Random Amplified Polymorphic DNA (RAPD) Analysis of an Easter Lily Chlorophyll Mutant

Kedong Da[1,2*] Samantha Smith[1] Nicole Miller[3]

[1]The Institute for Advanced Learning and Research, 150 Slayton Avenue, Danville, Virginia 24540.

[2]Department of Horticulture, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, Virginia 24061. [3] University of Virginia, Charlottesville, VA 22908. E-mail: kedong.da@ialr.org

Figure1. An Easter lily foliage mutant was generated through in vitro culture

INTRODUCTION

Lillium is one of the most important floricultural crops for pot and cut flower production. Asiatic, Oriental and Easter hybrids are among the premium potentials in lillium trade. Easter lilies are the most popular pot flowers among various lily forms, as their bulbs bloom in early spring and require special technology for their production. Limited cultivar selection restricted Easter lily market expansion. In an effort to establish an Easter lily tissue culture in vitro breeding system, we created a leaf color mutant (Fig. 1). Random Amplified Polymorphic DNA (RAPD) technology was preferred by many researchers as an effective method to use for identification of genetic variation within and among populations in plants. Additionally RAPD markers also show levels of polymorphism similar to iso-enzyme markers. RAPD analysis also can target amplify a large number of loci. Chlorophyll content test and RAPD analysis confirmed isolated plants have lower chlorophyll content and genetic differences in DNA level. Present results provide a new way for Easter lily breeding.

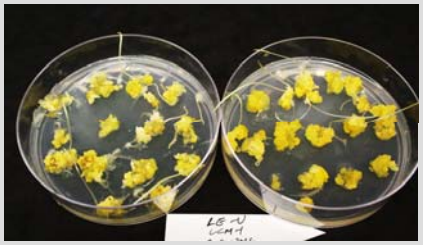

Figure 2. Callus induction and plant regeneration in Easter lily foliage mutant

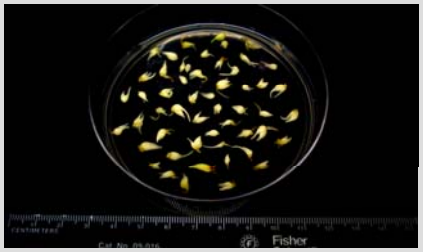

Figure 3. In vitro isolation of Easter lily foliage mutant

MATERIALS AND METHODS

Material: Immature flower bud from leaf color changed plant and wild type.

Sterilization and regeneration: flower buds from pot grow plants were collected in a clean hood and the surface was sterilized sequentially in tap water wash 1 hr → 75% ethanol 30" → 20% Clorox 20 min → sterile ddH2O wash 5 times → dip dry on sterile paper tower. Callus induction and regeneration media were MS basal medium (MSO) contain various level of BA, TDZ and NAA. Plantlet rooting medium was MS supplemented with 1g/L activated charcoal(MAC). Rooted plantlets were transferred to peat moss: pearlite: vermiculite=1:1:1 soil and acclimatized through chamber 30 days to greenhouse.

RAPD: DNA from regenerates of leaf color changed plant and wild type were subject RAPD analysis. The DNA was amplified by using a MJ Mini Thermal Cycler (BIO-RAD). The amplification was programmed at 45 cycles for 30 seconds of denaturation at 94°C, 30 seconds of annealing temperature at 36°C, 1 minute of primers extension at 72°C and final extension of 7 minutes at 72°C. PCR products were electrophoresed on 1.0% (w/v) agarose gel. Each primer was repeated three times.

RESULTS

Mutant plant exhibited growth slower compared to wild type. Various levels of yellow/white/green leaf color mutants were regenerated from the mutant. Only yellow green/green regenerates can survive through acclimatization and were transferred to the greenhouse(Fig. 4; Fig. 5). A mutant with yellow green leaf in greenhouse showed 22% less chlorophyll content(Fig. 6); RAPD analysis showed DNA level difference in primer P16-1(Fig. 7).

CONCLUSIONS

1. Easter lily foliage mutant was obtained (Fig. 1).
2. In vitro callus culture regeneration system was established in Easter lily (Fig. 2).
3. Mutant isolation system was established in Easter lily(Fig. 3).
4. Chlorophyll totally deficient type has difficulty surviving in nature.
5. RAPD results showed DNA level change of mutant compared to wild type (Fig. 7).
6. Mutant flower color identification in progress(Fig. 8).

ACKNOWLEDGEMENTS

This work was funded through operating funds provided by the Commonwealth of Virginia to the Institute for Advanced Learning and Research.

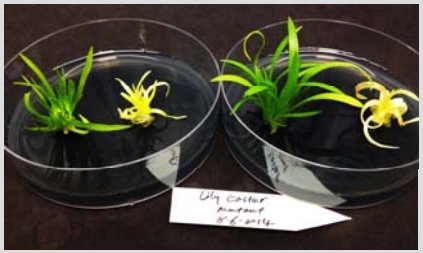

Figure 4. Yellow leaf mutant grows slower compared to yellow-green leaf mutant under in vitro condition.

Figure 5. Yellow leaf mutant has difficulty rooting in vitro and die in vivo

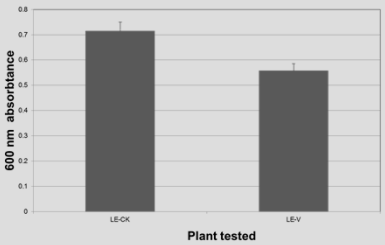

Figure 6. Chlorophyll content in control(LE-CK) and mutant(LE-V)

Figure 7. RAPD results for primer P16-1. 1. 1kb DNA ladder; 2. Easter lily wild type; 3. Easter lily mutant.

Figure 8. Easter lily chlorophyll mutants produced normal flowers.