Research Article

Inter simple sequence repeat (ISSR) based analysis of morphological variation of transgenic Kalanchoe (*Kalanchoe blossfeldiana*)

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Abstract

Inter simple sequence repeats (ISSR) analysis, using 10 primers was performed to examined morphological variation among 26 transgenic Kalanchoe (*Kalanchoe blossfeldiana*). The study of ISSR polymorphism was considered transgenic Kalanchoe into the polyphyletic group of its UPGMA tree, and analyzed with distance method (UPGMA) in PAUP 4.0b10, 5,000 replicates which comprised of 2 clades. Meanwhile, the first clade showed that sample 9 and 10 have close relationships support with 52% bootstrap, of their pointed tip. Simultaneously, the second clade revealed sample 18 and 19 have close relationships support with 76% bootstrap, of theirs oval-shaped petals and pointed tips, the sample 24 and 25 have close relationships support with 58% bootstrap that have close relationships with sample 21 support with 68% bootstrap, of theirs reddish pink flowers and pointed tips and the sample 22 and 23 have close relationships support with 75% bootstrap, with theirs reddish orange flowers, oval-shaped petals and pointed tips.

Keywords: Transgenic Kalanchoe (*Kalanchoe blossfeldiana*), inter simple sequence repeat, polymerase chain reaction

Introduction

Kalanchoe (*Kalanchoe blossfeldiana*) family Crassulaceae, It is a small shrub with a 0.50 to 1.50 feet height. The species is native to Africa and Asia. It is fast growing shrub, and the flowers are colorful and easy sprouting. Kalanchoe can help to absorb toxic substances in the air but at a low rate. It is suitable to be pot plants to decorate both indoor and outdoor and growing well in full sun. [1] The study was aimed to investigate transgenic Kalanchoe as Buddharak, et al., [2] and similar to Petchang, et al. [3] They were transformed with *DFR* gene from *Curcuma alismatifolia* 'Chiang Mai Pink' and have a morphological variation of Kalanchoe, which is different from the original Kalanchoe. Identify and analyze differences in the genetic level of Kalanchoe using Inter Simple Sequence Repeat (ISSR) markers. [4]

Material and Method

The 6 groups of transgenic Kalanchoe are as follows:

1) Red flowers, narrow petals, pointed tips include: K01, K09 and K21
2) Red flowers, Oval-shaped petals, pointed tips include: K16, K19 and K26
3) Reddish pink flowers, narrow petals, pointed tips include: K03, K07 and K24
4) Reddish pink flowers, Oval-shaped petals, pointed tips include: K02 and K25
5) Reddish orange flowers, narrow petals, pointed tips include: K05, K06, K08, K12, K13, K15 and K20
6) Reddish orange flowers, Oval-shaped petals, pointed tips include: K04, K10, K11, K13, K14, K17, K18, K22 and K23

The leaves of 26 transgenic Kalanchoe were grinding to a powder in mortar with pestle and liquid nitrogen.

All samples were DNA extraction with the NucleoSpin ® Plant II Kit, follow to the DNA extraction kit manual of MACHEREY-NAGEL.

DNA templates were amplified by PCR with ISSR technique, with a master mix of the set PCR; *i*-Taq™ plas DNA polymerase of iNtRON Biotechnology. Of 1 reaction include: 1) 10X PCR Buffer 2 µL, 2) 2.5 mM dNTPs 2 µL, 3) DNA template 1 µL, 4) Primer 2 µL, 5) Deionized water 12.7 µL and 6) 1 Unit Taq Polymerase 0.3 µL.

Total volume is 20 µL, using 10 ISSR primers, the sequence as follow:

ACGAATTCATGGTCCGGTGAAGTGTTCG,
TAGAATTCCCCGGTTCGCTCGCCGTTAC,
ATGCGATACTTGGTGTGAAT,
GACGCTTCTCCAGACTACAAT,
$T(GA)_8$,
$T(AG)_7A$, $A(AG)_8$,
$C(TG)_7GTG$, $(CA)_9$,
$G(AC)_8A$

Then the steps of PCR cycles were followed: initial denaturation 94°C, 2 min; denaturation 94°C, 30 sec; annealing 48, 45°C, 30 sec; extension 72°C, 2 min; and final extension 72°C, 7 min. It should be notes that the repeat steps 2 to 4 were performed for 35 rounds. PCR products were estimated DNA fragments by 1.5% agarose gel electrophoresis, 100 volts for 30 minutes and viewed under UV light. The DNA fragments were analyzed with the number “1” and “0” by appear and disappear of DNA banding. The data were analysed for the phylogenetic tree using the Distance method (UPGMA) in PUAP 4.0 b10 with 5,000 bootstrap replicates.

Results

The DNA fragments revealed 100% polymorphism. **(Figure 1)** The distance diagram of UPGMA tree revealed that 26 transgenic Kalanchoe were examined into a polyphyletic group that comprised of 2 clades support with 50% bootstrap. **(Figure 2)** The first clade sample 9 and 10 showed close relationships support with 52% bootstrap, of their pointed tip. Meanwhile, the second clade revealed sample 18 and 19 have close relationships support with 76% bootstrap, of their oval-shaped petals and pointed tips, the sample 24 and 25 have close relationships support with 58% bootstrap that have close relationships with sample 21 support with 68% bootstrap, of theirs reddish-pink flowers and pointed tips and the sample 22 and 23 have close relationships support with 75% bootstrap, with theirs reddish orange flowers, oval-shaped petals and pointed tips. **(Figure 3)**

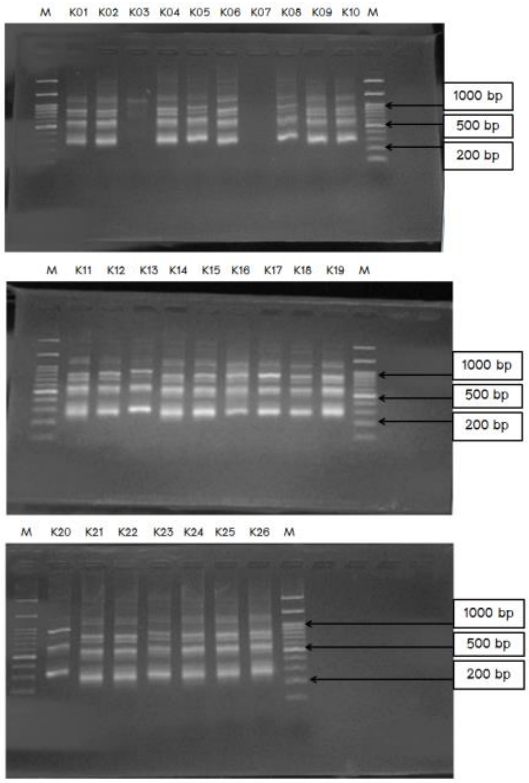

Figure 1 DNA fragments with primer $T(GA)_8$

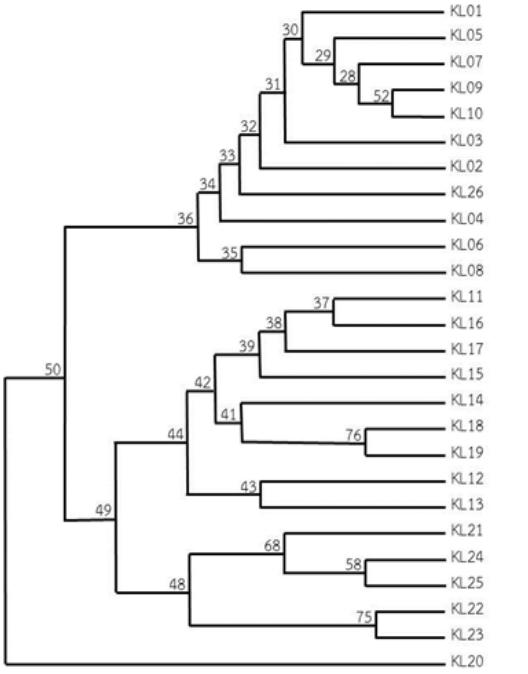

Figure 2 UPGMA tree of 26 transgenic Kalanchoe

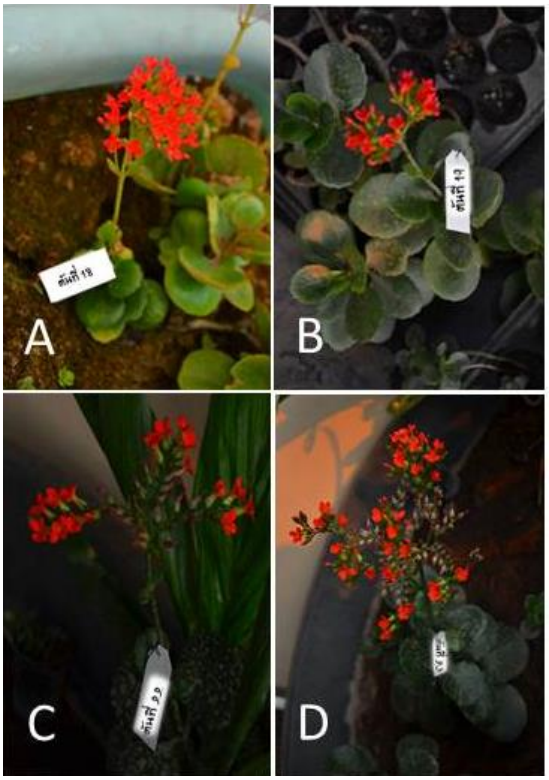

Figure 3 Morphology of Kalanchoe
A) K18, B) K19 C) K22, D) K23

Discussion

With the morphological variation of 26 transgenics, Kalanchoe could not clarify the relationship, classification of the Kalanchoe, and application of molecular biology techniques to examine the Kalanchoe. This study is consistent with a study in northeast Thailand. [5] ISSR molecular markers are capable of classifying the genetic relationship of organisms to a certain degree. Sometimes the results may not be specific. The results of this study can't be separated by geographical conditions, and consistent with a study in China, [6] using ISSR technique to study morphology to detect genetic variation of *Camellia sinensis* 39 species using 40 primers, there are only 15 primers that can distinguish similarly cultured *C. sinensis* strains, likewise in *Canarium album* and *Penthorum chinense*, [7,8] and could be

applied in the hop (*Humulus lupuls*). [9]. In the next trial, should be using more primers or other molecular biology techniques.

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