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Diversity analysis of Asiatic lily using sequence related amplified polymorphism (SRAP) markers

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Abstract
Genetic diversity within lily is the key to genetic improvement of this important ornamental species. In the present study, genetic diversity of 15 lily genotypes was assessed through Sequence Related Amplified Polymorphism (SRAP) markers. SRAP analysis was carried out using eighteen primer combinations out of which, ten primer combinations revealed a total of 122 scorable bands, among which 86 were polymorphic with an average of 8.6 polymorphic bands per pair of primers. The percentage of polymorphism ranged from 81.82 to 40.00. The similarity matrix coefficient ranged from 00 to 67 percent, suggesting a low to moderate genetic variation within lily genotypes. On comparing the genetic diversity as revealed by the dendrogram, it was evident that Telisker and Merluza were identified quite distinct from other genotypes. The SRAP profiles generated were evaluated for studying the Jaccard's similarity coefficient. The similarity matrix coefficient ranged from 00 to 67 percent, suggesting a low to moderate genetic variation within lily genotypes. On comparing the genetic diversity as revealed by the dendrogram, it was evident two genotypes were identified quite distinct from other genotypes.

Keywords: Genetic diversity, correlation, Asiatic lily genotypes, SRAP markers

Introduction
Lilium is one of the top ten cut flowers. In the language of flowers, lily is the symbol of chastity and innocence. In America *Lilium longiflora* is a national symbol of pride and hope. Although white lilies are associated with funerals, generally lilies are perfect flowers for many different festive occasions. Asiatic lily (*Lilium* spp.) which adds a touch of elegance to garden, belonging to the family Liliaceae, is one of the most important bulbous crops grown commercially in different parts of the world, including India for cut flowers, landscape perennials as well as potted plants. Lily color range has been widely extended to include a vast array of both bright and pastel hues as well as multicolored flowers often with darker colored spots on lighter base petals. The blooms are red, pink, orange, yellow, lavender or white in colour and also lilies like their feet in the shade and faces in the sun. All *Lilium* species are diploid (2n=2x=24), except some triploid forms of *L. tigrinum* and L. *Bulbiferum existing* in nature. The genome size of *Lilium* belongs to one of the largest in plant kingdom. There are about 7000 registered genotypes of lily. International Lily Register is maintained under international agreement by the Royal Horticultural Society in London (Singh, 2006) [10].
Traditionally the diversity in germplasm of crop species was estimated by morphological characters but these are few in number and highly influenced by environmental conditions. To overcome these influences there is a need to use molecular markers. Molecular approaches collectively represent a potential tool that can be applied for effective characterization of germplasm. It addresses the limitations associated with morphological and biochemical processes.
A common approach for assessing levels of genetic diversity is the use of molecular markers such as Sequence-Related Amplified Polymorphism (SRAP) is designed to amplify open reading frames (ORFs) based on specially designed primer pairs (Li and Quiros 2001) [4]. As one of the DNA-based markers, SRAPs is designed to amplify open reading frames (ORFs) based on specially designed primer pairs (Li and Quiros 2001) [4]. Compared with other marker systems, SRAP markers have been successfully used to study genetic diversity and relationships because of its simplicity, reproducibility and disclosure of multiple markers from a single two-primer reaction, when compared with other marker systems. Molecular markers have previously been used successfully to assess levels of genetic diversity genotype identification using various molecular techniques, including Amplified Fragment Length

Polymorphism (AFLP (Pragya *et al.*, 2010b) [7], Random Amplified Polymorphic DNA (RAPD) (Kanika and Krishan, 2014 and Takatsu *et al.*, 2001) [3, 11] and Sequence-Related Amplified Polymorphism (SRAP) (Geeta *et al.* 2014) [2].

Material and Methods

A total of 15 Asiatic lily genotypes representing majority under cultivation in India were analyzed using morphological traits and selected SRAP markers. The materials were planted in randomized block design with 3 replications at experimental block of Department of Floriculture and Landscape Architecture, College of Horticulture, Mudigere, India, during 2014–2015 for morphological characterization. The experimental site is situated at situated in the Western Ghats and represents the typical hill zone (Zone-9 and Region-V) of Karnataka and lies at $13^0$25' North latitude and $75^0$25' East longitude with an altitude of 980 m above mean sea level. The plant material used for the study comprised of 15 lily genotypes viz., Advantage, Bright Diamond, CEB Dazzle, Dazzle, Courier, Mestre, Telisker, Batistero, Pirandeu, Merluza, Fangio, Pavia, Ercolania, Tresor and Navona. The color characteristics of genotypes are presented in Table 1 and molecular analysis were carried out in Division of Biotechnology, Indian Institute of Horticultural Research, Bangalore.

Table 1: Colour characteristics of 15 Asiatic lily genotypes

Sl. No.	Genotypes	Colour
1	Advantage	Orange
2	Bright Diamond	White
3	CEB Dazzle	Yellow
4	Dazzle	Yellow
5	Courier	White
6	Mestre	Light pink
7	Telisker	Orange
8	Batistero	Red
9	Pirandeu	Dark pink
10	Merluza	White
11	Fangio	Red
12	Pavia	Yellow
13	Ercolania	White
14	Tresor	Orange
15	Navona	White

DNA extraction and SRAP-PCR analysis

Total genomic DNA from fresh young leaves was extracted using a modified cetyl trimethyl ammonium bromide (CTAB) method as per the protocol of Doyle and Doyle (1987) [1]. The quality of DNA was determined using agarose gel (0.8%) electrophoresis and stored at -20ºC until use. Polymerase chain reaction (PCR) for SRAP was carried out in 25 µl reaction mixture as described by Mishra *et al*. (2011) [5]. A reaction buffer consisting 40 ng/µl of genomic DNA, 3.0 unit of Taq DNA polymerase, 10mM of each dNTPs, 20 mM MgCl2 and 3pM each of forward and reverse primers. PCR amplification was performed under the following conditions: 7 minutes initial denaturation at 94 °C; 5 cycles consisting of 1 minute denaturation at 94 °C, 1 minute primer annealing at 35 °C and 1 minute extension at 72 °C followed by 30 cycles consisting of 1 minute denaturation at 94 °C, 1 minute primer anneling at 50 °C and 1 minute extension at 72 °C and final extension of 10 minutes at 72 °C. The amplified products were resolved by electrophoresis on 1.5% agarose gel and stained with 6 µg/100 ml ethidium bromide. Amplified bands were scored as '1' for presence and '0' for the absence of bands for all samples. The resulting presence/absence data matrix was analysed using NTSYS-PC Ver. 2.02 software (Rohlf, 2000) [9] to estimate the level of genetic diversity and the resultant similarity matrix was subjected to Unweighed Pair Group Method with Arithmetic Average (UPGMA) to construct the dendrogram.

Results and Discussion

The results of SRAP in this study show good amplification, stability and reproducibility with easily found polymorphism. Genetic diversity of 15 lily genotypes using eighteen primer combinations was screened. Out of eighteen primer combinations, ten primer combinations amplified among which, ten primer combinations (Table 2) were polymorphic that revealed a total of 122 scorable bands, among which 86 were polymorphic with an average of 8.6 polymorphic bands per pair of primers. The amplification products obtained with different primer combinations are illustrated in (Fig 1). Among ten combinations screened Me 1 +Em 13 scored maximum number of polymorphic bands (18) followed by Me 2 + Em 4 (16) and Me 5 + Em 3 (15 bands), while Me 5 + Em 1 (03), Me 5 + Em 1 (04), Me 2 + Em 2 and Me 2 + Em 3 (02 each) produced minimum number of bands. The combinations of primer Me 1 + Em 13, Me 2 + Em 4 and Me 5 + Em 2 produced highest polymorphism of 81.82, 80.00 and 80.00 percent respectively, followed by Me 5 + Em 3 (75.00%), where as primer combination Me 2 + Em 2 produced least polymorphism of 40 percent (Table 3). This was comparable with Geeta *et al.* (2014) [2] in *Gladiolus hybridus* Hort. and Rashmi *et al.* (2016) [8] in Gladiolus. The similarity matrix coefficient ranged from 00 to 67 percent (Table 4), suggesting a low to moderate genetic variation within lily genotypes. The highest genetic similarity of 67 percent was observed between Navona and Ercolania followed by Tresor and Advantage of 60 percent, while least genetic similarity index of 00 percent. Similar conclusion was drawn by Geeta *et al.* (2014) [2] reported greatest similarity was found between Charms Flow and Green Bay (Jaccard coefficient = 0.73), while Jester Gold was identified quite distinct variety among 15 gladiolus genotypes screened by SRAPs marker system and Pragya *et al.* (2010b) [7] reported greatest similarity was found between Pusa Lohit and Pusa Swarnima (Jaccard coefficient= 0.788), while Pusa Gunjan was found to be the most distinct genotype by AFLP marker in gladiolus.

Table 2: Sequences of SRAP forward and reverse primer and primer combinations used for fingerprinting and diversity

Forward primer		Reverse primer		Primer combinations	
Name	Sequence (3'-5')	Name	Sequence (3'-5')	Forward	Reverse
ME 1	TGA GTC CAA ACC GGA TA	EM 1	GAC TGC GTA CGA ATT AAT	ME 1	EM13/EM 14
ME 2	TGA GTC CAA ACC GGA GC	EM 2	GAC TGC GTA CGA ATT TGC	ME 2	EM2/EM3/ EM4
ME 3	TGA GTC CAA ACC GGA AT	EM 3	GAC TGC GTA CGA ATT GAC	ME 3	EM3
ME 4	TGA GTC CAA ACC GGA CC	EM 4	GAC TGC GTA CGA ATT TGA	ME 4	EM3
ME 5	TGA GTC CAA ACC GGA AG	EM 13	GAC TGC GTA CGA ATT CTG	ME 5	EM1/EM2/ EM3
		EM 14	GAC TGC GTA CGA ATT CTT		

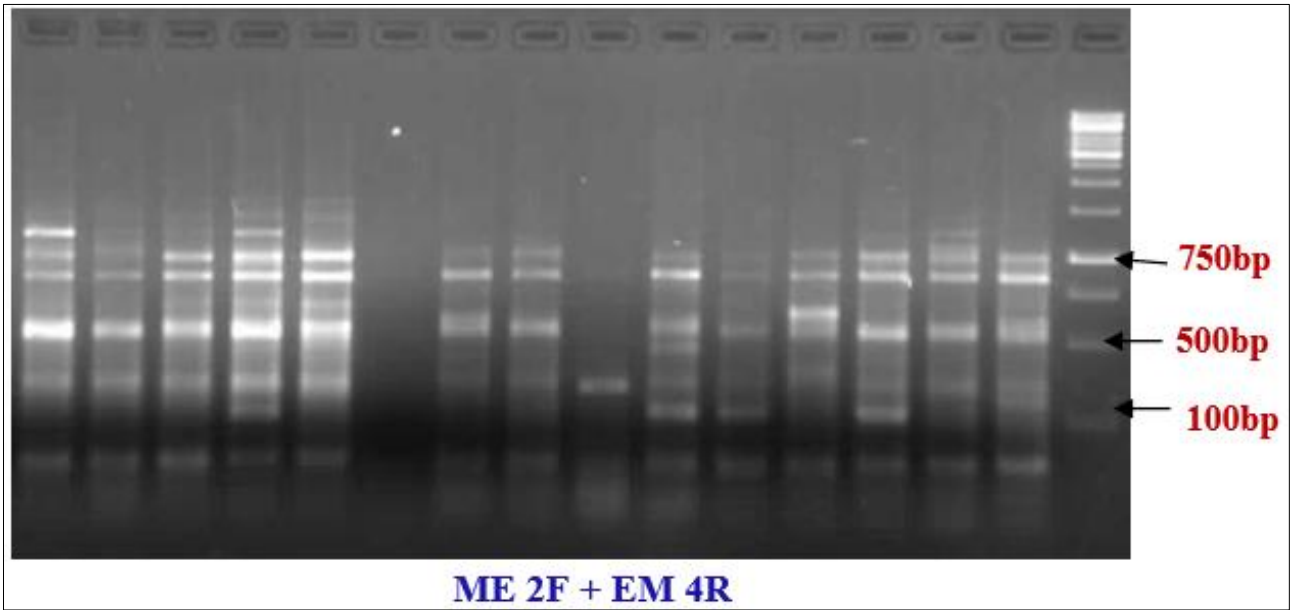

Fig 1: Gel profile of 15 lily genotypes using ME 2F + EM 2R SRAP primer combination

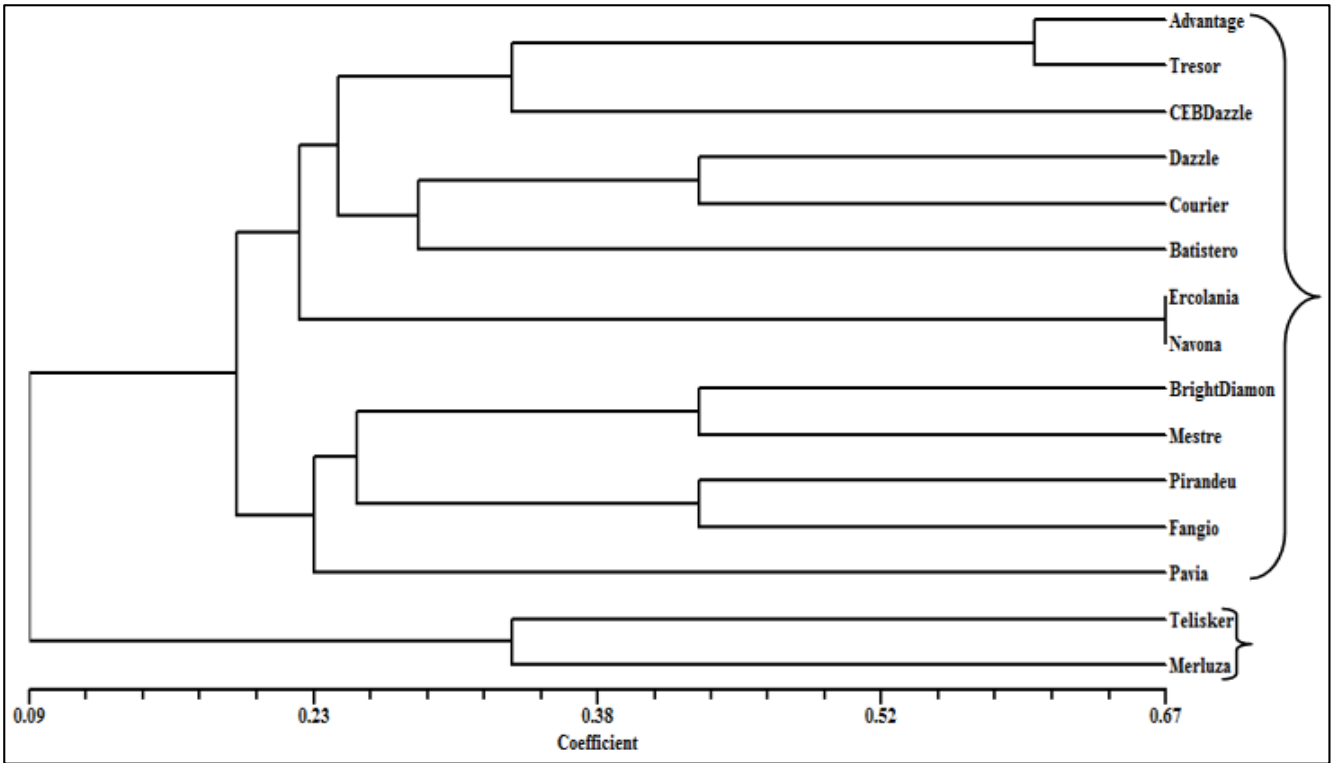

Fig 2: Dendrogram showing genetic relationship among 15 lily genotypes based on SRAP markers according UPGMA analysis

Table 3: Polymorphism rates for the 15 lily genotypes related to ten SRAPs primer combination

SL. No.	Primer combination	No. of Polymorphic bands	No. of Bands produced	Polymorphism (%)
1	Me 1 + Em 13	18	22	81.82
2	Me 1 + Em 14	9	14	64.29
3	Me 2 + Em 2	2	5	40.00
4	Me 2 + Em 3	2	4	50.00
5	Me 2 + Em 4	16	20	80.00
6	Me 3 + Em 3	10	16	62.50
7	Me 4 + Em 3	7	11	63.54
8	Me 5 + Em 1	3	5	60.00
9	Me 5 + Em 2	4	5	80.00
10	Me 5 + Em 3	15	20	75.00
Total		86	122	65.72
Mean		8.6	12.2	

Table 4: Similarity co-efficient of lily genotypes (listed in material and methods) by using SRAP marker

@	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.11	1.00													
3	0.33	0.11	1.00												
4	0.28	0.37	0.28	1.00											
5	0.12	0.22	0.28	0.42	1.00										
6	0.14	0.42	0.33	0.12	0.12	1.00									
7	0.20	0.14	0.00	0.16	0.00	0.00	1.00								
8	0.33	0.25	0.14	0.28	0.28	0.14	0.20	1.00							
9	0.12	0.22	0.28	0.25	0.11	0.28	0.16	0.28	1.00						
10	0.20	0.00	0.00	0.00	0.00	0.00	0.33	0.20	0.16	1.00					
11	0.12	0.22	0.28	0.25	0.25	0.28	0.16	0.12	0.42	0.00	1.00				
12	0.16	0.28	0.16	0.4	0.14	0.16	0.00	0.16	0.33	0.25	0.14	1.00			
13	0.16	0.12	0.40	0.33	0.33	0.16	0.00	0.16	0.14	0.00	0.14	0.20	1.00		
14	0.60	0.11	0.33	0.28	0.12	0.33	0.20	0.33	0.28	0.20	0.28	0.16	0.16	1.00	
15	0.20	0.14	0.20	0.16	0.16	0.20	0.00	0.20	0.16	0.00	0.16	0.25	0.67	0.20	1.00

Cluster analysis

The dendrogram based on UPGMA clustering (Fig 2) resulted in the identification of two major clusters. The major cluster I in dendrogram is further divided into two subclusters. The major cluster I in dendrogram is further divided into two sub clusters. Sub cluster I again divided into three groups; group I, group II and group III. Group I consists of genotypes Advantage, Tresor and CEB Dazzle. Group II includes Dazzle, courier and Batistero. Group III includes two genotypes Ercolania and Navona. Sub cluster II again divided into two groups; group I again divided in to two subgroups, sub group I of Bright Diamond and Mestre. Sub group II includes Pirandeu and Fangio. Group II consist a single variety Pavia. Major cluster II consists of two genotypes Telisker and Merluza. All genotypes share more than 15 percent similarity among them, this could have happened due to highly heterozygous nature of the crop. Similar results were reported by Pragya *et al.* (2010a) [6] in gladiolus reveled that in cluster analysis by RAPD marker, the cultivar 'Pusa Lohit' branched out from the dendrogram, confirming that it is quite different from all other genotypes. Although major emphasis of the present investigation was to characterize the genotypes using SRAP marker, the data also revealed the genetic relationship among the genotypes. Molecular markers have been proved to be a fundamental and reliable tool for fingerprinting of genotypes.

On comparing the genetic diversity as revealed by the dendrogram, it was evident that Telisker and Merluza were identified quite distinct among 15 Asiatic lily genotypes.

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