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Genetic Diversity in *Acalypha indica* L., (Copper leaf) through RAPD analysis

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ABSTRACT
In the present study, *Acalypha indica* L., plants were treated with SA & JA alone T2 (1.0mM SA), T3 (3.0mM SA), T4 (2.0 µM JA), T5 (4.0 µM JA) and combination of hormones like T6 (1.0mMSA + 2.0µM JA), T7 (1.0mM SA+4.0µM JA), T8 (3.0mMSA+2.0µM JA) and T9 (3.0mM SA + 4.0µM JA), T1 (control). The treatment was conducted for 25, 40 and 55 days and results were observed. After 55 days, genetic diversity was investigated using RAPD primers. All the numerical and taxonomical analysis was conducted using the NTSYS-PC software version 2.0 and cluster analysis of the complete data is done. The population which showed high percentage of polymorphism was selected. The samples from this population were chosen for further photochemical analysis and the active principle has been quantified. The population which exhibited both high percentage of polymorphism and high amount of active principle was considered to be the superior genotype.
Key words: *Acalypha indica* L., genetic diversity, RAPD primers, NTSYS-PC, JA (Jasmonic acid), SA (Salicylic acid).

Introduction:

Nature has a good source of medicinal components for ancient time and most of the drugs were isolated from the nature which is playing an important role in human health care. The majority ofthe world population based on mainly traditional medicine for primary health care's.

Plant based traditional medicine continue to play an important role in human health. The plant products can be traced back to over five thousand years ago as there is evidence of its use in the treatment of diseases and for revitalizing body systems in Egyptian, Chinese, Greek and Roman Civilizations Recently, the people of Asian countries like India were utilizing plants as a part of their routine health lives (Perumalsamy et al., 2008). The plant-based medicine was prominently used in India and China for curing diseases (Duraipandyan et al., 2007). In India, the plant derived traditional used in various methods like Ayurveda, Siddha,, Unani and homeopathy. Because, India has an abundant source of plant flora throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods by Ayurveda and Homeopathy (Srinivasan et al., 2007). Phyto-medicines area major component of traditional system of healing in developing countries, which have been an integral part of their history and culture (Arif et al., 2009).

Medicinal agents from the plants plays an important role on traditional and western medicine and plant extracts have been used from the ancient time. Some of the plant derivative drugs used for different diseases like analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardio tonics (digoxin) and antimalarials (quinine). The drug discovery from plants iscontinues to provide new drug which are used against different pharmacological diseases (Ramawat et al., 2009). The bioactive compounds have been emerging source for inventing new drug molecules and their chemical structure and ability acts on various diseases (Bhutani and Gohli, 2010). The natural products derived from medicinal plants have been using from thousands of years to treat the diseases. Even though, number of medicinal plants yet to be explored for their medicinal importance (Hassan, 2012).

Plants have wide variety of phytochemcials like, Terpinoids, Tannins, Alkaloids, Flavonoids, Saponins, Anthraquinones and which are having antimicrobial activities. Most of thephotochemical are synthesis from primary metabolic such as amino acids, carbohydrates andfatty acids and are generally categorized as primary metabolites and secondary metabolites are derived from products of primary metabolism (Ramawat et al.., 2009). The plants containmedicinal activities such as, antioxidant, antimicrobial and other properties due to presence ofsecondary metabolites in them (Adesokan et al., 2008).

The plant bio active compounds can help the plant to adapt with different conditions like biotic and abiotic stresses and also help in plant growth and development (Kessler and Kalske, 2018 ; Zaynab et al., 2018 ;). The antimicrobial activity is a key to know the ability of plants. The plants could be a prominent solution for different microbial diseases and they have active mechanism from pets and pathogens by producing bioactive compounds (Magallon and Hilu, 2009;). In the present work, we were focused mainly on the action of the commonly on genetic polymorphism by using RAPD Primers. RAPD markers are decamer (10 nucleotide length) DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way. It is used to analyze the genetic diversity of an individual by using random primers. In this paper, the principles, working mechanism, differences between standard PCR and RAPD -PCR, characteristics, laboratory steps, data analysis and interpretation, advantages and disadvantages and several of the most common applications of RAPD markers in biology are discussed. his technique always allows the examination of genomic variation without prior knowledge of DNA sequences7 and is especially useful for unzipping the variations in species with low genetic variability when other techniques such as isozyme analysis fail to reveal differences among the individuals8,9. Keepingall these in purview the present work was undertaken to estimate genetic variation in thegermplasm of six rice cultivars using RAPD technique for future use in selection, hybridization, biodiversity assessment, evaluation and conservation of diverse gene pools, etc.

MATERIALS AND METHODS:

Genetic diversity analysis of *Acalypha indica* by RAPD primersDNA isolation from *Acalypha indica* leaves:

Genomic DNA was isolated from leaf tissue by modified procedure of CTAB method as described by Murray and Thompson, (1980). Total genomic DNA was isolated from tender leaves of the eight diverse lines that were collected from different hormone generated plants which are maintained in botanical garden in Telangana University. After RNase treatment, DNA solution was purified with the standard phenol: chloroform method. DNA solution was mixed gently with phenol: chloroform (1:1) and centrifuged at 5000 rpm for 10 minutes at room temperature. The aqueous phase was separated and mixed with an equal volume of chloroform, mixed gently and centrifuged at 5000 rpm for 10 min at room temperature.

The aqueous phase was separated and mixed with two volumes of absolute ethanol and incubate at -200C for 20 min. The DNA pellet was spooled out with a glass hook, wash with 70% aqueous ethanol. The DNA pellet was air dried for 20 min and dissolved in an appropriate volume of sterile TE buffer (10 mMTris-HCl pH 8.0, 1 mM EDTA pH 8.0) and stored at 4oC. The concentration of DNA was determined spectrophotometrically and the quality of DNA was checked through agarose gel electrophoresis. DNA is quantified using double beam uv-visible spectrophotometer at 260 nm & 280 nm. The DNA samples were diluted to 10 ng/µl. and further used for PCR amplification of RAPD and ISSR primers.

Results:

RAPD studies

Primers obtained from Bioartist Private limited, Hyderabad, India. Were used to amplify DNA of

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Nine varieties plant hormone treated plants (which include parental line from Control). PCR reaction were carried out in 20 µl reaction made of 40 ng of DNA as template, 1xPCR buffer,250 µm of each dNTP, 5 Pico moles of primer and 1unit of tag DNA polymerase. A 40 cycles PCR reaction is set with all the above mixture programme is set with 95°C of initial denaturation for 5 minutes, followed by 40 cycles of 94°C of denaturation for 1 minute, 35°C of annealing temperature for 1 minute, 72°C of extension for 1 minute and finally final extension at 72°C for 10 minutes. The amplified product is resolved in 1.5% Agarose gel stained with ethidium bromide in 0.5xTBE buffer with 100 bp gene ladder at one end of the gel. The RAPD bands werescored on the basis of presence or absence of band.

Discussion:

SCORING THE DATA AND ANALYSIS

Reproducible bands were scored in all the samples for each of the primer separately. Each amplification was considered as a separate marker. The presence of the amplified product in each variety was recorded as 1 and absence as 0 respectively. All the numerical and taxonomical analysis was conducted using the NTSYS-PC software version 2.0 and cluster analysis of the complete data is done. Similarly matching (SM) dice coefficient values for pair wise comparison between accessions were calculated and a dice coefficient matrix was constructed using the SIMQUAL subroutine. This matrix was subjected to un weighted pair wise group method using arithmetic average analysis (UPGMA) to generate a dendrogram using SAHN sub routine and TREE PLOT of NTSYS-PC. The similarity indices were calculated across all possible pair wise comparisons of individuals within and among the population, following the method of Nei and Li (1979). The formula was: SI = 2 NXY / (NX + NY).

List of primers for RAPD analysis

S.No	Primers	Sequence	Tm (^{0}C)
1	OPA-18	AGGTGACCGT	**35**
2	OPB-12	CCTTGACGCA	**35**
3	OPB-17	AGGGAACGAG	**35**
4	OPC-04	CCGCATCTAC	**36**
5	OPC-16	CACACTCCAG	**35**
6	OPC-20	ACTTCGCCAC	**38**
7	OPD-06	ACCTGAACGG	**37**
8	OPD-20	ACCCGGTCAC	**35**
9	OPE-01	CCCAAGGTCC	**33**
10	OPG-04	AGCGTGTCTG	**34**
11	OPH-05	AGTCGTCCCC	**35**
12	OPK-16	GAGCGTCGAA	**36**
13	OPT-17	CCAACGTCGT	**39**
14	OPX-11	GGAGCCTCAG	**36**
15	OPY-11	AGACGATGGG	**35**
16	OPA01	CAGGCCCTTC	**37**

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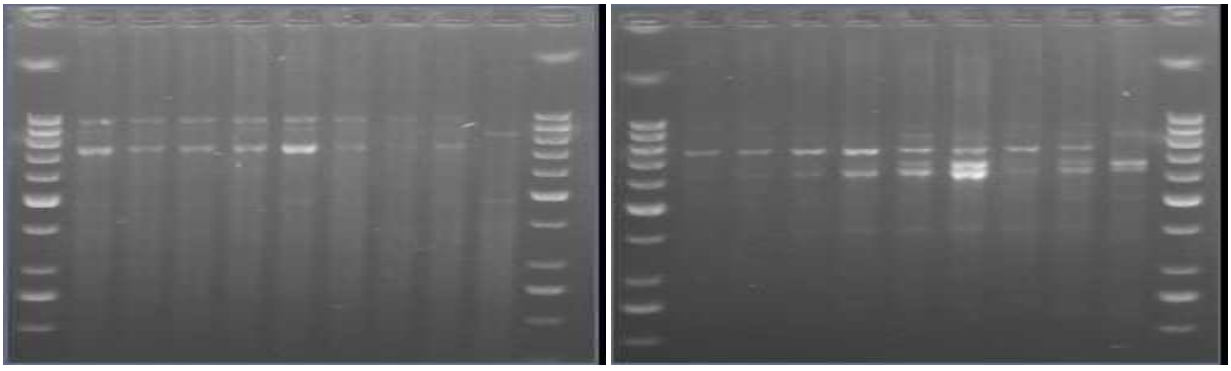
17	OPA02	TGCCGAGCTG	**35**
18	OPA03	AGTCAGCCAC	**37**
19	OPA04	AATCGGGCTG	**39**
20	OPA05	AGGGGTCTTG	**33**
21	OPA07	GAAACGGGTG	**34**
22	OPA10	GTGATCGCAG	**33**
23	OPA12	TCGGCGATAG	**35**
24	OPA13	CAGCACCCAC	**37**
25	OPB01	GTTTCGCTCC	**38**

50kb T1T2 T3 T4 T5 T6T7T8T9 50kb 50kb T1 T2 T3 T4 T5 T6 T7 T8 T9 50kb



a) OPA-01 **b) OPC-16**

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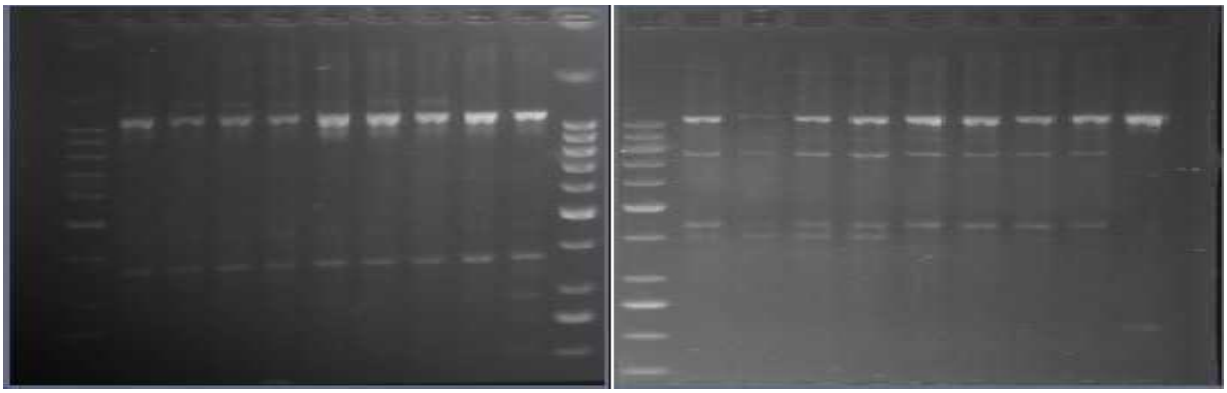

C)OPB-17 **D) OPA-18**

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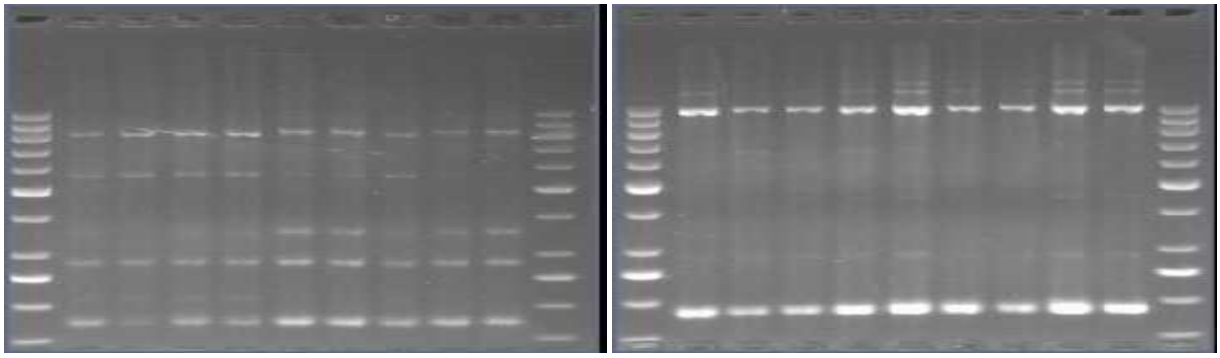

E) OPH-05 F) OPB-12

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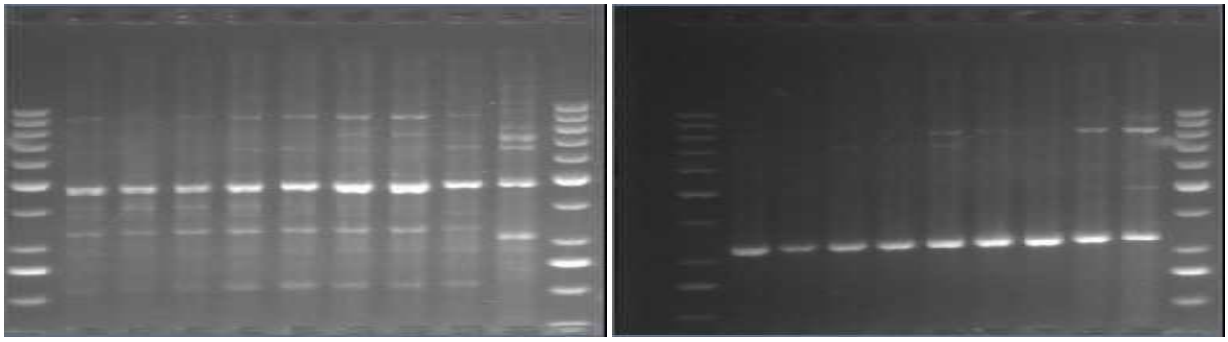

G)OPX-11 H)OPA-07

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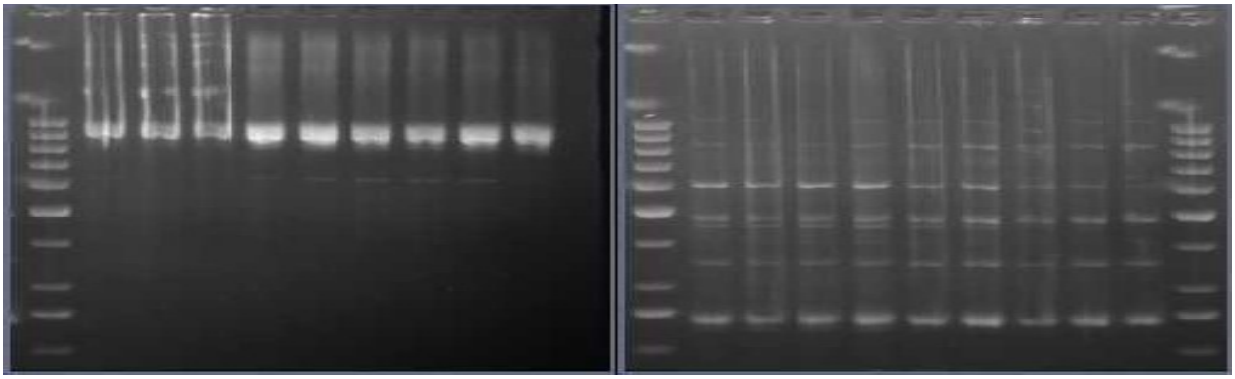

I) OPC-20 J)OPE-01

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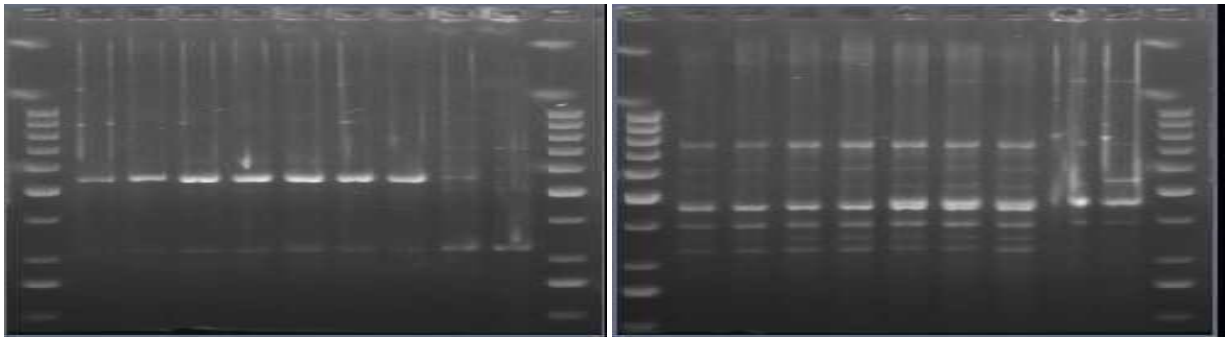
T9 50kb 50kb T1 T2 T3 T4 T5 T6 T7 T8
T9 50kb



K) OPG-04 L) OPK-16

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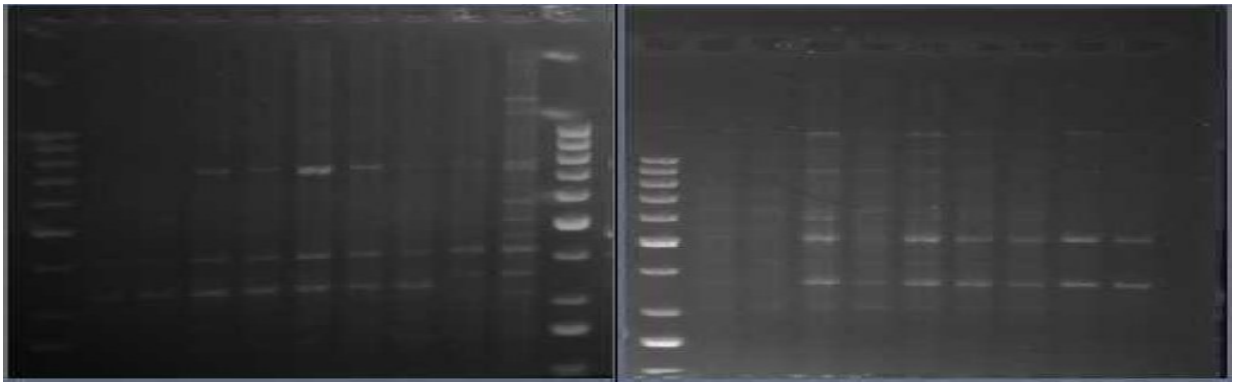
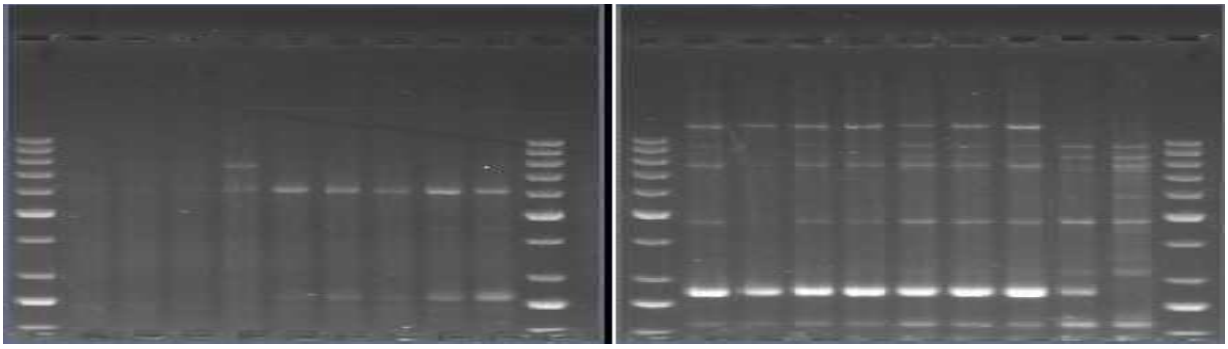


T2 T3 T4 T5 T6 T7 T8 T9 50kb

M) OPB-01 N) OPD-20

50kb T1 T2 T3 T4 T5 T6 T7 T8 T9 50kb 50kb T1 T2



T3 T4 T5 T6 T7 T8 T9 50kb

O) OPB-17 P) OPA-10

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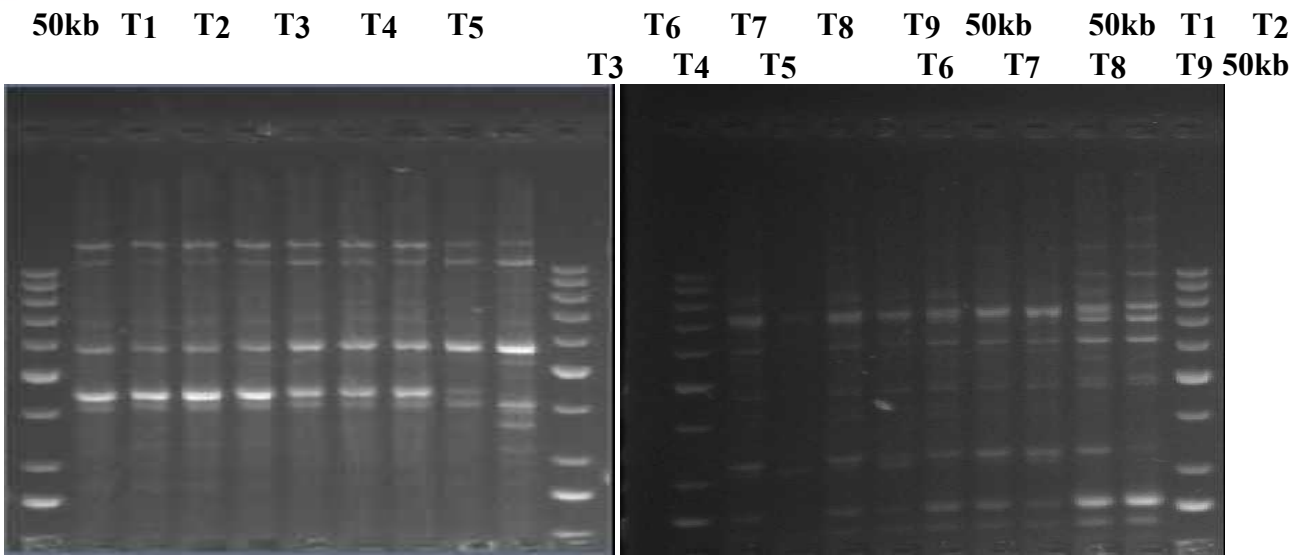

Q)OPA-01 **R) OPA-03**

DNA banding analysis

	T1	T2	T3	T4	T5	T6	T7	T8	T9
1OP1A	0	1	0	0	0	0	1	1	1
2OP1B	1	0	1	1	1	1	0	0	0
3OP2A	1	1	1	1	1	1	1	0	1
4OP2B	0	0	0	0	0	0	0	1	0
5OP3A	1	1	1	1	1	1	1	0	1
6OP3B	0	0	0	0	0	0	0	1	0
7OP4A	1	1	0	0	0	0	0	0	1
8OP4B	0	0	1	0	0	0	0	0	0
9OP4C	0	0	0	1	1	1	1	1	0
10OP5A	1	1	1	1	1	1	1	1	1
11OP6A	1	1	1	1	1	1	0	0	1
12OP6B	0	0	0	0	0	0	1	1	0
13OP7A	1	1	1	1	1	1	1	1	1
14OP8A	1	1	1	1	1	1	0	0	1
15OP8B	0	0	0	0	0	0	1	1	0
16OP9A	1	1	1	1	1	1	1	1	1
17OP10A	1	1	0	0	0	0	0	1	1
18OP10B	0	0	1	1	1	1	1	0	0
19OP11A	1	1	1	1	1	1	1	1	1
20OP12A	1	1	1	1	1	1	1	0	1
21OP12B	0	0	0	0	0	0	0	1	0
22OP13A	1	1	1	1	1	1	1	1	1
23OP14A	1	1	1	0	0	1	0	0	1
24OP14B	0	0	0	1	1	0	1	1	0
25OP15A	1	1	1	1	1	1	1	0	1

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26OP15B	0	0	0	0	0	0	0	1	0
27OP16A	1	1	1	1	1	1	1	1	1
28OP17A	1	1	1	1	1	1	1	0	1
29OP17B	0	0	0	0	0	0	0	1	0
30.OP18A	1	1	1	1	1	1	1	0	1
31.OP18B	0	0	0	0	0	0	0	1	0

3.Similarityindex table

	T1	T2	T3	T4	T5	T6	T7	T8	T9
T1	1.0000								
T2	0.9355	1.0000							
T3	0.8710	0.8065	1.0000						
T4	0.8065	0.7419	0.8710	1.0000					
T5	0.8065	0.7419	0.8710	1.0000	1.0000				
T6	0.8710	0.8065	0.9355	0.9355	0.9355	1.0000			
T7	0.6129	0.6774	0.6774	0.8065	0.8065	0.7419	1.0000		
T8	0.2903	0.3548	0.2258	0.3548	0.3548	0.2903	0.5484	1.0000	
T9	0.6129	0.6774	0.4839	0.4194	0.4194	0.4839	0.5484	0.6774	1.0000

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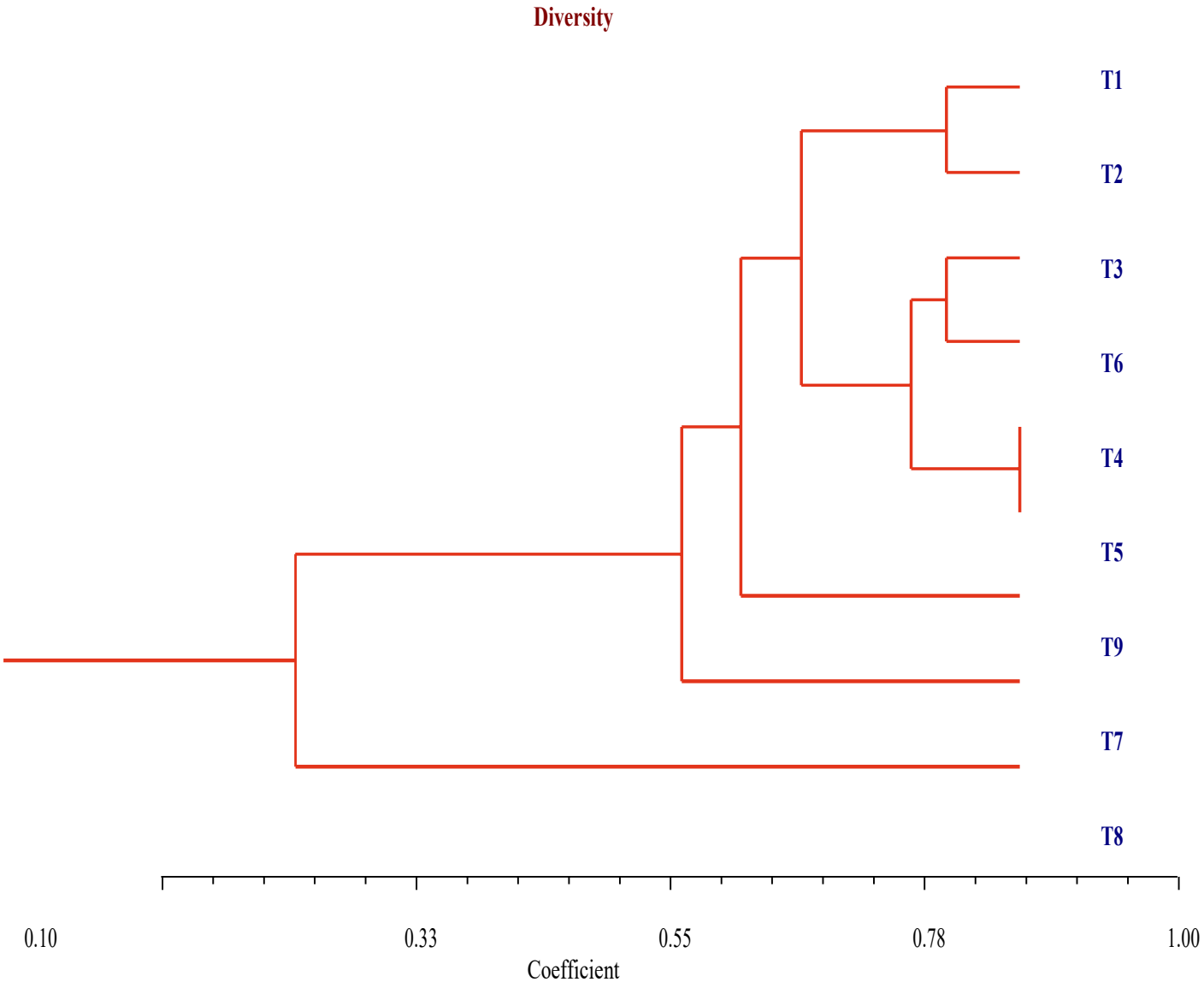

Fig. 5 Dendrogram construction between SA and JA treated plants along with control plants of *Acalypha indica* L.,

Despite most medicinal importance of *Acalifa indica* L., , research findings on genetic analysis of different hormonal treated plants were used for the genetic diversity by using molecular markers are scarce. RAPD revealed sample polymorphisms among 9 samples along with controlprofiled in this study. 24 primers displayed appreciable band resolution and substantial variations among different cultivars. Total 31 bands were observed of which 19 (53.85%) were polymorphic (either occurring in or absent in less than 95% of individuals). Primer OP7A produced maximum bands whereas OPD06 produced minimum bands (Table 1). Banding patterns of different cultivars in respect of three primers showed that primer OP18B produced maximum polymorphic bands (Fig. 1). Present study also indicated the effectiveness of RAPD indetecting polymorphism among different samples. RAPD polymorphism in a set of O. status accessions was also reported earlier , Similarities (Sij) between individuals of different cultivars showed highest value (93%) for TNDB 100 Vs Y 1281. Lowest similarity (Sij = 78%) was observed between Basmati 370 Vs Y 1281 cultivar pair (Table 3). Band-sharing based similarity indices between individuals of same cultivar were higher (average: 95.33%) than similarity indices (average: 86.47%) between individuals among different cultivars. This implies that individuals within each cultivar are genetically more similar to each other, as expected than to individuals from all other cultivars.

Nei's Gene Diversity Intra cultivar gene diversity values showed gene diversity across all populations for all loci was 0.21. High intra cultivar gene diversity was observed in TNDB 100 and Basmati 370 cultivars whereas the gene diversity value for Y 1281 was nil (Table 2). In respect of intra cultivar similarity indices (Si), the proportion of polymorphic loci and gene diversity11 , Basmati 370 was found as the genetically most diversified cultivar followed by TNDB 100 cultivar. Population Differentiation and Gene Flow Overall differentiation among six different cultivars was high (GST = 0.75). Estimated gene flow (Nm) across all cultivars was low(Nm = 0.16). GST and Nm values indicated that the cultivars are different from each other. Nm values between cultivar pairs (Table 3) indicate low level of gene flow between each pair of them possibly resulting from low level of cross-pollination between each pair of cultivars. Rice is a self pollinating plant, the pollen of which is short lived (5 min) and there are no known insect pollinators. It is, therefore, highly unlikely that crosspollination by wind and other rice plants outside of trial area will occur. Another reason behind the selfpollination is likely that since the florets of rice are adichogamous, most of the florets are self-pollinated at the time of floret opening18 . Synchrony between floret opening and anther dehiscence may contribute to the high rate of self-pollination19. Thus, selfpollination reduces the chance of inter mixing of genetic make up of different cultivars resulting in low level of both intra cultivar genetic variation and gene flow in different cultivar pairs. Genetic Distance Pair-wise comparisons of genetic distance12 between cultivars, computed from combined data for three primers, ranged from 0.07 to 0.37 (Table 5). Relatively low genetic distance was observed in TNDB 100 Vs Y 1281, IRATOM 24 Vs Binadhan 6 and Basmati 370 Vs DM 25 cultivar pairs comparative to the other cultivar pairs.

Genetic diversity is very important aspect in maintenance the developmental stability and biological potential of an organism. In the present work revealed ample genetic diversity and similarities among the 9 samples of *Acalifa indica* L., along with control. Low levels of genetic variation and high levels of genetic relatedness were found in *Acalifa indica* L., whereas significant and different levels of genetic diversity has been observed between each samples . Besides this, breeders could make a strategy for conservation of cultivars having diverse gene pools. As literature on genetic analysis of *Acalifa indica* L., present study could help the researchers in this regard in future. However, there were some lacunae in the present study) and 24 primers were used in RAPD analysis that reduces the chance to obtain a reliable knowledge precisely about the genetic structure of each cultivar of rice. Further studies involving large number of samples and primers need to be conducted to get more precise information.

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