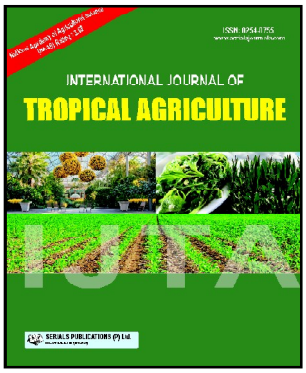


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# Cultural Analysis and Oligonucleotide Polymorphisms to Detect Diversity Among Indian Isolates of *Alternaria Solani* Causing Blight to Tomato

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***Abstract:*** Tomato is the most important vegetable crop in terms of quantities produced and consumed worldwide. It is the fast-growing major crop in the world with important economic impact on many resource-poor farming families. However, its production is currently threatened by a number of biotic and abiotic constraints. Tomato early blight disease, caused by *Alternaria solani* is the major bottleneck in tomato production in the world as well as in India. *Alterneria solani* from leaf spot infected tomato leaves were collected from four different location *viz.* Beed, Hingoli, Jalna and Latur of Maharashtra State. All these supported better growth on PDA medium. Wide variability was noted among isolates with respect to mycelial growth, spore size, colony, colour, mycelia width etc. The isolates of *Alternaria solani* observed with different rate of colony growth but 5 days later growth observed slowly. Spores of *Alternaria solani* are observed the condia contained 5 to 10 transverse septa and 1 to 5 longitudinal septa for molecular characterization eight primers were used i.e OPA-04, OPA-05, OPA-07, OPA-08, OPA-09, OPA-11, OPA-12, and OPA-13. These primers could generate total 110 polymorphic amplicons. The primer OPA-07 produced maximum polymorphism *i.e.* 84.61% while OPA-13 primer showed minimum 59.09%. The range of polymorphism among selected strains of *Alternaria solani* ie is 59.09 - 84.61%.

***Key words:*** Tomato, Alterneria solani, molecular variability, primers, PCR.

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill), $2n = 2x = 24$, *self-pollinated seasonal rich flavored fruit crop* and is one of the most remunerable and widely grown vegetables in *solanaceae* family. Among the vegetables tomato ranks second in world acreage and ranks first among the processing crops. Tomato cultivation has become more popular since mid of nineteenth century due to its varied climatic adaptability and high nutritive value, in the sense of source of dietary lycopene, β-carotene, carotenoids, vitamin C, potassium, fiber, color, flavor and antioxidant properties in a low energy dense food (Britt and Kristin, 2011; Rani and Khetarpaul, 2009). Several human studies indicated relationship between a high intake of tomato products and a decreased risk of several types of cancer, atherosclerosis and cardio-vascular disease (Svelander *et al*, 2010). Recently, this crop is recognized as a model crop for plant-pathogen interaction preeminent for genetic studies (Arie *et al*, 2007). Agriculture invention help in gradual expansion of area under tomato cultivation, but the production is being affected by several biotic and abiotic stresses such as insect pest, diseases and drought conditions which could hamper the yield of tomato.

Among this early blight disease of tomato causes due to *Alterneria solani* become most destructive in all over the world and causes yield losses in tomato to the extent of 80% (Singh *et al,* 1985; Mathur and Shekhawat, 1986; Chandravanshi *et al,* 1994, Pandey and Pandey 2002). It specifically occurs in tropical and sub-tropical region. Distinctive bulls-eye pattern of leaf spots with concentric rings of spores surrounded by a halo of chlorotic leaf area are the common. Leaves turn yellow and dry up when only a few spots are present (Gelson and Edmonds, 2006). Infected spotted leaves soon turn yellow, wither, and drop off. In severe cases plants can become completely defoliated late in the season. *Alternarias olani* exploited hyper variability at morpho-molecular level among its species. The modified polymerase chain reaction (PCR) with single primers of arbitrary nucleotide sequence and requiring no prior sequence information have proved useful in detecting intraspecific polymorphism among organisms (Welsh and McClelland, 1990 and Williams *et al.,* 1990).This amplification technique (arbitrarily primed PCR on RAPD) can generate specific DNA fragments useful for genome mapping, identification of isolates and application in molecular biology (Hadrys *et al.,* 1992). For plant pathogenic fungi, RAPD analysis can provide marker to differentiate isolates. Therefore, the present investigations were studied among four isolates of *Alterneria solani* collected from different agro climatic conditions in Maharashtra to explore the cultural and molecular variability among the four isolates of *Alterneria solani* isolated from the tomato crop. Considering the prevalence and losses caused by this disease and implementation of suitable management strategies a species specific molecular marker were exploited to undertake the extent of study in cultural and molecular diversity of *Alterneria solani* by isolation and purification.

## MATERIALS AND METHODS

The present investigation was undertaken to characterize leaf spot and blight pathogen morphologically and at molecular level by using molecular markers. This investigation was carried out at Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharshtra.

### Source of Organism

Four isolates of Isolated from leaf spot bits of infected tomato were selected for the present study. The leaf spot and blight pathogen were isolated from blight infected leaves collected from four different tomato orchids *viz.* Beed (ASB), Hingoli (ASH),Jalna (ASJ) and Latur (ASL).

## Isolation of *Alternaria Solani* from Infected Leaf of Tomato Leaves

The leaves of tomato showing typical symptoms of leaf spot and blight disease were cut into small pieces and surface sterilized with 0.01 % $HgCl_2$ for 1 min and then washed in three changes of sterile water to remove any traces of chemicals. The surface sterilized bits were inoculated on PDA plates. Inoculated plates incubated at 28°C and examined for growth of *Alternaria solani* and identified by microscopic observations. Xenic cultures of fungus were obtained by single spore suspension method. (Johnston and Booth, 1983).

## Study of Morphological Characteristics of Leaf Spot and Blight Pathogens

All of the isolates of *Alternaria solani* were grown on PDA for study various morphological characteristics. The inoculated plates (90 cm diameter) were incubated in incubator at 28 ± 2°C. The morphological growth of this pathogen was examined after 5, 10 and 20 days after inoculation. The colony diameter was calculated. Based on their growth, they have been categorized in slow moderate and fast growing species. The spore morphology of these fungus isolates was observed by slide culture technique. A bite of mycelium was teased with lactophenol dye and placed on clean glass cover slip and visualized under light microscope connected with camera. Spore morphology pertaining shape of spores, septation, beak, etc was examined.

## Molecular Characterization of *Alterneria Solani* by Using Molecular Markers

### *DNA isolation*

The genomic DNA was isolated from tomato leaf spot and blight pathogen by protocol developed and standardized by Chavan *et al.*, 2008. The extracted DNA was resolved on 0.8% Agarose gel. The quantification was done by spectrophotometer and stored at –20°C until further use.

### *Quantification of DNA*

Spectrophotometer was used for quantitative and qualitative analysis of DNA. One µl of DNA sample was added in Cuvette carrying 49 µl of sterile $H_2O$ and absorbance was measured at 280 nm wavelengths. Similarly the purity of DNA was checked by measuring the ratio of OD at A260/ A280 nm. The wavelength ratio higher than 2.0 is the indicator of impurity of proteins and less than 1.8 as an indication RNA impurity in sample. The quantification of DNA was calculated by using following formula

$$\text{DNA } (\mu g/\mu l) = \frac{\text{OD at 260 nm} \times \text{dilution factor}}{1000} \times 50$$

## Molecular Characterization

### *RAPD analysis*

Random primers derived from OPERON kit were used for RAPD analysis of four strains of *alterneria solani*. The PCR reaction of 25 µl volume was set for each strain by using an individual RAPD primer. All PCR reactions were carried out in a final volume containing 1 µl (10 pmol) of primer, 0.3 µl Taq DNA polymerase (1.5 U/µl), 2.5 µl PCR buffer, 1.5 µl 1.5 mM Mg $Cl_2$, 0.5 µl 0.2 mMdNTPs (for each), 1 µl of template DNA (approximately 25ng/µl) and 18.2 µl sterile distilled $H_2O$. The sequences of the RAPD primers used in this study are shown in Table 1. PCR reaction mixture was prepared with the above mentioned components and equally distributed (24 µl) into 5 PCR tubes. Genomics DNA (1µl) derived from 4 different strain of *Alterneria solani* were added. PCR tubes were placed in thermal cycler (Eppen drop) for amplification of the genomic DNA as per the standardized protocol. 94°C for 5 min as initial denaturation and 40 cycles of 94 °C for 0.5 min, 36°C for 1 min and 72°C for 1.5 min. This was followed by a 10 min final extension at 72°C.

**Table 1**
**List of RAPD primers used for Molecular characterization**

| *Sr. No.* | *Name of Primers* | *Sequences (5'-3')* | *No. of nucleotides (bases)* |
|---|---|---|---|
| 1. | OPA-4 | AATCGGGCTG | 10 |
| 2. | OPA-5 | AGGGGTCTTG | 10 |
| 3. | OPA-7 | GAAACGGGTG | 10 |
| 4. | OPA-8 | GTGACGTAGG | 10 |
| 5. | OPA-9 | GGGTAACGCC | 10 |
| 6. | OPA-11 | CAATCGCCGT | 10 |
| 7. | OPA-12 | TCGGCGATAG | 10 |
| 8. | OPA-13 | CAGCACCCAC | 10 |

### *Resolution of amplified product*

The PCR amplified product was resolved on 1.5 % agarose gel at 100 V for 2.5 h. The gel was stained with ethidium bromide at concentration 5 µg/µl and image was captured by Gel Documentation System (Alpha in notech).

## RESULTS AND DISCUSSION

Fungus is an opportunistic organism that is able to develop rapidly depending on favorable temperature and moisture. Among the different fungal diseases infecting tomato crop, early blight caused by *Alternaria solani* is most destructive and causes heavy losses to the extent of 78% (Datar and Mayee, 1981). Morphological and pathogenic variability among isolates of *Alterneria solani* has given rise to claims of the existence of races, although this remains unproven (Rotem, 1966). Few attempts have been made earlier to assess diversity between *Alternaria solani* (Babu *et al.*, 2000, Ahmed, 2002, Kumar, 2008, Naik, 2010).

### Morphology

Initially the experiment was conducted for finding out the best media suitable for growth of *Alternaria solani* isolates. These isolates were grown on PDA media and studied the following colony characteristics.

### Colony Characteristics

Colony of isolates *Alterneria solani* are observed in white creamy colour. These four isolates were found in varied colours from creamy white to dark greyish to brownish white colony. The isolates of *Alterneria solani* starting growth are seen in white then after 20 days later turn to its creamy blackish colour. The isolates of *Alterneria solani* colony growth was observed slowly after 5 days later. Different isolates of *Alterneria solani* are seen in diameter of 4 cm, 5 cm, 3.9 cm, 6.2 cm and colony diameter of each isolates were measured after 10 and 20 days. Spores of *Alternaria solani* are observed the condia contained 5 to 10 transverse septa and 1 to 5 longitudinal septa. In case of growth of four isolates ASB and ASH were found to be growing rapidly while the growth of isolates ASJ and ASL were found to be medium and late in respectively. Regarding the shape of the isolates of the colonies which were collected from the different four districts of Maharashtra were enlisted. The shape of the colonies of the isolates from ASB and ASL districts were observed in circular shape while the colonies of the isolates ASH and ASJ were reported in irregular shape. In case of spore

**Table 2**
**Colony characteristics of isolates of *Alternaria solani***

| | *Colony diameter (cm)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Isolates* | *5 days* | *10 Days* | *20 days* | *Average* | *Colour* | *Growth* | *Shape* | *Sporulation* |
| ASB | 4 | 7.6 | 9.5 | 7.03 | Grayish white | Rapid | Circular | Early |
| ASH | 5 | 8.4 | 9.4 | 7.6 | Dark grayish | Rapid | Irregular | Early |
| ASJ | 3.9 | 7.2 | 8.6 | 6.5 | Creamiest white | Medium | Irregular | Medium |
| ASL | 3.2 | 5.6 | 7.5 | 5.43 | Brownish white | Slow | circular | Late |

**Table 3**
**Polymorphism among various species of *Alternaria solani* on the basis of RAPD**

| | | *No. of polymorphic bands* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Sr. No.* | *RAPD primers/ Species* | *ASB* | *ASH* | *ASJ* | *ASL* | *Total no. of the bands* | *Total no. polymorphic amplicons* | *Polymorphism (%)* |
| 1. | OPA-04 | 6 | 6 | 5 | 6 | 9 | 15 | 60.00% |
| 2. | OPA-05 | 1 | 5 | 4 | 4 | 10 | 14 | 71.42% |
| 3. | OPA-07 | 7 | 5 | 6 | 3 | 11 | 13 | 84.61 % |
| 4. | OPA-08 | 5 | 3 | 2 | 5 | 9 | 11 | 81.81% |
| 5. | OPA-09 | 4 | 6 | 6 | 4 | 9 | 12 | 75.00% |
| 6. | OPA-11 | 3 | 1 | 6 | 1 | 8 | 11 | 72.72% |
| 7. | OPA-12 | 6 | 7 | 7 | 4 | 11 | 16 | 68.75% |
| 8. | OPA-13 | 8 | 7 | 3 | 4 | 13 | 22 | 59.09% |
| | | | | | | | 110 | |

formation it had been observed that in the isolates of the ASJ was found to be medium in range while that of other two isolates ASB and ASH were reported earlier in spore formation. According to morphological characters and phylogentic analyses, it was observed that *Alternaria solani* belongs to large long beaked and non catenated spore group of the genus *Alternaria* condiophores with short 60-90 µm and dark coloured.

**Table 4**
**Similarity matrix among four isolates of Alternariasolani based on RAPD primers.**

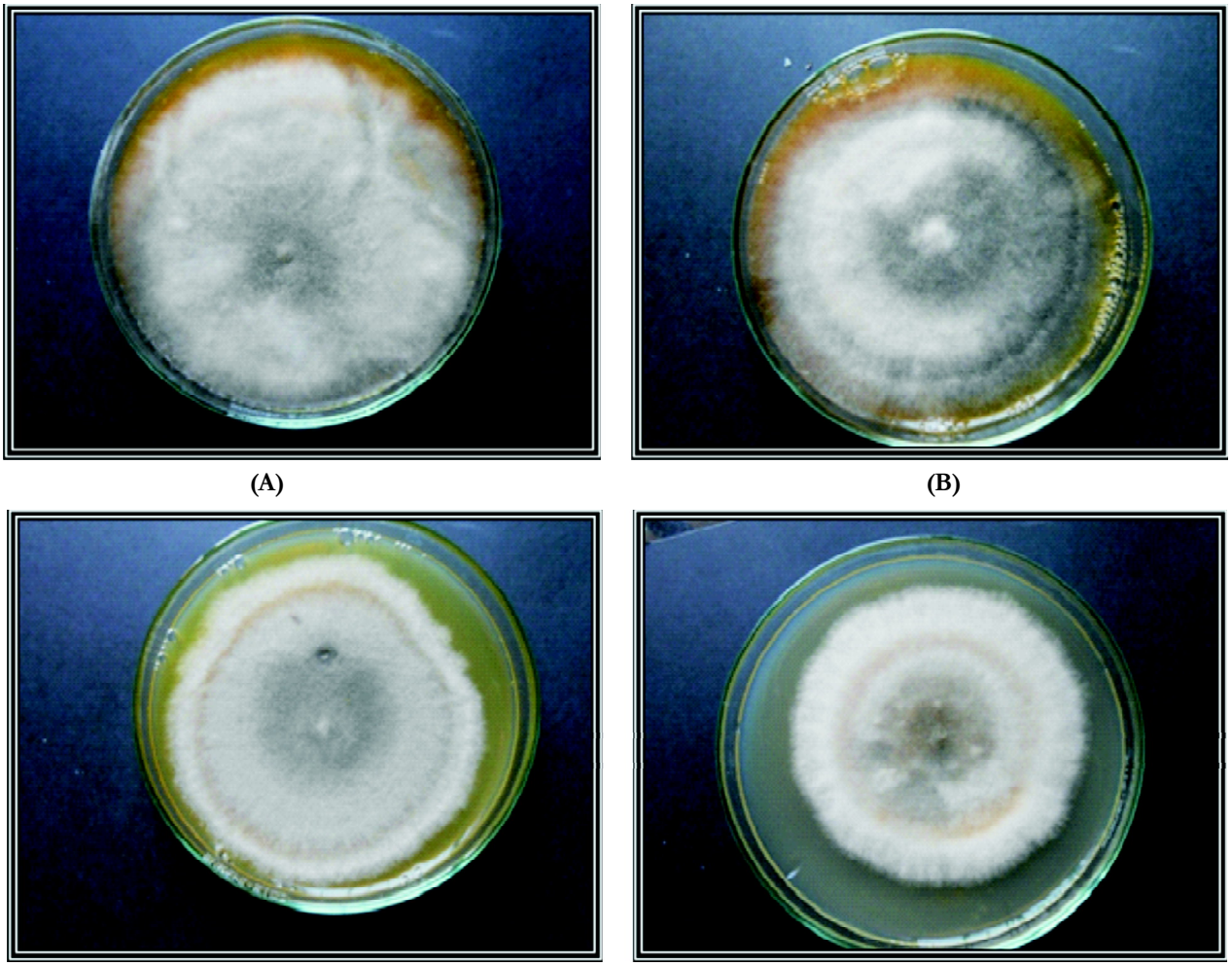
| | *ASB* | *ASH* | *ASJ* | *ASL* |
|---|---|---|---|---|
| ASB | 1 | | | |
| ASH | 0.66 | 1 | | |
| ASJ | 0.56 | 0.58 | 1 | |
| ASL | 0.41 | 0.29 | 0.41 | 1 |

## *Molecular characterization analysis*

Molecular markers, which detect variation at the DNA level overcome most of the limitations of morphological and biochemical markers. As demonstrated by their use in various plant species, molecular markers are best suited for estimation of genetic diversity and varietal identification. Besides their unlimited numbers, molecular markers are not affected by environmental and developmental stage; the technical simplicity of the RAPD technique has facilitated its use in the analysis of genetic relationship in several genera (Wilikie *et al.*, 1993). The major concern regarding RAPD-generated phylogenies, include homology of bands showing the same rate of migration, causes of variation in fragment mobility and origin of sequence in the genome (Stammers *et al.*,1995). RAPD markers has the greatest advantage of its capability to scan across all regions of the genome hence highly suited for phylogeny studies at species level (Demeke *et al.*,1992).The level and distribution of genetic diversity detected by RAPD are in overall agreement with recent studies in India (Chaurasia *et al.*,2009). RAPD, being a multi-locus marker with the simplest and fastest technology, have been successfully employed for determination of intra-species genetic diversity in several plant species (Gupta *et al.*, 2010).

## *DNA isolation and quality analysis*

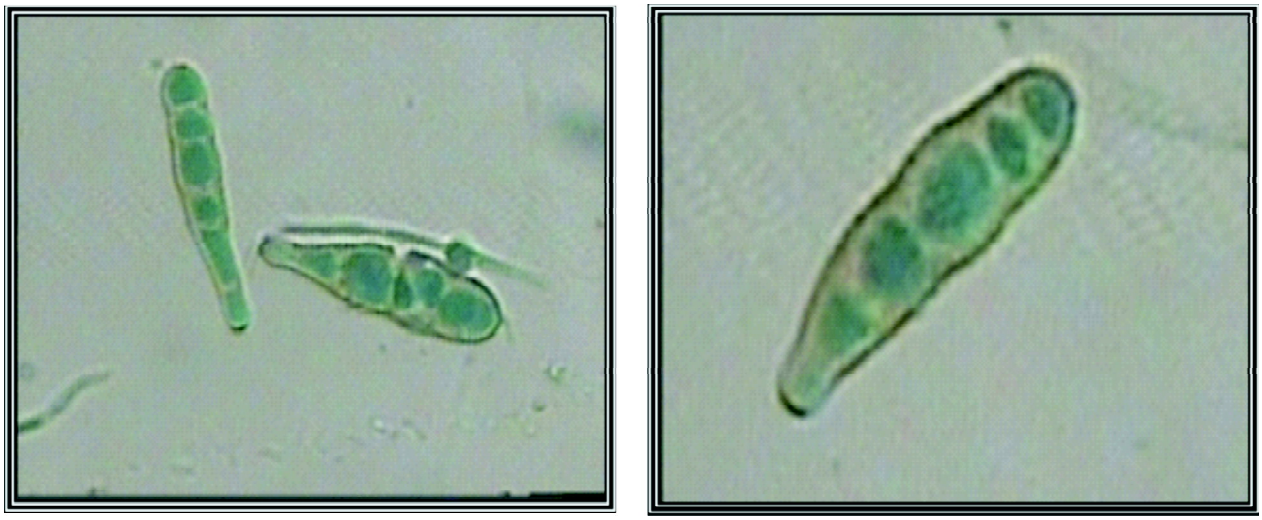
The first step of RAPD fingerprinting is the preparation of the target DNA template. The high molecular weight genomic DNA was isolated from



(A) (B)

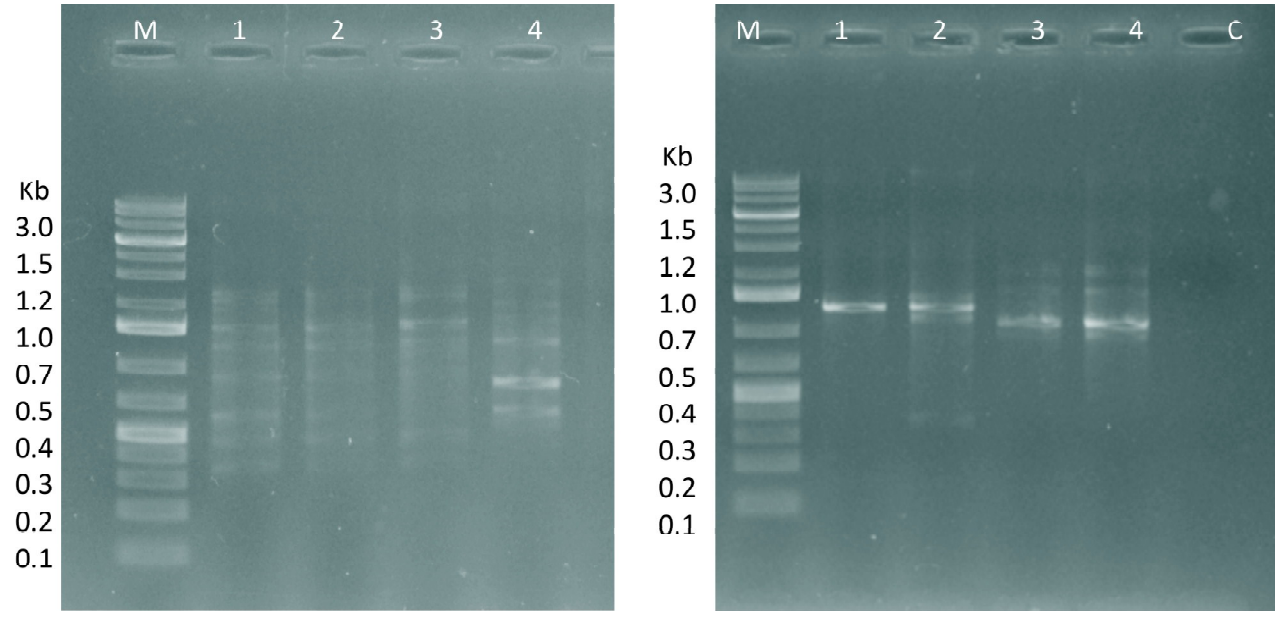
(C) (D)

**Figure 1: Colony morphology of Isolates of *Alternariasolani* Pure culture from Beed (A), Hingoli (B), Jalana (C), Latur (D).**



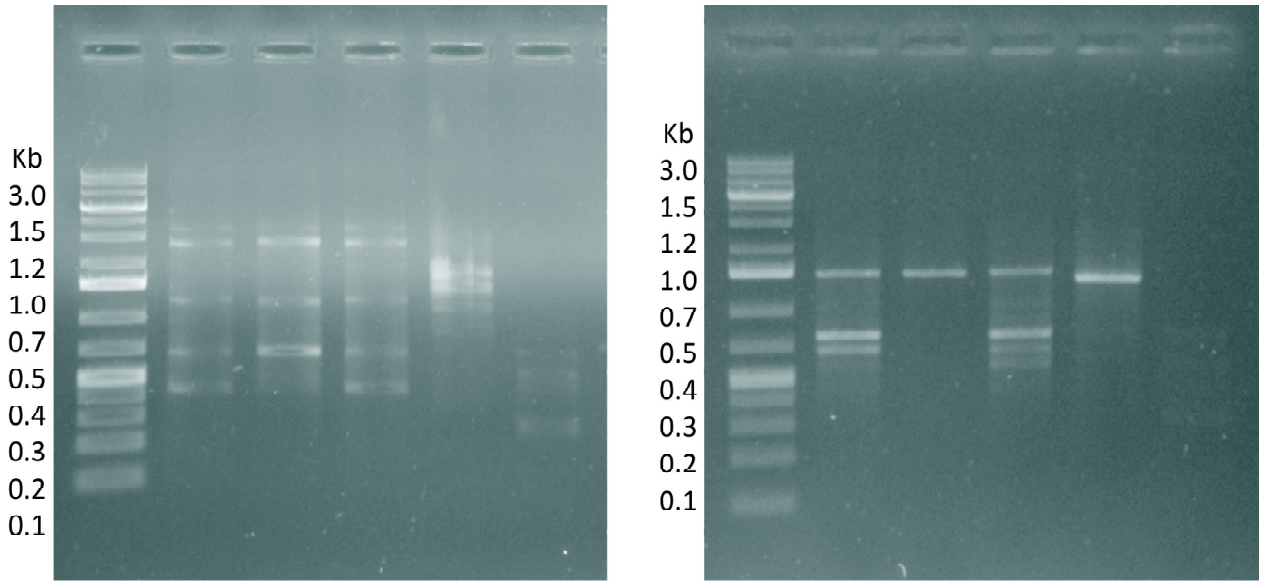
(A) (B)

**Figure 2: (A) Spores of *Alternariasolani* isolates of Latur. (B) Spores of *Alternariasolani*isolates of Beed**




**(A)** **(B)**

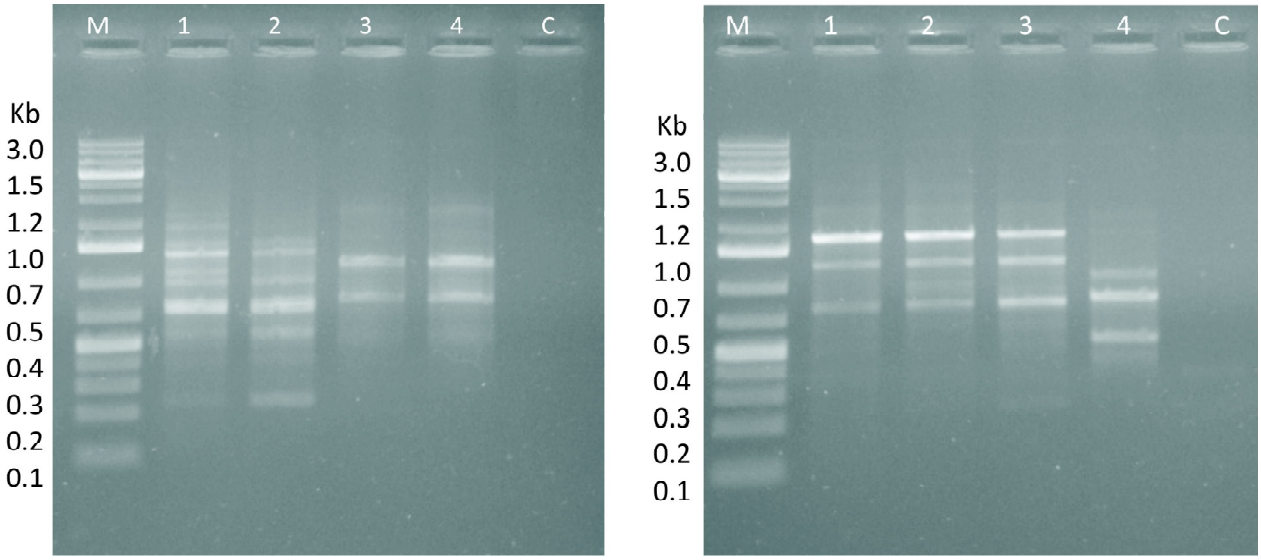
**Figure 3A and 3B: RAPD profile of four isolates of *Alternariasolani* by using primer OPA-04 (A) and OPA 05 (B); Lane M, Marker (1kb DNA ladder); Lane 1 – 4, isolates of *Alternariasolani*casuing bacterial blight disease as described in Table 1, Lane C – Controll.**




**(C)** **(D)**

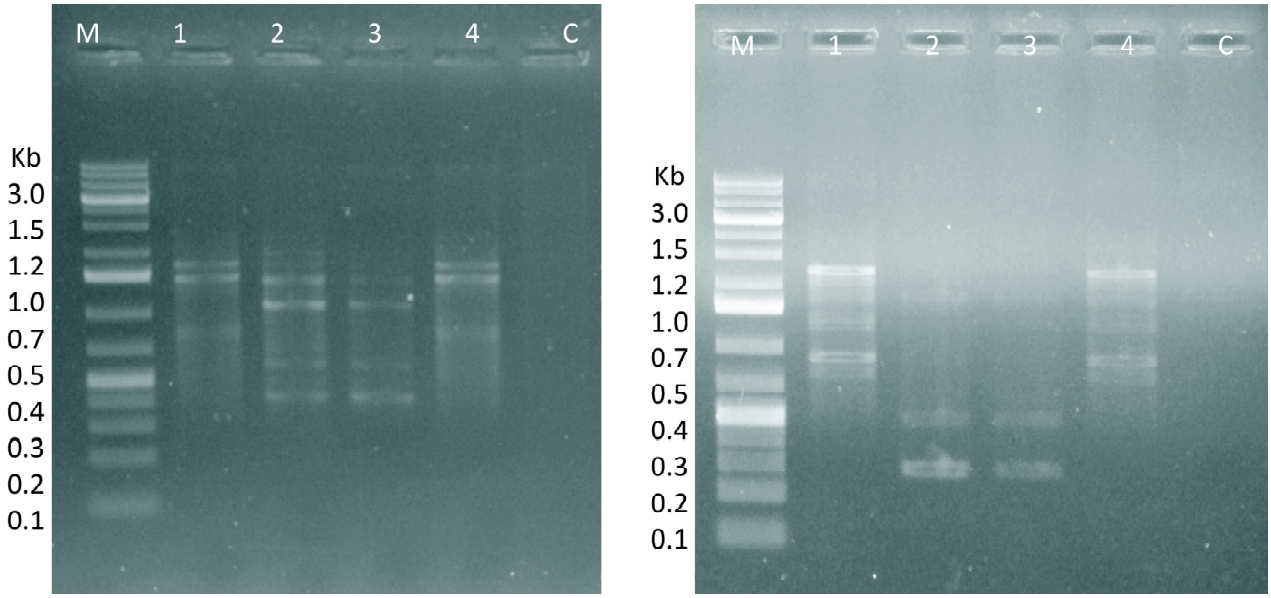
**Figure 3C and 3D: RAPD profile of four isolates of *Alternariasolani* by using primer OPA-07 (C) and OPA-11 (D); Lane M, Marker (1kb DNA ladder); Lane 1–4, isolates of *Alternariasolani*casuing bacterial blight disease as described in Table 1, Lane C–Controll.**

mat of *Alternaria solani* fungus by using protocol association of new fungal species with leaf spot and blight of sunflower and cloning their ribosomal RNA gene given by Chavhan *et al.* (2008). This method gives qualitatively and quantitatively pure genomic DNA. The quantification of isolated DNA was done by measuring absorbance at 260 nm wavelengths. Also qualitative analysis was done by resolving DNA on 0.8% Agarose gel. The concentration of four cultivars was ranged between 0.5-1µg/µl.




**(E)** **(F)**

**Figure 3E and 3F: RAPD profile of four isolates of *Alternariasolani* by using primer OPA-12 (E) and OPA-13 (4F); Lane M, Marker (1kb DNA ladder); Lane 1–4, isolates of *Alternariasolani*casuing bacterial blight disease as described in Table 1, Lane C–Controll.**




**(G)** **(H)**

**Figure 3G and 3H: RAPD profile of four isolates of *Alternariasolani* by using primer OPA-09 (G) and OPA-08 (H); Lane M, Marker (1kb DNA ladder); Lane 1 – 4, isolates of *Alternariasolani*casuing bacterial blight disease as described in Table -, Lane C–Controll.**

## *PCR cyclic parameters for RAPD reaction*

Annealing temperature is the critical parameter of PCR cyclic condition of RAPD fingerprint reaction. Four different levels of annealing temperature (35-38°C) of RAPD-PCR were set in RAPD fingerprint reaction. The annealing temperature 40°C could produce more distinct and clear RAPD fingerprint pattern.

## *RAPD fingerprint analysis*

*Alterneria solani* causing blight on tomato from four different locations was used for molecular

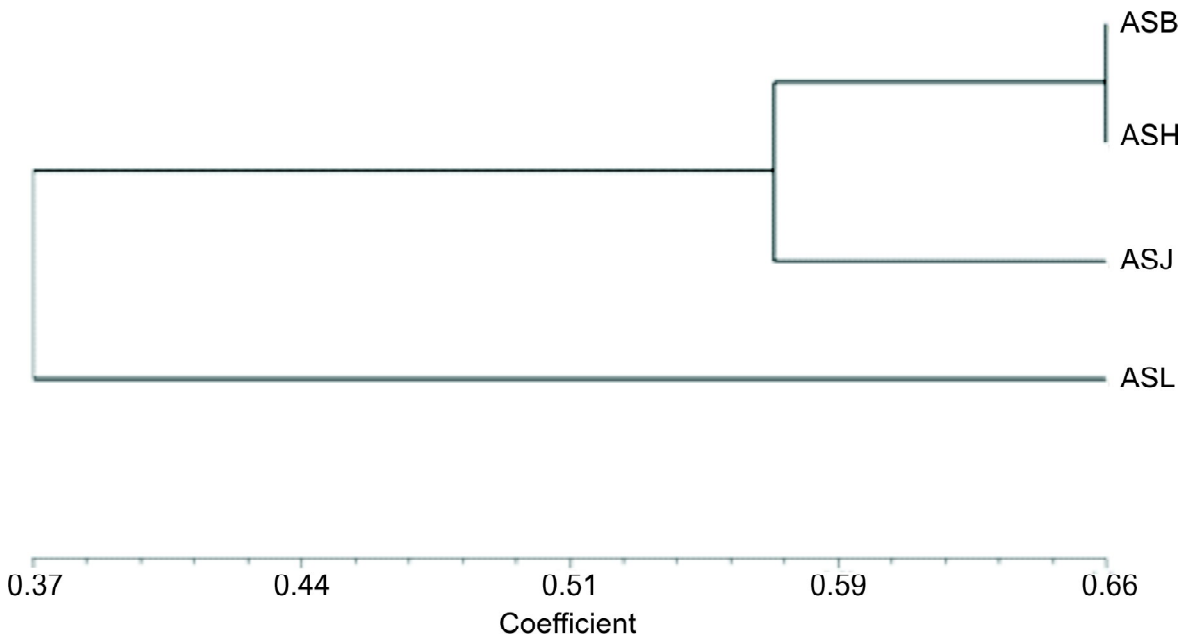



**Figure 4: UPGMA dendrogram of four isolates of *Alternaria solani* based on RAPD primers.**

characterization study. For this study eight primers were used *i.e.* OPA-04, OPA-05, OPA-07, OPA-08, OPA-09, OPA-11, OPA-12, and OPA-13. These primers could generate total 110 polymorphic amplicons. The primer OPA-07 produced maximum polymorphism *i.e.* 84.61% while OPA-13 primer showed minimum 59.09%. The range of polymorphism among selected strains of *Alternaria solani i.e.* 59.09-84.61%.The DNA fingerprint pattern generated by RAPD primer OPA-12 shows total number of 24 amplicons. Among these 7 bands was found polymorphic and % polymorphism is 68.75%. The amplicon of size 1kb and 600 bp was found common in four cultivars of *Alternaria solani*. Similarly the RAPD fingerprint profile generated by primer OPA-04 showed 60% polymorphism. The amplicon of size 600 bp and 900 bp was found common among four strain of *Alternaria solani*. The fragment having size 2300 bp was not found in latur sample. The dendrogram was decipited through UPGMA cluster analysis by using software NTSYSpc 3.2. The dendrogram analysis based on RAPD fingerprint profile of eight primers showed one major clusters. Cluster comprised four strain *i.e.* ASL and Sub cluster II of three strains having 37% dissimilarity. Sub cluster I comprised ASB and ASH. The members of cluster are shows 66% similarity and 34% dissimilarity among themselves. The members of sub cluster II has shown 61% similarity. Assessment of genetic diversity in *Fusarium solani is* needed to determine whether isolates constitute genetically distinct groups and to obtain molecular markers for differentiating them and also correlate their relationship with geographic distribution and/or pathogenic ability to cause quantum of disease. Polymorphism within an *Alternaria solani causing early blight* by RAPD molecular marker has been described by many workers (Weir *et al.*, 1998; Morries *et al.* 2002.,Wang and Zhang 2003., Verma *et al.*, Kumar *et al.*, 2008., Naik *et al.*).

## CONCLUSION

This disease blight on tomato causes yield loss to the extent of 80 %. Keeping view of this fact and importance of this disease present investigation carried out. Thus, this species specific primer could help in genetic variability and Species specific identification of *Alterneria solani* a blight pathogen of tomato by using primer PAlt. It can be used for

testing of seed samples infected by blight pathogen of tomato. Utility of this primer at quarantine centers to prevent dissemination of infected seed material at restricted areas of the country.

## REFERENCES


Ahmad, S., (2002), Conidial morphology of Alternaria solani, and its variation in tomato. *Annals of Agricultural Research* **23**: 514-515.

Arie, T., Takahshi, H., Kodama, M and Teraoka, T., (2007), Tomato as a model plant for pathogen interactions. *Plant Biotechnol.*, **24**(1):135–147.

Aritt, B. F. and Kristin, R. (2011), Tomato Consumption and Health Emerging Benefits. *Am. J Lifestyle Med.,* **8**: 182-191.

Babu, S., Seetharaman, K., Nandakumar, R., and Johnson, I. (2000), Variability in cultural characteristics of tomato leaf blight pathogen. *Plant Disease Research.*, **15**(1).

Chandravanshi, S.S., Singh, B.P., and Thakur, M.P. (1994), Persistance of different fungicides used against Alternaria alternata in tomato. *Indian Phytopathology*, **47**(3): 241-244.

Chaurasia, A. K., Subramaniam, V. R., Krishna, B., and Sane, P. V. (2009), RAPD based genetic variability among cultivated varieties of Aonla (Indian Gooseberry, Phyllanthus emblica L.). *Physiology and Molecular Biology of Plants*, **15**(2): 169-173.

Chavan, R. L., Chakrabarty, P. K., Patil, F. S., Patil. H. B., Singh, S. K. and Khadi, B. M. (2008), Association of new fungal species with leaf spot and blight of sunflower and cloning of their ribosomal RNA genes. *Indian Phytopath.*, **61**(1): 70-71.

Datar, V. V. and Mayee, C. D., (1981), Assessment of loss in tomato yield due to early blight, *Indian Phytopathology*, **34**: 191-195.

Demeke, T., Adams, R. P., and Chibbar, R. (1992), Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in Brassica. *TAG Theoretical and Applied Genetics.*, **84**(7): 990-994.

Gleason, M. L. and Edmonds, B. A. (2006).*Tomato diseases and disorders*. Iowa State University, University Extension, 1266-1277.

Gupta, R., Verma, K., Joshi, D. C., Yadav, D., and Singh, M. (2010), Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. *Genetic Engineering and Biotechnology Journal*, 1-9.

Hadrys, H., Balick, M. and Schierwater, B. (1992), Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular ecology.*, **11**: 55-63.

Johnston, A., and Booth, C. (1983), *Plant pathologist's pocketbook*. Commonwealth Agricultural Bureaux.

Kumar, V., Haldar, S., Pandey, K.K., Singh, R.P., Singh, A.K. and Singh, P.C. (2008). Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of Alternaria solani in India. *World Journal of Microbiology and Biotechnology*, **24**(7): 1003-1009.

Mathur, K., and Shekhawat, K. S. (1986), Chemical control of early blight in kharif sown tomato. *Indian Journal of Mycology and Plant Pathology.*, **16**(2): 235-236.

Naik, M. K., Prasad, Y., Bhat, K. V. and Rani, G. D., (2010), Morphology, physiologica pathogenic and molecular variability among isolates of *Alternaria solani* from tomato *Indian Phytopthol.*, **63**: 168-173.

Pandey, P. K., and Pandey, K. K. (2002), Field screening of different tomato germplasm lines against Septoria, Alternaria and bacterial disease complex at seedling stage. *J. Mycol. Plant Pathol.*, **32**(2): 234-235.

Rani, V., and Khetarpaul, N. (2009), Nutrient composition of tomato products prepared using tomato grown under sodic condition with gypsum and farmyard manure treatment. *Journal of the Science of Food and Agriculture*, **89**(15): 2601-2607.

Rotem, J., Cohen, Y., and Wahl, I. (1966), A new tomato foliage disease in Israel caused by Stemphylium botryosum. *Canadian Journal of Plant Science.*, **46**(3): 265-270.

Singh, B. M. (1967), Inducing sporulation in different strains of Alternaria solani. *Mycopathologia et mycologia applicata*, **32**(2): 163-171.

Stammers, M., Harris, J., Evans, G. M., Hayward, M. D. and Forster, J. W. (1995), Use of random PCR (RAPD) technology to analyse phylogenetic

relationships in the Lolium/Festuca complex. *Heredity.*, **74**: 19-27.

Svelander, C. A., Tiback, E. A., Ahrne, L. M., Langton, M. I., Svanberg, U. S., and Alminger, M. A. (2010), Processing of tomato: impact on in vitro bioaccessibility of lycopene and textural properties. *Journal of the Science of Food and Agriculture*, **90**(10): 1665-1672.

Varma, P. K., Singh, S. and Gandhi, S. K. (2007), Variability among Alternaria solani isolates causing early blight of tomato. *Indian Phytopathology.*, **60**(2): 180-186.

Wang. H. and Zhang. T. Y., (2003), RAPD analysis on small spored Alternaria species. *Mycosystema.*, **22**: 35-41.

Weir. T. L., Huff. D. R., Christ. B.J. and Romaine. C. P., (1998), RAPD-PCR analysis of genetic variations among isolates of *Alternaria solani*, and *Alternaria alternata* from potato, tomato. *Mycologia* **90**: 813–821.

Welsh, J. and McClelland, M. (1990), Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids research*, **18**(24): 7213-7218.

Wilkie, S. E., Isaac, P. G. and Slater, R. J. (1993), Random amplified polymorphic DNA (RAPD) markers for genetic analysis in Allium. *TAG Theoretical and Applied Genetics*, **86**(4): 497-504.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990), DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, **18**(22): 6531-6535.