

REVIEW

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A comprehensive review on conservation efforts of Shirui lily (*Lilium mackliniae* Sealy) of Manipur, India

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Abstract

Lilium mackliniae Sealy (Shirui Lily), the state flower of Manipur, is considered one of the most admired wild ornamental flowers around the world. Its modest beauty and rarity attract thousands of tourists through the difficult terrains of the Shirui hill ranges. With the introduction of the Shirui Festival in the state, its popularity is ever-increasing. However, there is a dearth of scientific work and research about this plant which will help explore the untapped potential in various sectors including horticulture, environment, tourism, rural development, etc. Hence, this review, through a comprehensive study of available literature, aims to highlight the different aspects of the plant including current status, botanical characteristics, challenges, conservation efforts and future directions for conservation. The rise in popularity of this species comes with increasing interaction with human activities and thereby compounding more challenges. The severity of the threat is reflected in being listed as an endangered species in the Red Data Book of Indian Plants. Climate change, forest fires, invasive species and habitat destruction through various human activities are posing serious challenges to the species. However, conservation approaches from various fields such as phenology, geospatial analysis, in vitro propagation, in situ and *ex situ* conservation, community efforts and government initiatives are in the right direction. A holistic approach with available resources and scientific advancement through various mechanisms of community and government sections must be implemented. There remains ample scope for further scientific research on this ecologically and culturally significant species.

Keywords Shirui lily, *Lilium mackliniae*, Conservation, Endangered species, Manipur

1 Introduction

Manipur, also known as the Jewel of India, holds a significant spot with regard to the diversity of fauna and flora. Numerous endemic and rare species such as *Lilium mackliniae* Sealy (Shirui lily), *Rucervus eldii eldii* (Sangai), *Citrus jambhiri* Lush. (Kachai lemon), *Oriza sativa* L (Chak-hao), etc. flourish only in the state and thereby remain a treasure house for scientific pursuits in the realms of botany, wildlife and ecology. *Lilium mackliniae* Sealy, Shirui lily (Pink Manipur lily), locally known as *Shirui Kashung Timrawon* in



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the Tangkhul dialect, is the state flower of Manipur. The flower is a rare and threatened species, endemic to Manipur [1]. Considering its heritage and uniqueness, the Shirui lily was declared the State flower of Manipur by the Government of Manipur, India, in 1989. A postal stamp was issued by the Indian Postal Department on March 31, 2000, in the denomination of 300 paise to commemorate this flower and to raise public awareness of the rich biodiversity of the Northeast region's flora and fauna (Fig. 1d). It was first reported by Frank Kingdon-Ward, a botanist from the New York Botanical Society, in 1946 from Siroi Kashong hill which is located about 10 km east of Ukhul district, Manipur [2]. The botanical name *Lilium mackliniae* was given in honour of his wife Jean Kingdon-Ward (née Macklin). In 1948, this beautiful flower won the prestigious merit award in the Chelsea Flower Show organized by the Royal Horticulture Society, London [3]. Indigenous people use this lily as remedies for digestive and skin issues. The blooming of Shirui lily signals the perfect time for the transplantation of paddy [4]. It is considered a culturally significant representation of success and happiness. For centuries, the people of Tangkhul tribe treasured the Shirui hill ranges as sacred and believed that all departed souls pass through these ranges. The mythical behind the origin and habitation of this flower is that plant comes out from the soil where the princess, lover of Shirui was buried and it is commonly believed that it cannot be grown outside of Shirui hill. Shirui lily is believed to represent kindness, protection, prosperity and happy life [5].

As a flagship species of Manipur's Indo-Myanmar hotspot region [6], the Shirui lily holds significant ecological and cultural importance. The popularity of this flower is attributed to its modest beauty, endemism, rarity, ecological significance and cultural association in the region. However, with the changing climate and raising anthropogenic impacts, Shirui lily is facing severe challenges and its conservation is utmost important, given the value of this species in the state. Although Shirui lily is widely recognised, research is scanty and there remain many promising areas of untapped potential. Hence, the need to comprehensively and systematically examine the current status and propose future strategies to be able to harness the best of the plant and the scientific research. This study, therefore, provides a concise overview of its botanical characteristics, challenges and its conservational efforts in the state of Manipur.

1.1 Distribution

Shirui lily was earlier described to be an endemic species of a small area of Shirui hills of Ukhul District of Manipur (Fig. 2) and then its occurrence was reported from other parts of the State with similar climatic conditions. Shirui hill ranges constitute a major



Fig. 1 *a* *Lilium mackliniae* plant, *b* Pods, *c* Seeds (Photo by Chuimaran Shangh), and *d* Postage stamp of *Lilium mackliniae* (Shirui Lily)

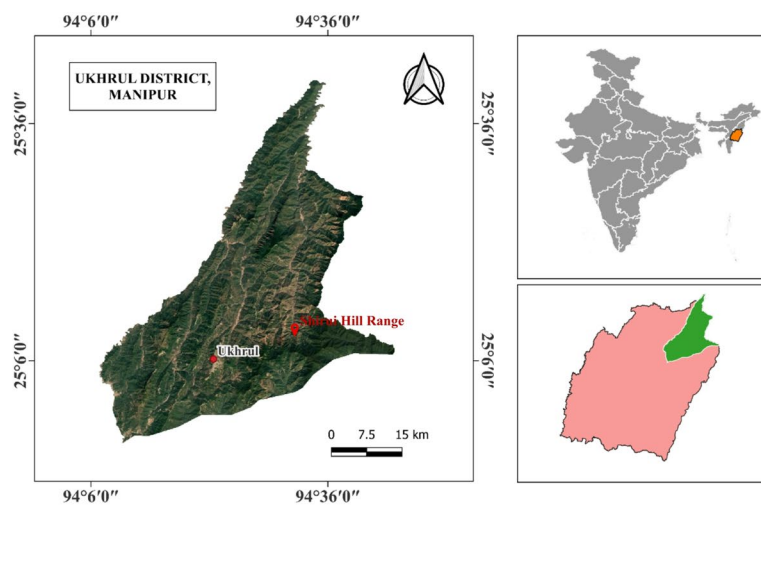


Fig. 2 Map of Ukhrul District of Manipur (India); the natural habitat of *L. mackliniae* (Shirui Lily) (constructed using QGIS)



Fig. 3 Tourist attractions of Shirui hill range: **a** Flowering of *L. Mackliniae* (Shirui lily) and **b** Flowering of Horamwon in Shirui Hill range (Photo credit: Chuimaran Shangh)

tourist attraction with the renowned Shirui lily and flowering of Himalayan Snow Anemone, locally called Horamwon (*Anemone elongata*), (Fig. 3). The occurrence of the lily in Dzukou Valley was discovered by researchers in 1994 [7]. Researchers have also revealed its presence in other two locations, Ngainoi hill and Ngair hill as its natural habitats [8]. There are reports of plant hunters from Europe and Japan having collected thousands of lily bulbs from Shirui hill and taken them to different countries for cultivation [8]. Shirui lily has also been planted at the Woodlands Compound of Botanical Survey of India, Shillong using bulbs collected from the natural habitat [1]. However, growth performance outside its natural habitat is to be assessed scientifically. Plantation outside its natural habitats can be made successful through the efforts of scientific communities.

1.2 Classification

The *Lilium* genus is a monocot, which belongs to the Liliaceae family and contains approximately 110–115 taxa that are found predominantly in the northern hemisphere's cold and temperate zones [9, 10]. India hosts a large population of *Lilium*, while some are endemic to the region. For instance, *Lilium polyphyllum* is listed as Critically Endangered in IUCN Red List, owing to habitat loss and unsustainable harvesting [11]. The

growth and development of *Lilium* can be greatly dependent on the climatic conditions especially temperature of the habitat [12, 13]. The ideal temperature for the growth of the genus *Lilium* is between 18 and 22 °C [14, 15].

1.3 Taxonomy

Kingdom: Plantae
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Liliopsida
Subclass: Liliidae
Order: Liliales
Family: Liliaceae
Genus: *Lilium*
Species: *Lilium mackliniae*

1.4 Botanical characteristics

L. mackliniae is an herbaceous perennial plant which is categorised under Asiatic lily species. It is a rare pinkish-white/pale bluish-pink flower but has seven colours when observed through a microscope [1, 8, 16]. The lily possesses an ovoid bulb, measuring 2–2.5 × 2–2.5 cm, with elliptic scales that are 1.5–2 × 1–1.5 cm and fleshy [8]. The stem is 1–3 feet (0.30–0.91 m) tall, terete at the base and angular above, with 3–4 leaves at the base [1, 8]. The leaves are sessile, irregularly spiral, linear-elliptic, 2.3–2.7 × 0.7–0.8, acute, 3-nerved, and glabrous, but hairy at the joints [1, 8]. The bell-shaped flowers are solitary and terminal, up to 3.5 cm long, pale purple, with a pedicel approximately 1.2–2.5 cm long, with the outer elliptic perianth of approximately 3.4 × 1.2 cm, acute, with greenish reflexed midribs and 3–4-paired nerves, and the inner perianth is similar to the outer [1, 8]. The nectarines are narrow channelled and glabrous, and there are 6 stamens in 2 rows, approximately 2.2 cm long, with a slender, greenish filament that is approximately 1.4 cm long, and deep brownish red, basifixed anthers that are approximately 0.8 mm long and open by longitudinal slits [8]. The ovary is oblong, approximately 8 × 2.5 mm, truncate, narrowly 3-lobed, with a terete, glabrous green style that is approximately 1.8 cm long and a shortly 3-lobed stigma with recurved, papillose lobes [1, 8]. It has one to seven flowers per plant. The flower blooms can be observed during the months of April to June every year; however, the peak period is from 15th May to 5th June.

The anatomical study of *L. mackliniae* reveals that its leaves have epidermis on both surfaces with differing cell sizes, anomocytic stomata on the lower side, homogenous bifacial mesophyll, collateral vascular bundles, and covered with a thin cuticle, while the midrib is poorly differentiated and surrounded by a parenchymatous bundle sheath [17]. Its pollen grains are medium-sized, yellow, elliptical and monocolpate with variable dimensions depending on habitat, featuring a distinctive exine structure [17].

1.5 Phytochemicals

Through chemical analysis, 27 compounds were identified and isolated from the bulbs of *L. mackliniae*, including nine new phenolics [18]. The new compounds, such as 4,4'-di-O-acetyl-3,6'-di-O-feruloylsucrose, 3,4'-di-O-p-coumaroylsucrose, 3,6'-di-O-p-coumaroylsucrose, 2'-O-acetyl-3,4'-di-O-p-coumaroylsucrose,

3'-O-acetyl-3,4'-di-O-p-coumaroylsucrose, (2S)-1-O-p-methoxycinnamoyl-2-O-β-D-glucopyranosylglycerol (methylregaloside D), (2S)-1-O-cinnamoyl-2-O-β-D-glucopyranosylglycerol (regaloside J), (2S)-1-O-caffeoyl-2-O-β-D-glucopyranosylglycerol (regaloside K) and (2R)-1-O-β-D-glucopyranosyl-2-O-caffeoylglycerol (regaloside L), have been identified through spectral analysis and chemical degradation. Additionally, previously reported phenolic glycosides, steroidal saponins, and a steroidal alkaloid have been discovered. The presence of the steroidal alkaloid suggests a taxonomic relationship between *L. mackliniae* and the genus *Fritillaria* [18].

1.6 Molecular attributes

DNA extraction from *L. mackliniae* has been successfully performed using the CTAB (hexadecyltrimethylammonium bromide) method, facilitating further genetic research to understand and preserve this species [16]. The presence of a distinct band indicates that the DNA extracted is of good quality and quantity, making it appropriate for subsequent molecular studies. These findings lay the groundwork for future research into the genetic characteristics of the Shirui lily, which is essential for its conservation and propagation efforts [16]. Molecular analysis of *L. mackliniae* proved that it belonged to clade I, based on the matK gene tree, although its sectional delimitation has yet to be determined [9]. C-band patterns of somatic chromosomes of *L. mackliniae* were examined and placed it tentatively in *Sinomartagon* [19], suggesting that this species should be placed in a new and separate section. Several researchers have deposited the genetic information of the plant using various genetic sequencing methods, to the NCBI (National Center for Biotechnology Information) with research publications [9, 20, 21] or direct submission. There are 7 nucleotide sequences extracted from chloroplast and plastids. Accession numbers in NCBI Genbank are AB030877, KC539824, LM993140, LM993253, LM993028, AF088211 and AB035286.

2 Challenges

L. mackliniae is facing numerous challenges that threaten its survival and hamper its conservation. This endangered species, listed in the Red Data Book of Indian Plants: Volume I, is endemic to the Shirui (Sirohi) Hills of Ukhrul district, Manipur, India [1, 3]. The combination of habitat degradation, climate change, invasive species, and human activities poses significant challenges to the survival of this endemic species.

Climate change poses a significant threat, altering the species' reproductive and adaptive fitness and impacting population stability [3, 22, 23]. Human activities further aggravate these threats through encroachment, exploitation of natural resources, uncontrolled trampling, plucking and uprooting by visitors [3, 22, 23]. This could potentially alter the lily's growth and distribution patterns, further threatening its long-term survival. There is rising pressure from human activities such as extensive slash-and-burn cultivation and conversion of forests for cropping contribute to habitat degradation [23, 24]. The rising tourism in the region, though important for awareness, popularity and eco-tourism, has not only resulted in physical damage to the plant through plucking and trampling but also in environmental pollution. Increased waste and plastic dumping near the lily's habitat have led to the degradation of the local ecosystem. The increased human intrusion into its natural habitat has led to frequent disturbances in its regeneration and growth [3].

Forest fires, often caused by human activities and visitor carelessness, present another significant threat to the Shirui lily's habitat. Periodic fires during the dry season have been reported to damage the species' natural environment [8, 23]. These fires not only destroy the lily population but also threaten other native species coexisting in these regions [8]. Furthermore, invasive species such as wild dwarf bamboo (*Arundinaria* sp.) and *Sinarundinaria* sp. pose a threat to the lily's habitat through rapid colonization [3, 8]. The dense root system of these bamboo species has encroached upon the lily's natural space, competing for essential resources and further limiting conservation efforts. This invasive species could impact the lily's population stability. The population dynamics of the Shirui lily have been significantly impacted by these threats. The previously widespread population has become fragmented, with declining number in natural habitats [22].

Conservation efforts for the Shirui lily face several challenges. In situ conservation is hampered due to the presence of invasive species [3]. Additionally, difficulties in plant multiplication from bulbs and/or seeds hinder *ex situ* conservation efforts [3]. These challenges call for the need for holistic conservation measures to protect the remaining populations of this endangered species.

3 Conservation efforts

With the increasing threats and challenges, it is of utmost importance to formulate strategic and holistic conservation measures. It has gained significant attention through community-led efforts, government initiatives and modern scientific approaches. These combined efforts emphasize a holistic approach to biodiversity preservation. Major conservation efforts taken up for this endangered species are discussed below (Fig. 4).

3.1 Community efforts

For centuries, the people of Tangkhul tribe treasured the Shirui hill ranges as sacred and believed that all departed souls pass through these ranges. A small species population has been conserved in the upper elevation range only by protected by the community. Sacred places, places of worship, and geographic regions are almost universal phenomena. It has been suggested that the belief in local gods and spirits at sacred places helps

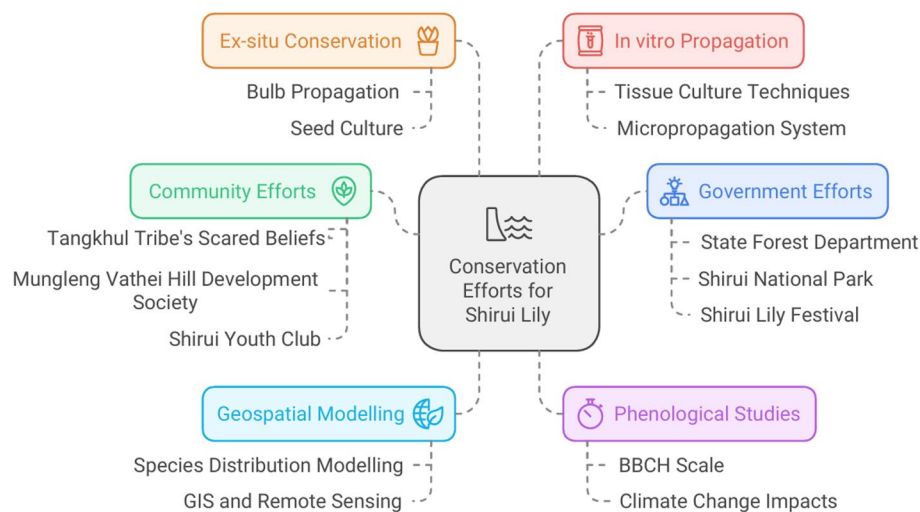


Fig. 4 Overview of conservation efforts for *L.mackliniae*



Fig. 5 Activities and initiatives of Shirui Youth Club (Photo credit: Chuimaran Shangh)

preserve unique and rare species of plants and animals in the region [25]. Several studies have shown the importance of sacred sites and socio-cultural practices in biodiversity conservation in Manipur [26–28]. The Shirui Youth Club of Shirui village has undertaken several initiatives for the conservation of Shirui lily and its habitat. These initiatives include prevention of illegal removal of plants or plant parts, maintaining a sustainable tourist practices and ensuring proper garbage disposal (Fig. 5). One of the key initiatives of the club is the plantation of Shirui lily plants. Volunteers collect seeds after the flowering season and preserve them until the next season, at which point they plant the seeds in the Shirui hill range. Additionally, they clear weeds, particularly invasive species like dwarf bamboos, either by cutting or burning them. The club also enhances the beauty of the Shirui hill through decorations, placard and directional signs. Every January, villagers burn the main grassland area to reduce the growth of competing species of *L.mackliniae*, which leads to a decline in other plant species. The society has also tried to enclose the main park with a fence and prohibited burning the mountain sides. The Mungleng Vatheh Hill Development Society (MVHDS) [29] is also a known organization responsible for the monitoring of Shirui hill. To prevent the continuous decline of various plant species, more effective forest management measures by the indigenous communities are needed [30]. Community based conservation practice does not only ensure the said ecosystem alone, but all aspects of it such as biodiversity, ecosystem services and indigenous knowledge associated with it [24].

3.2 Government efforts

The State Forest Department of Manipur, with financial assistance from the Central Government, has implemented local conservation measures, such as awareness programs and hiring local volunteers to protect the lily [29]. In 1982, the Shirui National Park was established with an area of 0.41 km² to preserve the natural habitat of the lily. Efforts by individuals like Shajin Jinks, head of Goa's Department of Environment and Heritage, and the National Institute of Bio-resources and Sustainable Development (NIBSD), have

raised awareness about the Shirui lily's endangered status. In 2015, NIBSD launched a campaign to restore the lily's habitat, including lab cultivation and distribution to nurseries for further propagation [5]. Indian Council of Agricultural Research (ICAR), in collaboration with Krishi Vigyan Kendra (KVK), Ukhrul and the Shirui Village Authority, also initiated campaigns to transplant tissue cultured Shirui lily plants in Shiroy peak.

One of the most prominent awareness initiatives is the annual Shirui Lily Festival, launched in 2017 by the Department of Tourism, Government of Manipur. Celebrated in April-May, this festival promotes sustainable and responsible tourism while increasing awareness of the rare flower. The festival features a range of cultural and traditional activities, including live music, folk performances, indigenous games, exhibitions, and adventure activities like trekking and camping. The event draws thousands of tourists, allowing them to explore the Shirui hills and witness the rare lily in its natural habitat while fostering community participation and promoting local heritage [5].

3.3 Geospatial modelling approach

Geospatial modelling plays a crucial role in the conservation of endangered plant species by enhancing our understanding of their distribution, habitat requirements, and potential reintroduction sites [31]. This approach integrates various technologies and methodologies to inform conservation strategies effectively. Species Distribution Modelling (SDM) helps identify areas with high likelihoods of species occurrence, guiding conservation efforts and resource allocation [32]. The application of geospatial technologies, such as GIS and remote sensing, allows for precise mapping and monitoring of threatened plant species, facilitating better management decisions [31].

Researchers assessed the potential distribution and habitat suitability of *L. mackliniae* Sealy, identifying the limiting geospatial and bioclimatic variables for the species' survival using Maximum entropy-based modelling [22]. The response curve confirmed the sensitivity of the predicted habitat for the elevation between 2000 and 2781 m, temperature annual range between 19.25 and 20.25 °C, aspect > 75°, precipitation of warmest quarter of > 1120 mm, and precipitation of the coldest quarter (55 mm) [22]. The response curve for the LULC suggested the rangeland class as more suitable than the other classes, viz. water-bodies, trees, flooded vegetation, crops, urban built area and bare grounds [22]. The rangelands on an elevation higher than 2000 m were projected to be suitable habitats for the species [22]. The present habitat of the species was predicted to remain suitable for a long [22]. Furthermore, the study predicted another site in the south of the species population suitable for the species. This site is situated 20 km away from its present population in Kamjong region and may be considered for introducing the species as a new habitat for conservation [22].

3.4 Phenological studies

Phenology, the study of the timing of recurring biological events, has gained significant attention in the scientific community, particularly in understanding the impacts of climate change on ecological systems. Phenology serves as a vital tool for monitoring global environmental changes, particularly in mid-latitude regions. For instance, the "spring green wave" phenomenon illustrates how phenological data can be generalized from local to global scales, aiding in the detection of biosphere changes [33]. Around the world, phenological data is increasingly recognized as essential for natural resource

management. It helps address climate-induced changes, guiding conservation strategies and adaptive management practices [34].

The use of BBCH scale is not only limited to defining the phenology of plants but is also approved as a standard for species protection by the European and Mediterranean Plant Protection Organization (EPPOC), the Global Phenological Monitoring Network, and the European Phenology Network. In agriculture, phenological studies are useful in various cultural practices, orchard management, protection measures, optimizing crop yields and resource use [35].

Phenological stages of *L. mackliniae* was described the using the extended BBCH scale, consisting of 9 principal growth stages and 42 secondary growth stages [23]. Principal growth stage observed in *L. mackliniae* include germination, leaf development, stem elongation, booting, inflorescence emergence, flowering, development of fruit, fruit maturity and senescence. Various challenges affecting the phenology and population of the plant were observed. The study served as a tool for developing a consensus-based unified method for standardising phenological stages as well as implementing conservation strategies such as accurate timing for dwarf bamboo and control fire management [23]. Phenological stage identification is important for characterisation of germplasm and endemic plant enhancement programmes.

3.5 *Ex situ* conservation

Shirui lily can be propagated using the bulbs under proper care and successfully through in vitro propagation techniques [1, 3, 36]. An attempt was made by collecting the *L. mackliniae* and planting in pots with the available soil without treatments and special nutrient supplementation [20]. The plant survived and bloomed as in their natural habitat indicating that plant might have undergone the required changes to acclimatize the existing changed environment which outside their natural endemic habitat. Though facing stress condition in their natural habitat, it acquires more resilient characters to adapt to new habitats, thus from endemism it may become cosmopolitan in the near future [20]. Bulbs collected from the natural habitat and planted at Woodlands Compound of Botanical Survey of India, Shillong have survived and flowered indicating the possibility of further *ex situ* conservation measures. An attempt can be made to multiply it on large scale by seed culture, to meet demands [1].

3.6 In vitro propagation

In vitro propagation is one of the *ex situ* methods used for enhancing biomass and conserving germplasm of rare and threatened plants [37]. In vitro propagation can be successfully used for wild plants which are difficult to propagate through other conventional methods, when the population in nature is very less, or when a species has poor reproduction capability [37]. In vitro tissue culture techniques provide useful tools for rapid mass propagation and plant genetic resource conservation of rare and endangered *Lilium* species [38]. The development of a high-throughput micropropagation system for the production of healthy clonal plants is required for the sustainable conservation of such rare bulbous plants [39]. In vitro regeneration protocol will be helpful to augment breeding strategies in this rare endangered species for wide adaptation under changing climate conditions [36]. Many workers have carried out in vitro propagation in various *Lilium* species like *L. akkusianum* [40], *L. longiflorum* [41], *L. davidii* [42], *L.*

monadelphum [43], *L. candidum* [44], *L. polyphyllum* [45], etc. Bulb scales are the most preferred explants [40, 46, 47], however filaments, ovules [48], nodal segments [41] and leaf segments [49] have also been used.

An efficient *in vitro* regeneration protocol in *L. Mackliniae* was developed for its conservation [36]. Callus cultures were established in Murashige and Skoog (MS) medium containing different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) (1–2 mg/l). The regeneration ability in friable callus was 12-fold higher as compared to the compact callus. Direct regeneration and somatic embryogenesis were achieved in friable callus at 6–8 weeks of subculture in MS with benzyladenine (BA, 1.0 mg/l). Multiple shoot proliferation and rooting were achieved in MS + 0.5 mg/l BA and 0.5 mg/l naphthalene acetic acid (NAA), respectively. Hardening under mist house resulted in higher survivability (85%) over polyhouse (62.2%) and shed net (55.5%).

A protocol for direct and indirect shoot regeneration and proliferation was established from bulb scales of Shirui lily [3]. The researchers found that the optimal combination of growth regulators for direct shoot regeneration was obtained with MS medium supplemented with 0.5 mg/l 6-Benzylaminopurine (BAP) yielding the highest shoot induction (3.5 shoots per scale; a 96.7% response). For indirect *de novo* organogenesis, optimum callus induction was achieved with 2.0 mg/l 2,4-D and shoot organogenesis was higher (16.2) when sub-cultured onto 0.5 mg/l BAP medium. The highest shoot proliferation (10.1) occurred with 0.5 mg/l BAP and 1.0 mg/l gibberellic acid (GA3). Rooting response was 96% with 0.5 mg/l (NAA), with multiple roots per shootlet. Acclimatization using hydroponics with Hoagland's solution in a mist chamber maintained a survivality up to 92.5%. Clonal fidelity was confirmed through random amplified polymorphic DNA (RAPD) analysis.

4 Future prospects and conclusions

The rarity, endemism and rising threats to the population of Shirui lily have kept the species in a very critical condition. However, conservation approaches from various fields such as phenology, geospatial analysis, *in vitro* propagation, *in situ* and *ex situ* conservation, community efforts and government initiatives are in the right direction. Role of community in the conservation of this precious lily must be the frontline, guided by innovative scientific investigations. It is important to provide warm hospitality to visitors and proper cooperation with researchers. Sustainable tourism can aid conservation by promoting responsible visitation and generating revenue for local communities. The scenic beauty of the Shirui hill range and the renowned Shirui lily make it an ideal tourist destination. In addition to the Shirui lily, the flowering of Himalayan Snow Anemone, locally called Horemwon (*Anemone elongata*), characterized by large bloom of beautiful white flowers resembling snow-covered hills, is also a major tourist attraction in this hill track. Moreover, the Tangkhuls are known for their simplicity, hardworking and hospitality [49]. The future focus for the conservation of *L. mackliniae* should stress on holistic approach including enhancing awareness, research and conservation efforts, as well as promoting sustainable tourism and strengthening government policies. Public awareness campaigns and educational programs like poster campaigns, inclusion in academic curriculum, etc. can help expand conservation initiatives. The research is at a nascent stage and further research should investigate climate change impacts, genetic diversity and improved propagation techniques. Conservation strategies should encompass both

in situ and *ex situ* methods, such as expanding protected areas and rewilding suitable habitats. Lastly, government policies should offer stronger legal protections, increased funding, proper implementation and encourage collaborations between public, private, and community stakeholders for long-term preservation.

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Author contributions

Conceptualization and designing of the research work (WAM and NZ); Execution and data collection (WAM and NZ); Analysis of data and interpretation (WAM); Preparation of manuscript (WAM and NZ); Proofreading of manuscript (WAM and NZ).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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