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Study of Molecular Interaction Between *Cissus quadrangularis* Linn. and *Bacillus subtilis*

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Abstract: *Cissus quadrangularis* is an ancient medicinal plant native to the hotter parts of Srilanka and India. *Cissus quadrangularis* has been used by common folk in India for promoting the fracture healing process. *Cissus quadrangularis* belongs to the family, Vitaceae and genus *Cissus Linn*, used for the treatment of antihelmintic, analgesic in eye and ear diseases and in the treatment of irregular menstruation, asthma. The present paper deals with the phytochemical screening, Antimicrobial, Biochemical and molecular assay of *Cissus quadrangularis* L. The present investigation deals with before and after inoculation of pathogen *Bacillus subtilis* into *Cissus quadrangularis* at different concentrations of inoculam (0.5 ml, 1 ml and 1.5ml). After 24, 48 and 72 hours incubation the plant parts are cut, dried and powdered. The powdered samples taken for molecular assay. The molecular characterization of DNA over the period of incubation shows that the number of bands increases with time in each of the species and are more when compared to control readings. Comparative study of the molecular assay of DNA profile of the control and the treated one was done to locate the specific site of DNA that could be targeted for Drug designing in future.

Key words: *Cissus quadrangularis* · *Bacillus subtilis* · RAPD

INTRODUCTION

Cissus quadrangularis is a genus of approximately 350 species of woody climber in the grape family (Vitaceae). In India the plant has several other names in different languages such as in Oriya: Hadjodi; Hindi: Hadjora; Bengali: Harjora; Gujrati: Hadasankala; Punjabi: Hadjjor; Malayalam: Piranta; Tamil: Pirantai; Telgu: Nalleru and English: Edible stemmed vine [1]. This plant grows in Africa & Sri Lanka. This plant is used to treat anorexia, asthma, sickle cells, colds, pains, malaria, asthma and as an analgesic [2]. The stem pulps of this plant is commonly consumed in Indian diet, which have been used for fracture healing, eye diseases, chronic ulcer, tumors, asthma and piles [3], hemorrhoids, irregular menstruation and accelerates healing of bone fracture [4]. The methanolic extract of *Cissus quadrangularis* (CQE) produced healing effect on aspirin induced gastric mucosal damage in rats through its antioxidative mechanism [5]. The plant extracts also exhibit cardiotonic property [4]. The plant is reported to have antibacterial and antioxidant activities. The whole plant is used in oral re-hydration, while the leaf, stem and root extracts of this plant are important in the management of various ailments. The antiosteoporotic activity of *C. quadrangularis* may be justifiably is attributed to the steroids present which probably act as phytoestrogens to effectively prevent or reduce bone loss [6].

MATERIALS AND METHODS

Ten *Cissus quadrangularis* plants were selected for the experiment. One plant was kept as control and rest of the plants were incubated by injecting 2 ml culture each with three human pathogenic microorganisms (*B. subtilis, P. aeruginosa and E. coli)* at different time intervals.

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The organisms were inoculated in proper culture for a specific period of time. After appropriate incubation time leaves were collected and DNA was isolated by two different methods i.ec TAB method and phenol: isoamylalcohol method. The isolated DNA was run on gel and its purity and DNA content was checked via Nanodrop technique. Molecular characterization was done of the obtained samples via RAPD and verified by running the gel, developing matrix chart and dendogram.

Isolation of Dna from Plant Sample: Two g of sample + 5 ml of homogenizing buffer(grind 5 minutes). Add 15ml of lysis buffer (grind 5 minutes). Incubate at 65°C for 20 minutes. Centrifuge at 8000 rpm for 10 minutes. Take 500 ul of supernatant + equal volume of Phenol: Chloroform: Isoamyl Alcohol (25:24:1). Centrifuge at 12000 rpm for 10 minutes. Take supernatant + double volume of child ethanol. Centrifuge at 12000 rpm for 10 minutes. Collect pellet + 25 ul TE.

Gel Electrophoresis

Gel Used: 1% Agarose (Isolated DNA); 2% Agarose (RAPD/ PCR). Load the gel and buffer in electrophoretic chamber. Pre run the gel before loading the sample at 50V for 5 minutes. Load the sample into wells. Run at 50V for 10 minutes. Then run at 100V for 30 minutes.

PCR(RAPD): All chemicals for RAPD analysis were obtained from Merck genei.

The sample was prepared using, sterile water; Taq buffer; dNTP; Primers; DNA Template; Taq polymerase. The cycles were adjusted and the sample was run for obtaining PCR products. The cycles were set for 40 cycles in order to allow proper amplification of DNA template.

RESULTS

The molecular characterization of DNA over the period of incubation shows that the number of bands increases with time in each of the species and are more when compared to control readings.

B. subtilis didn't showed results for primer 1-5 for the sample for 24 hours of incubation. There was a mild increase in the band number with the species as incubation period increased as compared to control readings. It showed minimum deviation from the control bands. Primer 6 and 8 showed maximum similarity among the bands. Rest all the primers showed variations in bands for different incubation time.

DISCUSSION

The advent of PCR technology led to important achievements in genome analysis. Several molecular methods have been used to analyze the diversity of plant pathogens at the genome level, such as RAPD [7,8], AFLP [9,10] and ISSR [11,12].

The extracted DNA from injected samples showed greater variation in 260/280 as well as 260/230 ratio. This samples were required to be treated with proteinase and RNAse to bring the ratio of samples near to 1.80. This might be due to the fact that after injection, more RNA & proteins were produced in samples after injection of microbes.

Increase in bands as in case of injected samples as compared to control might be due to the integration of microbial DNA, annealing of primers with microbial DNA also. Exact reason can only be concluded after genomic sequencing of plants.

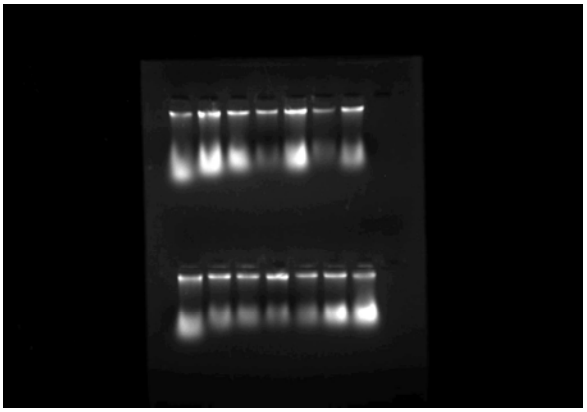
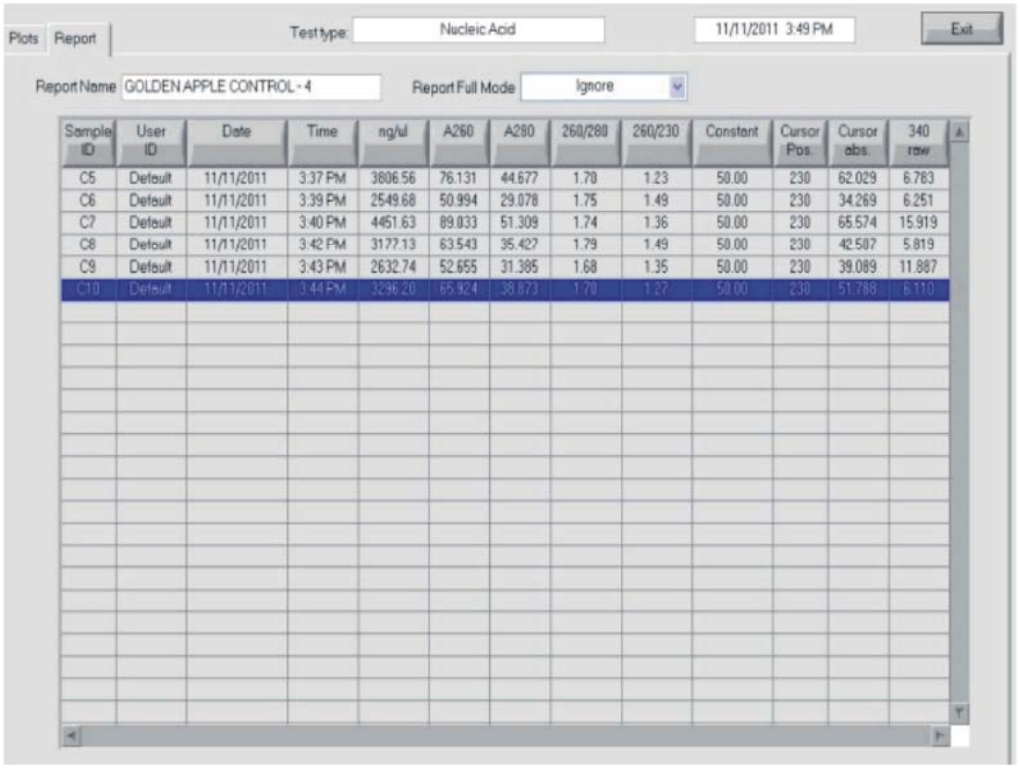
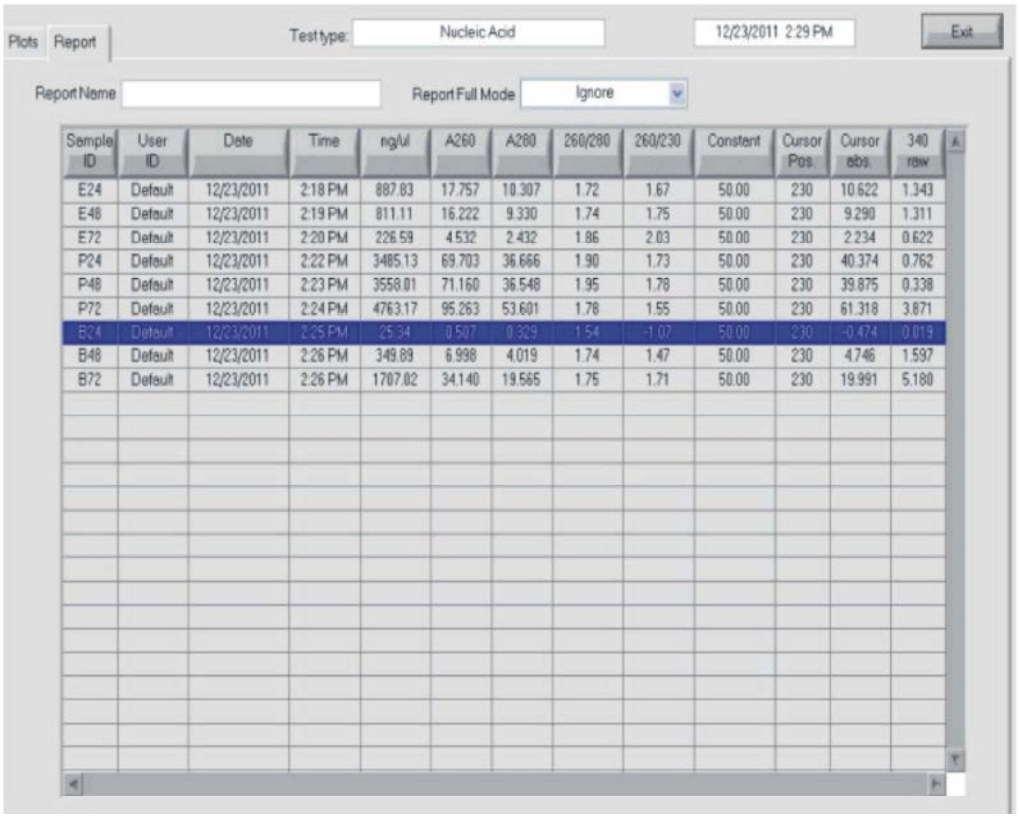


Fig. 1: Gel showing isolated DNA of control and injected samples



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
C5	Default	11/11/2011	3:37 PM	3806.56	76.131	44.677	1.70	1.23	50.00	230	62.029	6.783
C6	Default	11/11/2011	3:39 PM	2549.68	50.994	29.078	1.75	1.49	50.00	230	34.269	6.251
C7	Default	11/11/2011	3:40 PM	4451.63	89.033	51.309	1.74	1.36	50.00	230	65.574	15.919
C8	Default	11/11/2011	3:42 PM	3177.13	63.543	35.427	1.79	1.49	50.00	230	42.587	5.819
C9	Default	11/11/2011	3:43 PM	2632.74	52.655	31.385	1.68	1.35	50.00	230	39.089	11.887
C10	Default	11/11/2011	3:44 PM	3296.20	65.924	38.873	1.70	1.27	50.00	230	51.788	6.110

Fig. 2: Nanodrop image of control samples



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
E24	Default	12/23/2011	2:18 PM	887.83	17.757	10.307	1.72	1.67	50.00	230	10.622	1.343
E48	Default	12/23/2011	2:19 PM	811.11	16.222	9.330	1.74	1.75	50.00	230	9.290	1.311
E72	Default	12/23/2011	2:20 PM	226.59	4.532	2.432	1.86	2.03	50.00	230	2.234	0.622
P24	Default	12/23/2011	2:22 PM	3485.13	69.703	36.666	1.90	1.73	50.00	230	40.374	0.762
P48	Default	12/23/2011	2:23 PM	3558.01	71.160	36.548	1.95	1.78	50.00	230	39.875	0.338
P72	Default	12/23/2011	2:24 PM	4763.17	95.263	53.601	1.78	1.55	50.00	230	61.318	3.871
B24	Default	12/23/2011	2:25 PM	25.34	0.507	0.329	1.54	-1.07	50.00	230	-0.474	0.019
B48	Default	12/23/2011	2:26 PM	349.89	6.998	4.019	1.74	1.47	50.00	230	4.746	1.597
B72	Default	12/23/2011	2:26 PM	1707.02	34.140	19.565	1.75	1.71	50.00	230	19.991	5.180

Fig. 3: Nanodrop image of injected samples

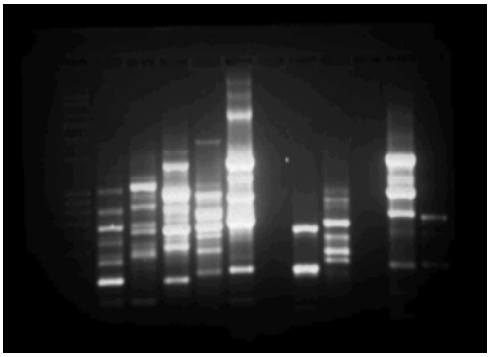


Fig. 4: RAPD of control with 10 primers

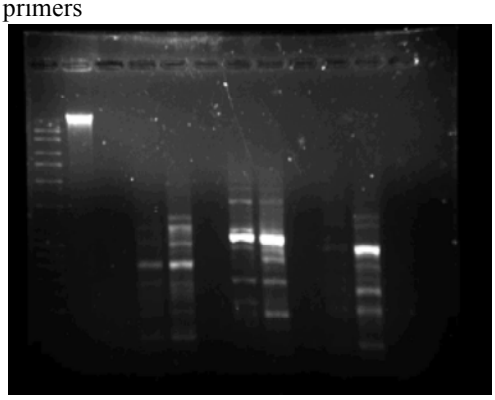


Fig. 5: RAPD of plants injected with *B. subtilis* for 24,48,72 hours with Primer 1,2,3

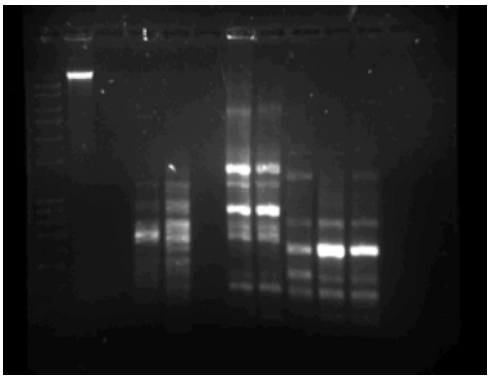


Fig. 6: RAPD of plants injected with *B. subtilis* for 24,48,72 hours with Primer 4,5,6

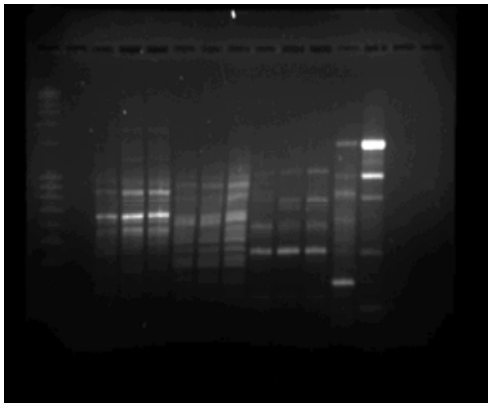


Fig. 7: RAPD of plants injected with *B. subtilis* for 24,48,72 hours with Primer 7,8,9,10

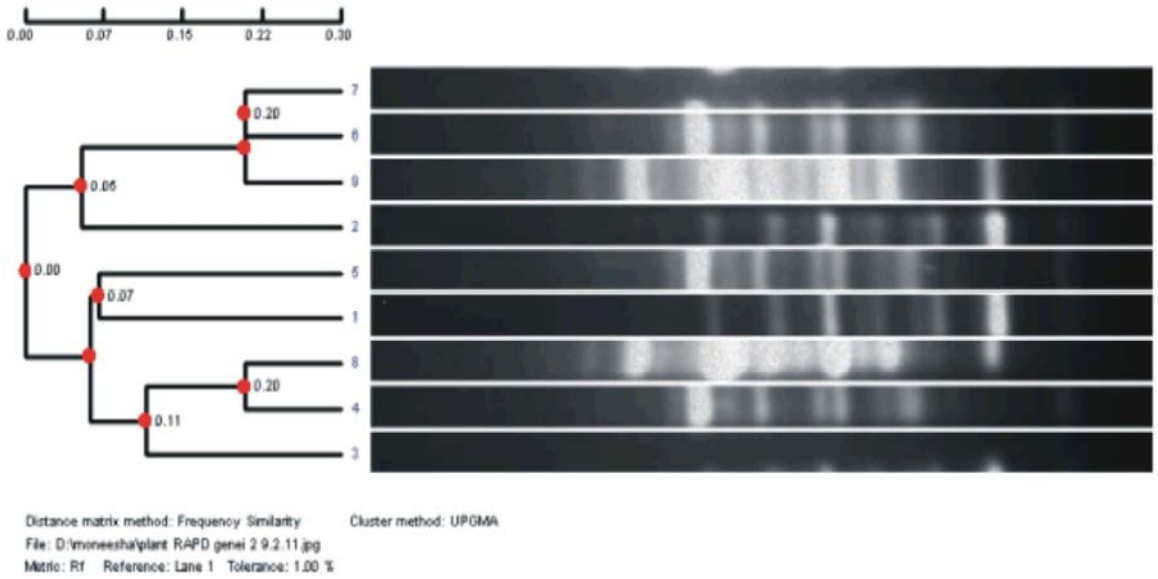


Fig. 8: Dendogram for control primers

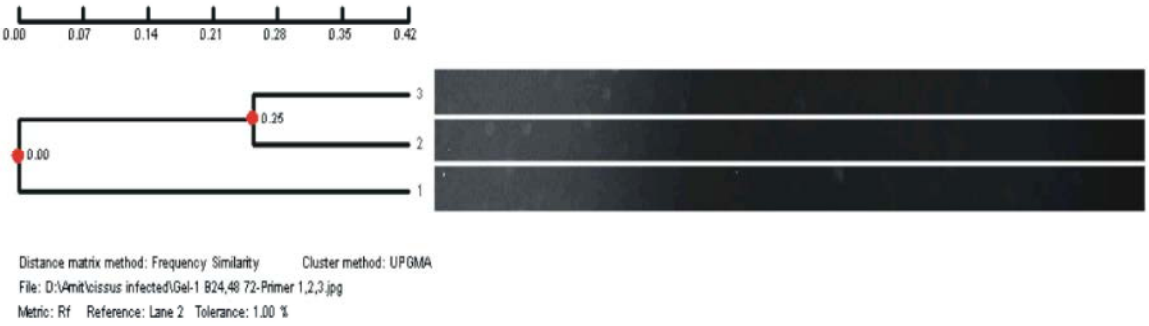


Fig. 9: Dendogram for *B. subtilis* for 24,48,72 for Primer 1

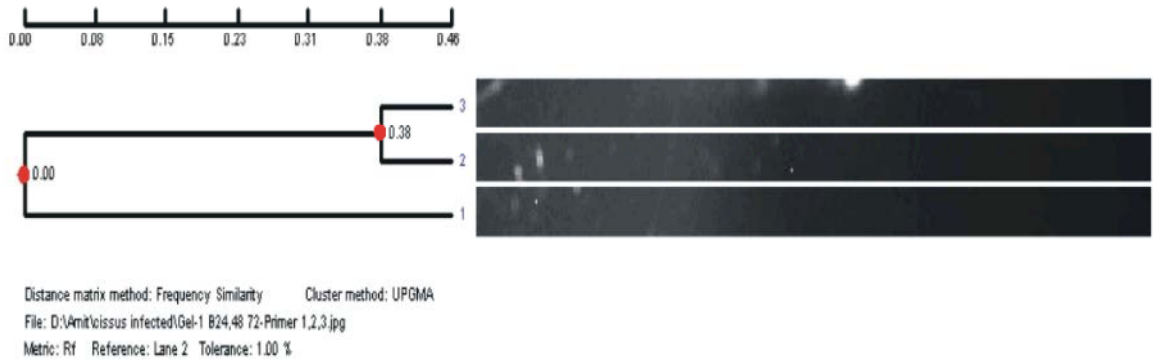


Fig. 10: Dendogram for *B. subtilis* for 24,48,72 for Primer 2

Various samples didn't showed any band formation as compared to control. The probable reason for it may be, interference of microbial DNA, disintegration of DNA samples, removal of sequence specific site.

Dendogram and matrix analysis of RAPD samples showed that as there is large dissimilarity among the samples over the incubation period for *Bacillus* injected samples. This shows that increasing the incubation period has effect on the samples as in case of *Bacillus* with time.

The samples can be used to check the anti microbial activity of the plant. Also, sequencing of plant genome can be carried out for further analysis.

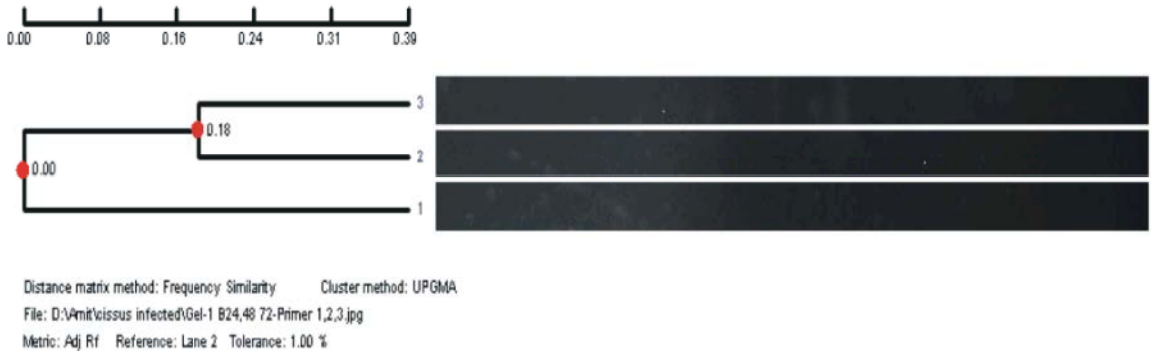


Fig. 11: Dendogram for *B. subtilis* for 24,48,72 for Primer 3

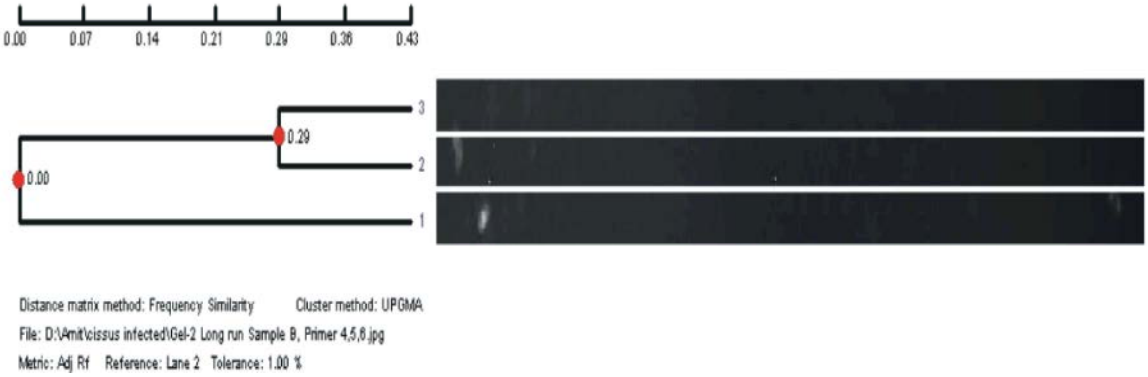


Fig. 12: Dendogram for *B. subtilis* for 24,48,72 for Primer 4

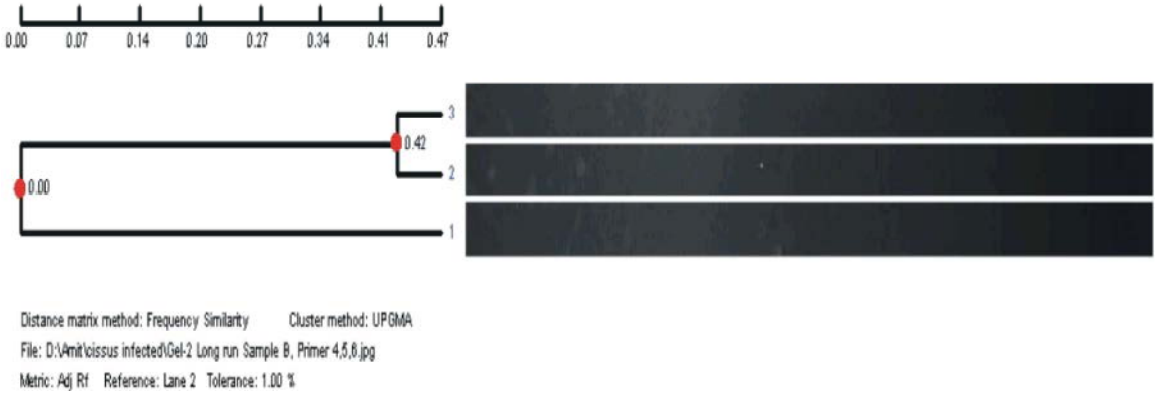


Fig. 13: Dendogram for *B. subtilis* for 24,48,72 for Primer 5

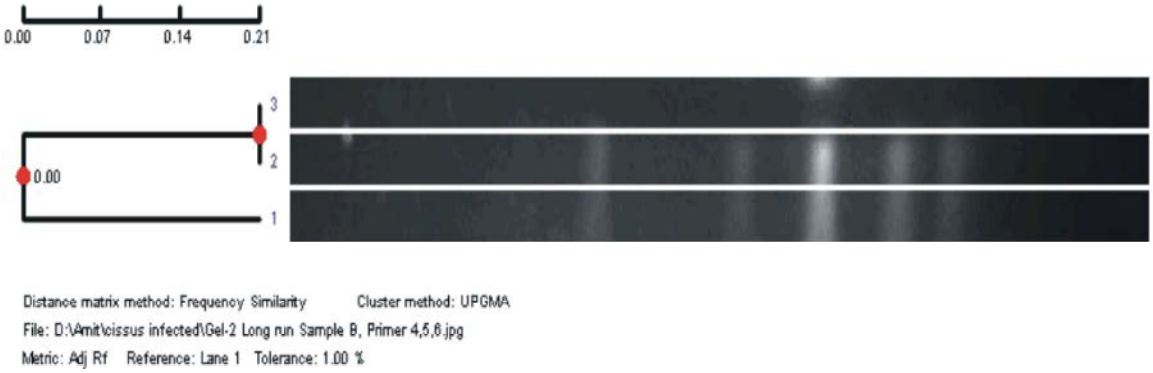


Fig. 14: Dendogram for *B. subtilis* for 24,48,72 for Primer 6

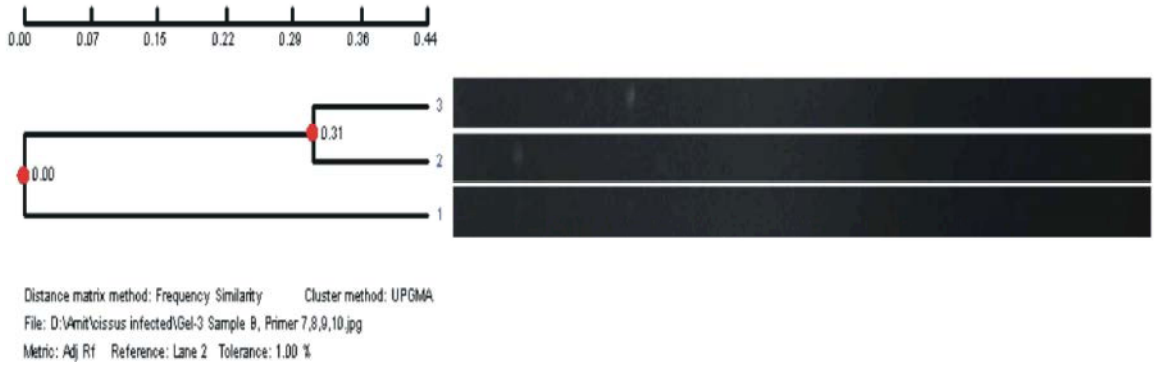


Fig. 15: Dendogram for *B. subtilis* for 24,48,72 for Primer 7

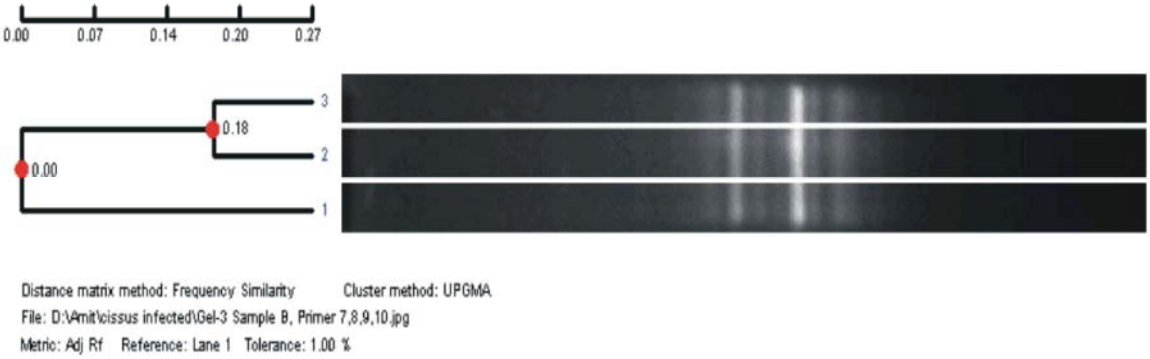


Fig. 16: Dendogram for *B. subtilis* for 24,48,72 for Primer 8

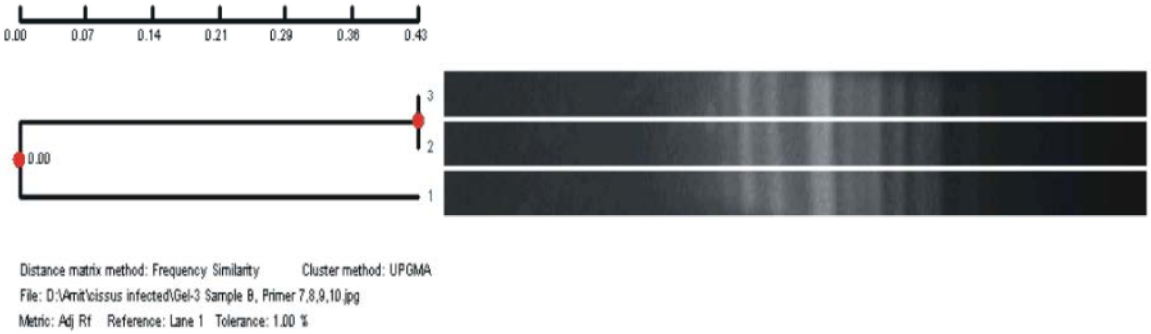


Fig. 17: Dendogram for *B. subtilis* for 24,48,72 for Primer 9

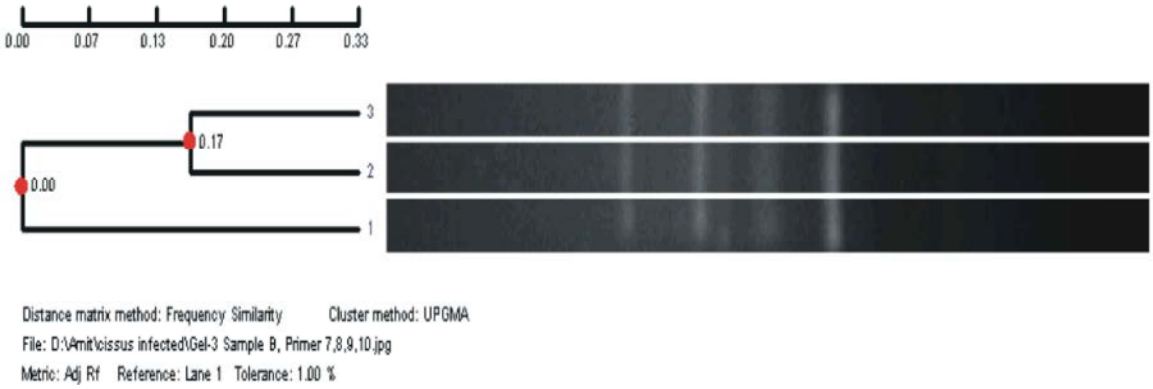


Fig. 18: Dendogram for *B. subtilis* for 24,48,72 for Primer 10

CONCLUSION

The RAPD products of the plants showed increase in the banding of products with the bands showing maximum similarities. This can be the result of plants having goodanti microbial activity. The plant can be evaluated to check the response in clinical trials. Also, the compound isolation form plant using GC-MS can give better insight of the effectors molecule carrying the response to the pathogens. Such, studies can be useful for development of drug at commercial level as well as it can also be used to study various interaction patterns.

Conflict of Interest: There is no Conflict of Interest among the authors of the paper.

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