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European Journal of Experimental Biology, 2016, 6(4):38-40

ISSN: 2248 –9215
CODEN (USA): EJEBAU

DNA Extraction from Siroi lily(*Lilium mackliniae*) - an endangered species using CTAB method

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ABSTRACT

Shirui lily or Siroi lily (Lilium mackliniae) is an endangered species of lily found only in the Ukhrul district of Manipur. CTAB method is used with little modification for the extraction of DNA from the young leaves of the plant. The electrophoresis result shows good quality of DNA. Further research is going on for the genetic study of this endangered lily.

Key words: Siroi lily(*Lilium mackliniae*), endangered species, CTAB method, genetic study.

INTRODUCTION

The Shirui lily or Siroi lily (*Lilium mackliniae*) is a rare Indian species of plant found only in the upper reaches of the Siroi hill ranges in the Ukhrul district of Manipur, India, at an elevation of 1,730–2,590 metres above sea level.

This shade-loving lily has pale bluish-pink petals but has seven colours when observed through a microscope. In the wild it flowers in the monsoon months of June and July. They are seasonal flowering plants and at their best in May and June when it blooms. The peak season of its bloom is May 15 to June 5. The height of the plant is 1–3 feet (0.30–0.91 m) and has one to seven flowers per plant.

Its unique characteristic is that one can't plant the flower in any part of the world besides the Siroi hill. During the British rule in Manipur, the British repeatedly attempted to propagate the flower to other places only to be faced with utter failure. After a prolonged effort, they gave up the attempt to do so. They felt Siroi lily was the daughter of the goddess, which rules the Siroi hill. Hence, one cannot separate the Siroi lily from the Siroi hills (truly endemic). It is the state flower of Manipur, but unfortunately it has become an endangered species in India. A postal stamp was issued by the Indian Postal Department to commemorate this flower.

There are no published documents about the molecular studies for the development and mass propagation of this rare lily. So far no scientific research have been reported for the genetic study of this lily.

Figure 1. Shirui Lily Flower

MATERIALS AND METHODS

DNA Extraction

The majority of DNA extraction methods from plant leaf tissue are derived from the original hexadecyltrimethylammonium bromide (CTAB) based method, described by Doyle and Doyle in 1987 [2]. Samples are collected and stored at 4ºC. Young leaves are used for the isolation of DNA.

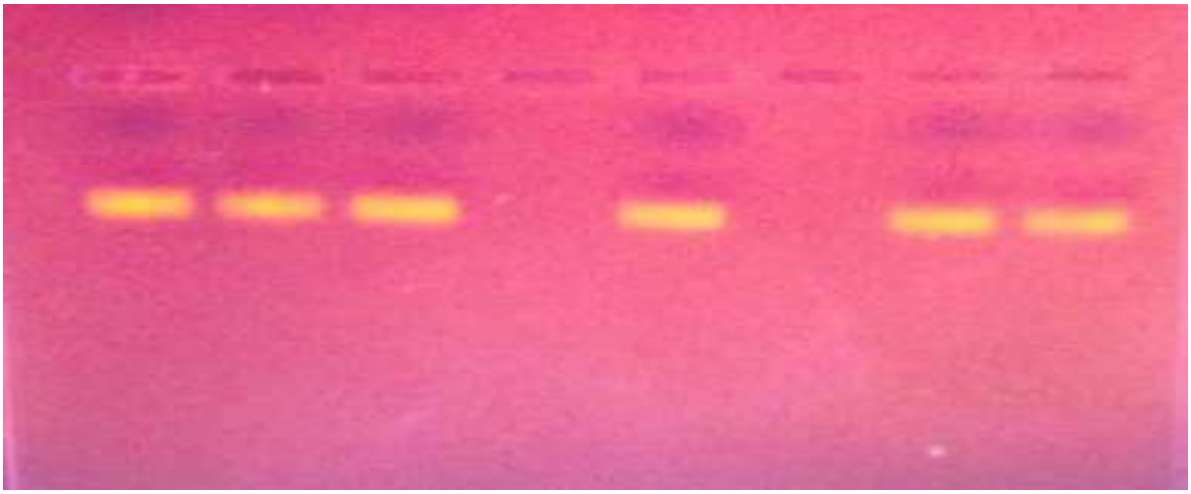
9ml of prewarmed CTAB Extraction Buffer is added to 0.3 – 0.4 mg of the lyophilized ground tissue in a sterile centrifuge tube. The samples are incubated for 60- 90 minutes, with occasional inversion at 65^0C. The samples are allowed to cool by keeping it at room temperature for 5 minutes. Then 5 ml of Chloroform: Octanol(24:1) is added and mixed gently by inverting the tubes for 5 minutes. The samples are spinned at 850 x g (≈2300 rpm) for 2 minutes at room temperature. The top aqueous layer is transferred into a fresh tube and 25 µl of RNase A is added. The sample is mixed gently by inversion and incubated for 30 minutes at room temperature. 6ml of isopropanol is added to each tube. The samples are mixed gently by inversion until a white fluffy DNA precipitate appears (it should appear within 1 minute of addition of isopropanol). Then centrifuge at 850 x g (≈2300 rpm) for 5 minutes to pellet the DNA. The supernatant is discarded. The pellet is resuspended in 8ml of cold CTAB Wash Buffer and incubated at room temperature for 20 minutes. It is then centrifuged at 850 x g (≈2300 rpm) for 5 minutes. The supernatant is discarded. 8ml of cold 70% ethanol is added to the tube containing DNA and centrifuged at 850 x g (≈2300 rpm) for 5 minutes. The supernatant is discarded. the pellet is air dried to remove the traces of ethanol. The DNA sample is transferred to a tube containing 1 ml of Elution Buffer (ET). The pellet is dissolved gently by pipetting.

DNA quantification

DNA was quantified in 4% polyacrylamide gels. Electrophoresis was conducted in a 1X TBE buffer [100 mL 10X TBE (0.89M Tris base, 0.89M Boric acid, 20 mM EDTA pH 8.0) and 900 mL distilled water]
at 60 V for 30 min and then at 120 V for 1.5 h[1].

RESULTS AND DISCUSSION

Electrophoresis was performed at constant power of 100 Watt for around 2 hrs. And when run on 1% agarose, a clear bright band (Figure 1) which shows that the DNA quantity and quality is good for further usage.



This is only the preliminary experiment of the genetic study of the Siroi lily. Further studies regarding the genetic diversity and other related research is going on using PCR amplification and other molecular tools.

Acknowledgement
We are grateful to the Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India, New Delhi, for providing official support and laboratory facilities at DBT-Biotech Hub, Pettigrew College, Ukhrul, Manipur, S. Kula Women's College, Nambol, Manipur and Kamakhya Pemton College, Hiyangthang, Manipur.

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