening for analysis of genetic diversity among Scutellaria tuvensis (Lamiaceae) populations. *BIO Web of Conferences, 38*, 82. https://doi.org/10.1051/bioconf/20213800082

Muthusamy, S., Kanagarajan, S., & Ponnusamy, S. (2008). Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. *Electronic Journal of Biotechnology, 11*(3).

Oyuntsetseg, B., Baasanmunkh, S., Nyambayar, N., Shiga, T., Yano, O., & Choi, H. J. (2022). *Illustrated Flora of Mongolia.* (2nd ed.). Geobook Publishing.

Oyutolgoi. (2022). *Cap Priority Plant Survey 2022 - Interim report*. Retrieved from https://www.ot.mn/mn/report

Parasher, K., Sharma, C., Sharma, S., Mukherjee, S., & Mukherjee, P., (2024). Cryopreservation and assessment of genetic fidelity *Acorus calamus* Linn., an endangered medicinal plant. *Cryoletters, 45*(2), 122-133. https://doi.org/10.54680/fr24210110412

Rai, M. K. (2023). Start codon targeted (SCoT) polymorphism marker in plant genome analysis: current status and prospects. *Planta, 257*, 34. https://doi.org/10.1007/s00425-023-04067-6

Sandag, Z., Balchin, D., Enkhtuya, A., Myagmar, N., Oyunbat, N., Buyanbat, K., Batnasan, S., Badam, N., Sandagdorj, J., Tsendsuren, O., & Davaakhuu, G. (2021). Assessment of genetic diversity in sea buckthorn (Hippophae rhamnoides L.) accessions from Mongolia based on RAPD markers. *Proceedings of the Mongolian Academy of Sciences, 61*(01), 1-9. https://doi.org/10.5564/pmas.v61i01.1556

Serrote, C. M. L., Reiniger, L. R. S., Silva, K. B., Rabaiolli, S. M. D. S., & Stefanel, C. M. (2020). Determining the Polymorphism Information Content of a molecular marker. *Gene, 726*, 144175. https://doi.org/10.1016/j.gene.2019.144175

Sharma, V., Belwal, N., Kamal, B., Dobriyal, A. K., & Jadon, V. S. (2016). Assessment of genetic fidelity of *in vitro* raised plants in *Swertia chirayita* through ISSR, RAPD analysis and peroxidase profiling during organogenesis. *Brazilian Archives of Biology and Technology, 59*, e16160389. https://doi.org/10.1590/1678-4324-2016160389

Shiirevdamba, T. (2013). *Mongolian Red Book*. (2nd ed.). Admon printing.

Soliman, M. I., Zaghloul, M. S., & Heikal, Y. M. (2014). Genetic variation within and among three Egyptian *Mesembryanthemum* species using different genetic markers. *Egyptian Journal of Basic and Applied Sciences, 1*(3-4), 127-135. https://doi.org/10.1016/j.ejbas.2014.08.001

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research, 18*(22), 6531-6535. https://doi.org/10.1093/nar/18.22.6531

Wu, B., Zhang, N. S., Dixon, B., Sierra, I., Kan, S., Layton, A., Gu, M., Pooler, M. R., Duan, H., & Qin, H. (2024). Reliable callus-induced plantlet regeneration from leaf explants of *Lagerstroemia speciosa* and genetic fidelity assessment through ISSR markers. *Plant Cell, Tissue and Organ Culture (PCTOC), 157*, 76. https://doi.org/10.1007/s11240-024-02801-w

Yao, R., Heinrich, M., & Weckerle, C. S. (2018). The genus Lycium as food and medicine: A botanical, ethnobotanical and historical review. *Journal of Ethnopharmacology, 212*, 50-66. https://doi.org/10.1016/j.jep.2017.10.010

Zhang, L., Zhang, E., Wei, Y., & Zheng, G. (2024). Phylogenetic analysis and divergence time estimation of *Lycium* species in China based on the chloroplast genomes. *BMC Genomics, 25*, 569. https://doi.org/10.1186/s12864-024-10487-9

Zhurba, M., Vergun, O., Klymenko, S., & Szot, I. (2021). Biochemical characterization of fruits of *Lycium* spp. in Ukraine. *Regulatory Mechanisms in Biosystems, 12*(1), 71-77. https://doi.org/10.15421/022111

Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics, 20*(2), 176-183. https://doi.org/10.1006/geno.1994.1151