Potential of Seaweed Liquid Fertilizer on Lycopersicum esculentum Mill. and utility of PCR-RAPD analysis

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Abstract- *Objective :The present investigation has been carried out to assess the effect of different concentrations of 10%, 25%, 50% and 75% seaweed liquid fertilizer SLF from Sargassum wightii Greville ex J. Agardh on Lycopersicum esculentum Mill. and genotypes using RAPD analysis. Methods: The molecular characterization of SLF treated plants were estimated by morphometric analysis Random amplified polymorphic DNA through PCR. Results: SLF treated plants expressed significant changes in the morphological characters as well as possessed good breeding value at low (10%) concentration. The high quality of genomic DNA was isolated using modified CTAB method and five primers were screened and amplified 105 bands used to generate Jaccard's similarity coefficients and constructed a dendrogram by means of UPGMA and out of which 10% of SLF treated plants products (83.33%) were polymorphic DNA. Due to the physico-biochemical indices changes and environmental adaptations may occur in particular changes in plants to cross pollination may have chance to genetic variations were occurred in 10% concentration of SLF treated tomato. Molecular characterization to assess the morphological characters and documented PCR-RAPD can be find the genetic diversity and analysis of phylogenetic variations of the plants. conclusions: The results of the study offer a platform of using Seaweed liquid fertilizer provide an important input to developed management strategies and would help the breeder for improvement program in L.esculentum.*

Keywords- Sargassum wightii, Seaweed liquid fertilizer, Lycopersicum esculentum, morphometric analysis and PCR-RAPD..

I. INTRODUCTION

Tomato (Lycopersicum esculentum Mill.) belong to the night shade a member of solanaceae family and most human consumption important vegetable crops grown widely all over the world.Tomato is richest source of nutrient dietary fibers, antioxidant, beta carotene (Habson and Grierson 1993; Beecher 1998), vitamin C and minerals such as iron, phosphorus (Kallo, 1991); as well as carbohydrates and phenolic compounds such as flavonoids, polyphenolic (Campbell et al., 2004) and organic acid such as naringenin and chloragenic acid (Knetkt et al., 2002). This vegetable eaten directly or consumed in a variety of product such as sauce, chutney, juice, soup, ketchup and puree.

Seaweeds are used for sustained crop improvement and great attention for their potential uses for all over the decades. However, the agricultural industry uses less than 1% out of the overall value seaweeds used in the current seaweed industry (Craigie, 2011). According to (Edmeades and Mcbride 2012), Seaweed extracts have been roved better than water. Seaweed extract was studied to improve vegetative and fruit quality, increase yield , adopt to climate change, resistance to insect pests and prolonged fruit preservation (Hong et al., 2007). Many reports supported using seaweeds as bio fertilizer which strongly contributes towards agricultural sustainability Herrera et al., 2014. Seaweed extract influence nutrient uptake, chlorophyll quality. Seaweed extracts showed resistance against fungal attack and sucking insect attack. Some studies have confirmed the favorable outcome of seaweed liquid fertilizer applications on plant growth and yield (Selvam and Kumar, 2015).

Seaweed extract was commonly studied in Sargassum wightii Spann and little 2011. Seaweed liquid fertilizer were used for crop improvement and quality of food processing without reducing nutritive values. The evaluation of genetic diversity may be done within and between population level by using various techniques like allozymes or DNA isolation method (Mondini et al.,2009). Morphometric description and Taxonomy is a old method used to calculate the genetic variances, and its habitually used for phylogenetic analysis (Nilkoumanesh et al., 2011). However, agronomic traits are simply changed by environmental adaptations; thus, quantification of genotypic differences is not always favorable (Cooke et al., 2003). By evaluation, genetic markers are reliable technique for the identification of genotypes, such as amplified fragment length polymorphism (AFLPs) (Park et al., 2004), randomly amplified polymorphic DNA (RAPD), Cao et al., 2015 and simple sequence repeats or microsatellites (SSRs) Wohrmann et al., 2011. Among these molecular

markers were very standard due to their individualities of high reproducibility, co-dominance, and polymorphism (Powell et al., 1996). EST-SSRs the SSRs derived from expressed sequence tags (ESTs), are fast developing markers and have been used for genetic diversity analysis of a wide range of plant species Korir et al., 2014. Moreover, using a both morphological and molecular markers to find plant genetic diversity has to be become popular Nilkoumanesh et al., 2011. The main objectives of tomato breeding is increasing yield, disease resistance, ripeness and improvement of fruit quality characteristics and TSS (Total soluble solids) (Foolad 2007). Systematic study and evaluation of germplasm is of great status for current and future agronomic and genetic enhancement of the crop (Reddy et al. 2013). In the present study attempt has been made to evaluate the molecular characterization of different concentrations of SLF treated L.esculentum using genomic PCR-RAPD.

II. MATERIALS AND METHODS

2.1 Collection of seaweeds

The marine brown seaweed Sargassum wightii was collected from Nochiyurani coast (09º 16.16ºN, 78º 02.43ºE) is located near madapam coast in the Gulf of mannar. The seaweeds were handpicked and cleaned thoroughly with seawater to eliminate all the unwanted sand particles and epiphytes.

2.2 Preparation of seaweed liquid fertilizer

The cleaned and washed seaweeds were shade dried for 10 days. After the dried material was taken grounded with the help of mixi grinder (Preethi ECO chef) .the powdered seaweed samples were stored in the airtight container for the future use. 500 g of seaweed powder added to 5 L of water and heated for 45mintutes at 60ºC in plugged conical flask. After cooling the contents were filtered through four muslin cloths layers. The filtrate was centrifuged the supernatant collected was used as concentrated SLF. From the supernatant different concentrations (control, 10%, 25%, 50% and 75%) of SLF were prepared using distilled water.

2.3 Experimental plantation and treatments

The certified seeds of L.esculentum PKM1 variety were procured from Agricultural research station in palur, Cuddalore (Dist) Tamil Nadu. The Tomato seeds were surface sterilized by 0.1 mercuric chloride and were sown in earthenware pot in (10 X10 feet). The sterilized seeds were soaked in different concentrations (10%,25%, 50% and 75%) of SLF for 5 hours while control was maintained by soaking the seed, in a beaker containing equal volume of distilled water. All the pot studies were done in the Botanical garden, Department of Botany, Annamalai University, Tamil Nadu.

2.4 Measurements and observations of growth parameter

Measured variable included germination percentage (GP) and retained per pot for the study of vegetative parameters ie., plant shoot root length, fresh weight, dry weight, total height numbers of branches was estimated by electronic balance and reproductive parameters number of flowers, number of fruits was recorded in 90th days. The present results were statistically analyzed using ANOVA.

III. MOLECULAR CHARACTERIZATION ANALYSIS

3.1 Isolation of genomic DNA

Conventional high quality of genomic DNA extraction protocol was essential for many molecular studies of different plant groups. The occurrence of secondary metabolites causes prevention in isolation and makes the whole procedure tedious. The fresh leaf material was harvested from three week old plants different concentrations of SLF (Seaweed liquid fertilizer) treated Lycopersicum esculentum. DNA was extracted by modified CTAB method with some modifications (Doyle and Doyle 1987). Extraction buffer wasincluded :1) CTAB (Cetyl trimethyl ammonium bromide 2.5ml, 2% β-mercapto ethanol and 2% PVP (Poly vinyl pyrrolidone) prepared by grinding with help of mortar and pestle. 2) After the solid substances transferred to the centrifuge tube and incubated for 1hr at 60º C and centrifuged at 10000 rpm for 10 minutes.3) The supernatant was collected and an equal volume (24:1) chloroform: isoamyl alcohol was added and centrifuged. 4) To the aqueous phase equal volume of isopropanol was added and stored at 20º C for overnight and centrifuged. 5) The pellet was dissolved in 200mM TE Buffer (pH:8.0) and 3µl of RNAse was added and keep at room temperature for 30 minutes. 6)Finally concentration of 3 molar solution of Sodium acetate or ammonium acetate centrifugation performed and collect the pellet 70% cold ethanol (200µl) with centrifuge tube. 8) The DNA was precipitin with 2 volume of 70% cold ethanol and left at 3 minutes and partially air dried and dissolved in50µl TE buffer solution.

3.2 Quantitative analysis of extracted DNA using Nanophotometer

The DNA extracted from fresh leaf tissue of the different concentrations of SLF treated and control plants

were measured using Nanophotometer (Implen, P360 version 1.2.0) at 260nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm.

3.3 PCR-RAPD analysis

Molecular method based on universal technique have been successfully applied to five different Oligo nucleotide operon primers (OPA-03, OPC-19, OPE-04, OPN-06, and OPW-04 for amplification process. Each replicate reaction volume containing primer 10mM Tris Hcl (pH.8.3), 2.5 mM MgCl2, 25 mM dNTPs mix, 0.2 mM of each primer, 10X Taq DNA polymerase and 50 µl of template DNA. PCR-RAPD was done in master cycler mexus (Eppendarf) 40 cycles involving of denaturation at 94º C for 45 sec, annealing at 38ºC for 50 sec and extension step conducted at the same temperature for 10 minutes and hold temperature of 4ºC at the end. The PCR amplified products were electrophoresed on 2% (w/v) agarose gel in 1X TAE buffer at 70 v for 3 h and then stained with ethidium bromide (0.5 µl/ml) gels with amplification fragments were visualized and photographed under UV gel documentation system (Alpha Innotech). Lambda DNA was used as molecular marker to know the size of the fragments (GeNei, Bangalore).

3.4 Data analysis

The analysis revealed that the PCR primer sets amplified from six primers and data scored for treated plants and also control plant. Amplified agarose gel pictures were compared with each other and data were scored as the absence (0) or presence (1) of a DNA band for each of the primer accession combination. The primary data was manipulative to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The size of amplified DNA fragments was estimated by comparison with the molecular weight marker 10000 bp DNA Ladder. Pair – wise comparisons of all the SLF treated tomato bands were used to make similarity matrix coefficients. The consequent similarity coefficients were employed to evaluate the genetic variations among the tomato accessions. These data were used to make dendrogram for cluster analysis based on un weighted pair group method with arithmetical average (UPGMA). Percent of polymorphism was calculated by using the following formula.

$$\text{Percent of polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

IV. RESULTS

Morphometric analysis

The present study was exposed to different concentrations of SLF treated L.esculentum exhibited the morphological variations in the crop plant. Various agronomic parameters such as germination percentage, root and shoot length, number of lateral roots, fresh weight, dry weight, total height and yield per plant were recorded. Morphological appearances was investigated to screen the molecular characterizations. Development process of active metabolism manifested in visible growth parameter used to measure the response of plant to SLF treatment. The late germination might be delay in metabolic process. The effect of different concentrations of S.wightii liquid fertilizer tested on Lycopersicum esculentum data were recorded in Table 1. The finding of the results noted lowest germination (43%) was found at 75% SLF. The Highest germination (99%) of tomato was recorded with 10% low concentration of SLF.. The maximum fresh and dry weight 0.98±0.02 and0.26±0.01 g/plant were observed in 10% concentration treated plants, while decreased in fresh and dry weight 0.22± 0.01 and 0.06± 0.001 g/plant were observed in 75% concentration of seaweed liquid fertilizer. The effect of SLF on growth parameters of L.esculentum studied in Table-1.The lowest root length 4.0±0.12 cm was observed in high concentration of SLF. The maximum shoot length was observed at 10% SLF (6.5±0.57 cm/plant). The lowest shoot length of 75% SLF 4.5±0.39. The maximum number of lateral roots observed in the low concentration of seaweed liquid fertilizer (28±1.52) and minimum number of lateral roots observed in the 75% SLF (7±1). The general observations of different concentrations of SLF have been some kind of changes in morphological characters in 60th days such as , number of lateral branches, number of main root branches, number of fruit per plant, fruit strings, fruit size and shape table -2.. The observations of seaweed liquid fertilizer treated plants were more number of lateral root branches are formed. Similar growth increases was reported in the present study at lower concentration of Sargassum wightii.

b)Molecular studies

PCR-RAPD amplified products were represented in [Table 1-5]. The primers code and sequences were listed in Table 4. [Fig1-5] showing the different pattern of monomorphic and polymorphic bands with changes in their intensity and all these bands has been noted in Table 5. A total number of 105 bands were gained in different concentrations of SLF treated tomato and control plant. Out of 105 bands 64 polymorphic bands and 41 monomorphic bands were

observed. Generally the levels of polymorphism were varied with different primers among the different concentration of SLF treated tomato. These polymorphic bands were ranging from 7 to 10 numbers in all the primers. Highest number of polymorphic bands was 10 as found in OPW-04 and lowest was 1 in OPE-04 and the average of the polymorphism percentage was highest 83.33% in primer followed by primer 66.65% OPA-03 polymorphism and lowest polymorphism percentage 25% was produced by the primer OPE-04.

Pair wise comparisons by evaluating the coefficients of similarity shows that different concentration of the SLF treated tomato plant. The genetic distance within five different concentrations of SLF treated L.esculentum is reflected by the UPGMA dendrogram Fig-6. The different concentrations of SLF treated plants were divided in to three main clusters or main groups consisting of five PCR- RAPD marker. First cluster comprising 10% of SLF 0.258+a2 similarity cluster (Table 6, 7) control and 25% of SLF (0.156 +a1 and 0.258+a3) third cluster included that 50% and 75% of SLF treated plants (0.150+a4, 0.056+a5) similarity. The highest genetic distance from 10% of SLF treated tomato plant 0.150:a2, while the closest correlation between the 25% of SLF and control, 50% and 75% of SLF (80%) similarity.

The analysis of the differences in phylogenetic tree and the identification of molecular traits particular markers are vital role for generating the statements for crop improvement. In this sense the phonetic methodology differs from the genetic method that RAPD profiles are not considered as genotypes. In this study dealt when markers reached the end of RAPD analysis done at the plants exhibited better agronomic traits and it would-be tested polymorphism between treated and control. In the dendrogram prepared according to RAPD markers the genetically distance to control and treated to different concentrations of SLF in 10% were rather variation with control plants. Dendrogram constructed based on genetic similarity coefficients the SLF treated were gathered in three main clusters. A) 10% of SLF alone. B) Control and 25% of SLF treated plants were clusters together. C) 50% and 75% of SLF treated plants were clusters together. The SLF treated showed the differences in morphological traits showed DNA polymorphism in PCR profile amplified by RAPD marker.

V. DISCUSSION

Morphometric analysis of SLF treated plants

The different concentrations of SLF (Seaweed liquid fertilizer) treated L. esculentum variations observed in the morphological characteristic features. Seed germination percentage reduction/encourage might have been due to effect of SLF on meristem cells of the seed. The decrease the germination at higher concentrations of SLF may be attributed to difficulties at cellular level (caused either at physiologic or physical level). The applying of seaweed extract on plants may response to the soil water deficits nutrients take from 'Slow-fast-slow' shaped curve in terms of main physico-biochemical indices change at the plant growth. Accordingly more number of research articles published in genetic analysis study depends upon the selecting different localities, taxonomically plant from same genus and different in species for the evaluating the genetic variations. Most of the seaweed articles statement proved only the crop improvement, increasing the biochemical constituents and resistance to the pathogens. In this preliminary screening of the research work SLF treated plants can also exhibited the physiological pathway at the level of photosynthesis reaction, RNA and protein synthesis of complete accumulation to the molecular level may chances to occurring the variations in the plants.

The first Indian study the seaweed as a manure for vegetable and crops was Thivay (1961) using Hypnea compost with cow dung and as on bhendi which showed increased yield seaweed fertilizer was found to be superior to chemical fertilizer because of high level of organic matter acids retaining moisture and minerals in the upper soil level available to the root. The increase in the yield of black gram, potato, coconut, palms citrus was reported by Mehta and Gaur 2001 extracts treatment was gradually decreased with increasing concentration of seaweed extract from S.wightii the growth of L.esculentum. Kannadhasan and Subramanian 2009 also reported similar effect in red gram linear growth of both shoots and roots in Vigna unguiculata and Phaseous mungo.

This results in better soil aeration and capillary activity of soil pores which in turn stimulate the growth of the plant root system as well as boost soil microbial activity. Seaweed constituents include macro-and micro element, nutrients, amino acids, vitamins, cytokinins, auxins and absicsic acid that effect cellular metabolism in treat plants most important to enhanced growth and crop improvement Strik et al., 2004. Seaweeds contain precursors of elicitor mixtures that promote germination (Stephenson 1974) growth and maintenance of plant condition (Kloareg et al., 1996). Presence of polysaccharides in SLF as sugars that are known to improve plant growing in a similar way to hormones (Rolland et al 2002). Furthermore, brown and green seaweed extracts contain betain compounds, it's may work as a nitrogen foundation when treated in low concentrations and work as an osmolyte at higher concentrations (Naidu et al., 1987). The presence of Phosphorus in SLFs, can help to stimulate root proliferation and enhance root-to-shoot length, making the plants more able to mine adequate nutrients from

the deeper soil layers and influence crop maturity as whole. Meriestamatic growth translocation of photosynthesis and disease resistance are also influenced by the presence of K (Potassium). The seaweed extracts contain Ca (Calcium) its helps in enzyme stimulations cell elongation, and cell stability. The organic components of seaweeds extract include growth regulators which promote strong physiological reactions in low concentrations (Pramanick et al., 2013). In this results shows that only a small amount of seaweed liquid fertilizer to improve the plant growth. SLF provide a powerful and Eco-friendly approach to nutrient management.

RAPD marker analysis in the SLF treated L.esculentum

This study evaluate the DNA polymorphism through RAPD markers in Lycopersicum esculentum agronomic traits variations expressed by low concentrations of SLF. RAPD is a common marker and used to analyze the genetic mapping, taxonomic phylogenetic studies, detect DNA alteration and mutation William et al., 1990. The polymorphism in genomic DNA was identified by RAPD profiles through randomly amplified PCR reactions. In this sense, the clear disappearance of normal bands, and appearance of new bands generated from the plants exposed to different concentrations of SLF in comparison to the untreated control. The disappearance of unclear bands (loss of bands) may be connected to the DNA damage (E.g single and double strand breaks modified bases, abasic sits, oxidized bases, bulky adducts). When Taq DNA polymerase come across a DNA adduct there are a number of possible outcomes including blockage, bypass and the possible disassociation of the enzyme / adduct –complex, which will cause the loss of bands Atienzar and Jha 2006. New PCR amplification products may reviled a change in some oligonucleotide priming spots due to annealing process. The use the molecular markers such as RAPD, AFLP, MASP, RFLP and ISSRs are being rabidly integrated as routine laboratory tools available for quick assessment of the genetic stability of plants Saker et al., 2006.

Cluster analysis clearly exposed that some genotype is closely related while some as significantly distinct. The study did not show the separation of individuals on the basis of their response towards morphometric analysis. The statistical analysis was carried out the pair wise genetic similarities among all concentrations of SLF treated L.esculentum was estimated with Jaccard's coefficient. The genotypes OPW-04 and OPA-03 showed maximum genetic similarity and the genotypes OPN-06 and OPC-19 showed significant lowest genetic diversity with the coefficient value of 0.056.

VI. CONCLUSION

The results of the present work revealed that based on morphological traits, high genetic variation was observed in different concentration of Seaweed liquid fertilizer treated L.esculentum. A number genotypes had quantitative and qualitative characteristics better than the commercial cultivars. The brown alga Sargassum wightii cell wall possess alginates derivatives perhaps its consuming the cell wall as mixed with salt in the major cations. Salts of alginic acid combine with the metalic ions in the soil to form high molecular weight complexes that absorbs moisture, swell, retain soil moisture and improve crumb structure. This results in improved soil aeration and capillary act of soil gaps which in turn motivate the growth of the plant root structure as well as increase the soil microbial association. While the brown seaweed Sargassum wightii have the environmental sustainability.

On the molecular level, five primers were used to differentiate between these different concentrations of SLF treated and gave reproducible results with wide variations in their band numbers. The molecular markers obtained by RAPD technique revealed a remarkable molecular discrimination between the five different concentrations of SLF treated tomato plants under the study. The phylogenetic analysis on the basis of RAPD derived a dendrogram revealed almost same cluster pattern that obtained from the combined markers of morphometric traits and confirm the molecular variations occurred in the treated plants. It conclude both SLF (morphometric traits) and RAPD marker are important for genetic analysis and confirmation a great approach of genetic diversity between the different studied of Lycopersicum esculentum.

The aim was to test the applicability of PCR-RAPD markers in tomato genetic diversity assessments and explain the variability of different concentrations of SLF treated L.esculentum the agronomic and molecular level and suitable genotypes for fresh and processing industry use, improve tomato production and use in future breeding programs.

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Table 1 :Seaweed liquid fertilizer of *Sargassum wightii* on growth parameters of *Lycopersicum esculentum* on 20th day seedlings.

Treatment	Germination percentage (%/)	Shoot length (cm/per plant)	Root length (cm/ per plant)	Fresh weight gm/per plant	Dry weight (gm/per plant)	Total height (cm/per plant)	No.of lateral roots
Control	88±2.6	5.2±0.156	4.7±0.41	0.55±0.03	0.12±0.456	9.5±0.20	14±1.52
10%	99±2.94	6.6±0.198	6.2±0.186	0.98±0.02	0.26±0.015	13.1±0.35	28±1.52
25%	90±2.7	5.0±0.15	4.5±0.135	0.078±0.02	0.12±0.01	10.8±0.15	24±2.0
50%	45±1.35	4.4±0.132	4.3±0.129	0.40±0.01	0.09±0.02	9.2±0.1	11±1
75%	43±1.29	3.9±0.117	4.0±0.12	0.22±0.01	0.06±0.01	7.4±0.1	7±1

The results are mean of triplicates determination ± Standard deviation.
Data are means ± SD (n=3).

Table 2: values of five different concentrations of SLF treated *L.esculentum* for morphology characters of genotypes

Treatment	Shoot length per/plant	No. of lateral branches per/plant cm	No. of root branches (main) per/plant	No. of fruit per/plant	No. of fruit strings per/plant	Fruit size	Fruit shape
Control	95cm	3.5-73cm	3-22.4cm	8	5 strings	Medium	round
10%	120cm	6.5-90cm	4.2-27.5cm	17	6-8 strings	Large	round
25%	107.2cm	4.3-78cm	4.0-22.9cm	11	6-7strings	Large	round
50%	67.3cm	3-48cm	3.0-19cm	6-7	5 strings	Small	flat
75%	52cm	3.0-34cm	3.0-14cm	5	5 strings	small	flat

Table.3 Quality and Quantity of genomic DNA isolated from different concentration of SLF treated *Lycopersicum esculentum*

Modified CTAB method	DNA yield (µg/g)	A260/280
Control	64.14±1.92	1.61±0.04
10% of SLF	84.11±2.52	1.89±0.05
25% of SLF	78.56±2.3	1.77±0.05
50% of SLF	70.64±2.17	1.65±0.04
75% of SLF	27.58±0.82	1.57±0.047

The results are mean of triplicates determination ± Standard deviation.
Data are means ± SD (n=3).

Table 4: useful RAPD primers: sequences and % (G+C) Content

S.No	Primer	Sequence	% GC	OD	Yield	Molecular weight	Temperature	Date
1	**OPA-03**	5'-AGTCAGCCAC-3'	60%	5.3	30.8 µg	2997 g/mole	32ºC	15.06.2018
2	**OPC-19**	5'-GTTGCCAGCC-3'	70%	5.4	33.98 µg	3004 g/mole	32ºC	15.06.2018
3	**OPE-04**	5'-GTGACATGCC-3'	60%	5	141.8 µg	3028.1 g/mole	32ºC	15.06.2018
4	**OPN-06**	5'-GAGACGCACA-3'	60%	5.7	153.2 µg	3046 g/mole	32ºC	15.06.2018
5	**OPW-04**	5'-CAGAAGCGGA-3'	60%	5.7	28.95 µg	3086 g/mole	32ºC	15.06.2018

Table 5. Code and sequence of the five DNA random primers used for identifying the SLF treated tomato and types of the amplified DNA bands.

a) List of bands produced after PCR amplification of 10% SLF Treated *Lycopersicum esculentum*

S.No	Primer code	Sequence 5' - 3'	Total No. of bands	No. of monomorphic bands	No.of polymorphic bands	Polymorphism %	size of bands scored bp
1	**OPA-03**	5'-AGTCAGCCAC-3'	8	3	5	62.5	1500-1200bp
2	**OPC-19**	5'-GTTGCCAGCC-3'	4	2	2	50.00	1000-300bp
3	**OPE-04**	5'-GTGACATGCC-3'	3	1	2	66.65	900-300bp
4	**OPN-06**	5'-GAGACGCACA-3'	5	1	4	80.00	1000-500bp
5	**OPW-04**	5'-CAGAAGCGGA-3'	12	2	10	83.33	1400-300bp
	Total		32	9	23	342.49	
	Average		6.4	1.8	4.6	68.49	

b) List of bands produced after PCR amplification of 25% SLF Treated *Lycopersicum esculentum*

S.No	Primer code	Sequence 5' - 3'	Total No. of bands	No.of monomorphic bands	No. of polymorphic bands	Polymorphism %	Size of bands scored bp
1	**OPA-03**	5'-AGTCAGCCAC-3'	5	2	3	60.00	800-500bp
2	**OPC-19**	5'-GTTGCCAGCC-3'	3	1	2	66.66	800-300bp
3	**OPE-04**	5'-GTGACATGCC-3'	3	2	1	33.30	600-100bp
4	**OPN-06**	5'-GAGACGCACA-3'	5	3	2	40.00	800-600bp
5	**PW-04**	5'-CAGAAGCGGA-3'	8	2	6	75.00	900-400bp
	Total		24	10	14	274.96	
	Average		4.8	2	2.8	54.99	

c) List of bands produced after PCR amplification of 50% SLF Treated *Lycopersicum esculentum*

S.No	Primer code	Sequence 5' - 3'	Total No. of bands	No. of monomorphic bands	No. of polymorphic bands	Polymorphism %	size of bands scored bp
1	**OPA-03**	5'-AGTCAGCCAC-3'	3	1	2	50.00	600-700bp
2	**OPC-19**	5'-GTTGCCAGCC-3'	4	3	1	25.00	700-300bp
3	**OPE-04**	5'-GTGACATGCC-3'	4	2	2	33.30	500-300bp
4	**OPN-06**	5'-GAGACGCACA-3'	3	2	1	66.60	800-400bp
5	**OPW-04**	5'-CAGAAGCGGA-3'	5	1	4	60.00	800-400bp
	Total		19	9	10	234.95	
	Average		3.4	1.8	1.6	46.99	

d) List of bands produced after PCR amplification of 75% SLF Treated *Lycopersicum esculentum*

S.No	Primer code	Sequence 5' - 3'	Total No.of bands	No.of Monomorphic bands	No.of polymorphic bands	Polymorphism %	size of bands scored bp
1	**OPA-03**	5'-AGTCAGCCAC-3'	3	2	1	33.30	900-800bp
2	**OPC-19**	5'-GTTGCCAGCC-3'	2	1	1	50.00	700-800bp
3	**OPE-04**	5'-GTGACATGCC-3'	1	1	0	0	500bp
4	**OPN-06**	5'-GAGACGCACA-3'	3	1	2	66.60	500-300bp
5	**OPW-04**	5'-CAGAAGCGGA-3'	5	2	2	50.00	700-300bp
	Total		13	7	6	199.95	
	Average		2.6	1.4	1.2	39.99	

List of bands produced after PCR amplification of control plant *Lycopersicum esculentum*

S.No	Primer code	Sequence 5' - 3'	Total No. of bands	No. of monomorphic bands	No. of Polymorphic bands	Polymorphism %	size of bands scored bp
1	**OPA-03**	5'-AGTCAGCCAC-3'	3	2	1	33.33	900-800bp
2	**OPC-19**	5'-GTTGCCAGCC-3'	3	0	3	100	600bp
3	**OPE-04**	5'-GTGACATGCC-3'	3	2	1	33.35	500-300bp
4	**OPN-06**	5'-GAGACGCACA-3'	3	1	2	66.67	700-500bp
5	**OPW-04**	5'-CAGAAGCGGA-3'	5	1	4	80.00	1000-500bp
	Total		17	6	11	313.35	
	Average		3.4	1.2	2.2	62.7	

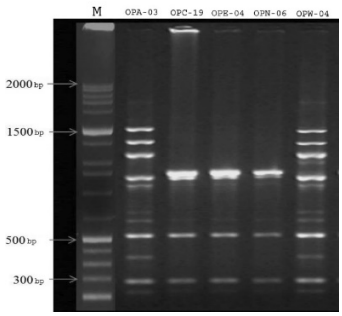
Table. 6 Similarity matrix contributed with Jaccard -Coefficient

	Control (a_1)	10% (a_2)	25% (a_3)	50% (a_4)	75% (a_5)
Control (a_1)	1	0.486	0.500	0.594	0.889
10%(a_2)		1	0.452	0.700	0.500
25%(a_3)			1	0.517	0.467
50%(a_4)				1	0.613
75%(a_5)					1

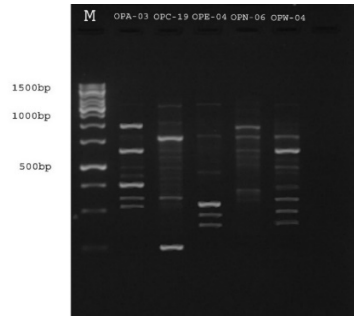
Table. 7 Distance matrix based on Jaccard -Coefficient

	Control (a_1)	10% (a_2)	25% (a_3)	50% (a_4)	75% (a_5)
Control (a_1)	0	0.514	0.500	0.406	0.111
10%(a_2)		0	0.548	0.300	0.500
25%(a_3)			0	0.483	0.533
50%(a_4)				0	0.387
75%(a_5)					0

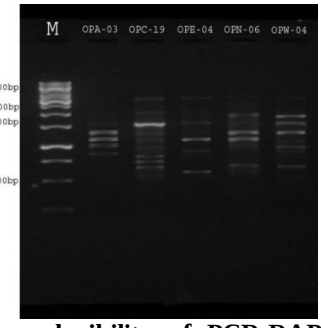
Fig:1 a-e DNA band profile of the different concentrations of SLF treated *L.esculentum* using RAPD with primer OPA-03, OPC-19, OPE-04, OPN-06, OPW-04. (M-Marker-2 kb ladder).



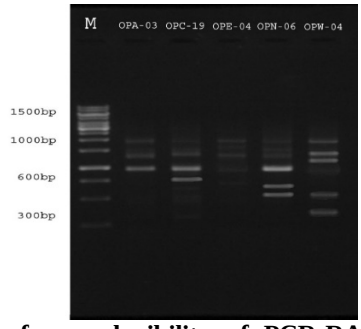
a)Assessment of reproducibility of PCR-RAPD formed on multiple, identical DNA samples from 10% SLF treated *L.esculentum*. Mill



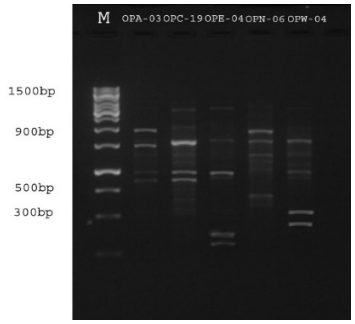
b) Assessment of reproducibility of PCR-RAPD formed on multiple, identical DNA samples from 25% SLF treated *L.esculentum*. Mill



c) Assessment of reproducibility of PCR-RAPD formed on multiple, identical DNA samples from 50% SLF treated *L.esculentum*. Mill



d) Assessment of reproducibility of PCR-RAPD formed on multiple, identical DNA samples from 75% SLF treated *L.esculentum*. Mill



e) Assessment of reproducibility of PCR-RAPD formed on multiple, identical DNA samples from control plant of*L.esculentum*. Mill

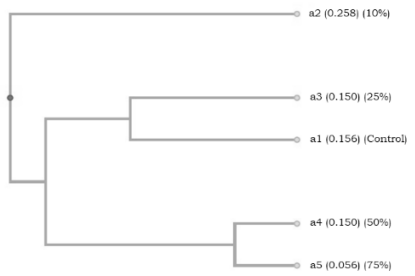


Fig 2: Dendrogram of SLF treated *L.esculentum* and control contruted with UPGMA method based on molecular characters.

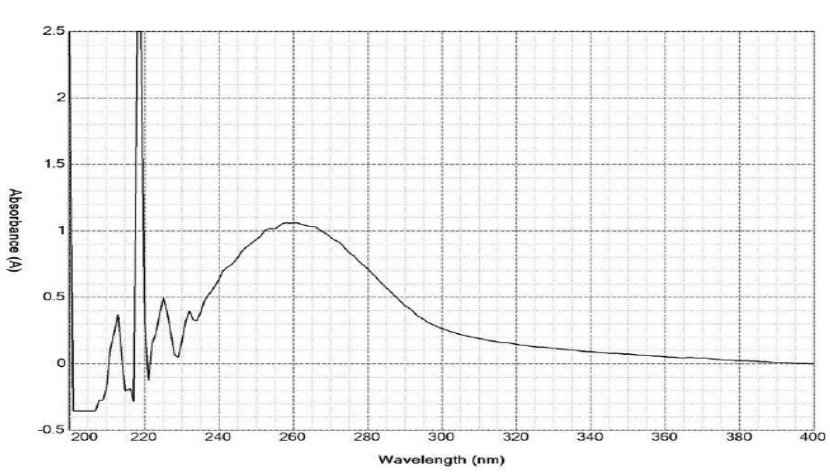


Fig 3: Genomic DNA of SLF treated *Lycopersicumesculentum*resolved in 0.8% of agarose gel electrophoresis