The Pharma Innovation Journal 2021; 10(1): 279-284

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277- 7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.03**
TPI 2021; 10(1): 279-284

www.thepharmajournal.com
Received: 19-11-2020
Accepted: 21-12-2020

**Mudasir Hassan**
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore, Jammu and Kashmir, India

**Vaseem Yousuf**
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore, Jammu and Kashmir, India

**Roaf Ahmad Rather**
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore, Jammu and Kashmir, India

**ZA Bhat**
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore, Jammu and Kashmir, India

**MA Khan**
Division of Genetics and Plant breeding, Faculty of Agriculture, Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore, Jammu and Kashmir, India

**Farkhandah Jan**
Division of Genetics and Plant breeding, Faculty of Agriculture, Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore, Jammu and Kashmir, India

**Corresponding Author:**
**Mudasir Hassan**
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir, University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore, Jammu and Kashmir, India

# Genetic diversity of *Stemphylium vesicarium* causing Stemphylium blight of onion from different geographical regions of Kashmir

**Mudasir Hassan, Vaseem Yousuf, Roaf Ahmad Rather, ZA Bhat, MA Khan and Farkhandah Jan**

**DOI:** https://doi.org/10.22271/tpi.2021.v10.i1d.5526

**Abstract**
Stemphylium blight is the most serious and devastating disease of onion limiting the quality and quantity of both bulb and seed. Molecular analysis using 9 RAPD primers was done to assess the genetic diversity among the 36 *S. vesicarium* isolates from onion obtained from different geographical areas of Kashmir valley. All the 9 RAPD markers used during the present study revealed a moderate level of polymorphism in different isolates of *S. vesicarium*. In the present study maximum scored bands were 160 in SV-A04 marker with maximum polymorphic bands (10) showing 6.25 per cent polymorphism. The lowest scored bands were 7 in marker "SV-F02" with only two polymorphic bands showing 28.57 per cent polymorphism. In total, 579 bands were scored of which 54 were polymorphic, thereby showing 9.32 per cent polymorphism. Genetic dissimilarity of the isolates varied from 1.00 to 0.14.The present study provides insights into the extent of genetic diversity available in 36 *S. vesicarium* isolates from onion obtained from different geographical areas of Kashmir valley.

**Keywords:** Stemphylium blight, *S. vesicarium*, RAPD markers, polymorphism, genetic diversity

## Introduction
Onion (*Allium cepa* L.) is one of the most important crops in the world that belongs to the family Alliaceae. It is also used as a common favourite spice, salad and vegetable in many countries of Asia. It is also used as a condiment for flavoring a number of foods and medicines [1, 2]. Many fungal, bacteria, viral and nematodes diseases besides abiotic factors effect onion crop [3]. Among the fungal diseases stemphylium blight is the most serious and devastating resulting in yield loses [4].

The disease is characterized by the appearance of small yellow to orange streaks which soon develop into elongated, spindle shaped to ovate elongated diffusate spots surrounded by pinkish margins. Losses of about 80-85% occours to crop due to severe demage from disease on leaves and seed stalk [5].

The most efficient and economical method to manage plant diseases is the use of resistant varieties. Cultivation of resistant varieties can be an effective approach to reduce the cost of cultivation, risk of development of resistance in pathogen, risk to human health and environmental pollution. However, knowledge regarding diversity of the pathogen population is prerequisite for success in disease resistance breeding and development of management strategies [6].

Molecular analysis can be applied to estimate genetic diversity of the pathogen population so that the germplasm of national importance is tested for resistance against all different pathogen genotypes occurring across the diverse onion growing areas of the State.

Among the molecular markers, RAPD (random amplified polymorphic DNA), which depends on random amplification of DNA with short primers, offers a cheap and simple DNA-based marker that is quick and easy to assay.

This procedure requires only small quantity of DNA and doesn't need primer construction, since random primers are already commercially available. In general, valuable data regarding genetic variations within and among populations of a species can be generated by using RAPD markers [7]. Keeping the above facts in view, the present study involved RAPD markers was undertaken to estimate the genetic diversity of S. vesicarium isolates collected from different geographical regions of Kashmir valley.

## Material and Methods

### Isolation and pathogenicity of S. vesicarium isolates

Onion leaves bearing the typical symptoms of Stemphylium blight, collected from thirty-six onion fields from four districts of the Kashmir valley, *viz*., Baramulla, Srinagar, Budgam and Anantnag during surveys were immediately brought to laboratory for isolation of the pathogen. The isolations of the pathogen from the diseased samples collected from different onion fields during survey were carried out by tissue bit transfer method [8]. The pathogenecity of the isolates of causal fungus was established by proving the Koch's postulates on potted onion plants (cv. Yellow Globe) as per the method adopted by Bassalote-Ureba *et al*. [9]. In all thirty six cultures, proved pathogenic on susceptible host "Yellow Globe" and were named as Sv-01, Sv-02, Sv-03; Sv-04, Sv-05, Sv-06 ; Sv-07, Sv-08, Sv-09 from three different onion fields from wadura, Achabal and Pattan of district Baramulla respectively, Sv-10 Sv-11 Sv-12; Sv-13, Sv-14, Sv-15; Sv-16, Sv-17, Sv-18 from Shalimar, Danderkah, and Noorbagh of district Srinagar, respectively, Sv-19, Sv-20, Sv-21; Sv-22, Sv-23, Sv-24; Sv-25, Sv-26, Sv-27 isolates from Chadoora, Magam, and Ompora of district Budgam, respectively, Sv-28, Sv-29, Sv-30; Sv-31, Sv-32, Sv-33; Sv-34, Sv-35, Sv-36 from Sangam, Bijbhera and Larnoo of district Anantnag, respectively and were used for study of genetic diversity. The cultural, morphological and pathogenic variability of these isolates is discussed in Hassan *et al.* [10].

### Molecular variability of pathogen isolates

The genetic diversity of thirty-six pathogenic *S. vesicarium* isolates associated with Stemphylium blight of onion collected from different was studied by using random amplified polymorphic DNA (RAPD) markers [11]. The details of RAPD markers used during the present study is available in Table 1.

### Genomic DNA extraction

CTAB method [12] was used for isolation of genomic DNA of collected isolates of *S. vesicarium*. Assessment of quality and quantity of the DNA was checked in 1% agarose gel by staining DNA with ethidium bromide (0.1µg/ml). Marker of known concentration was also loaded along with DNA samples and the gel was run at 100V for 1 hour in 0.5xTBE buffer. DNA concentration and quality of each sample was ascertained by comparing with that of standard marker.

### Primer selection

A set of eleven decamer oligonucleotide primers (Integrated DNA Technologies) were screened with two randomly selected isolates for polymorphism [13]. The primers showing consistency in polymorphism among isolates were selected for RAPD profiling of all the thirty-six *S. vesicarium* isolates. The base sequence and annealing temperature of the primers used for screening are listed in Table 1.

### PCR amplification

Polymerase chain reaction (PCR) [14] for RAPD analysis of each isolate was performed in volume of 25 µl reaction mixture in 0.2 ml PCR tubes containing 125ng genomic DNA, 1.5mM $MgCl_2$, 0.2mM each of dNTP's, 0.4pmol of each primer, 1U *Taq* DNA polymerase and 2.5µl of 10X PCR buffer. PCR was performed in a T-Gradient Whatman Biometra thermal cycler. The reaction mixture in PCR tubes were given short spin or vortexed in microcentrifuge (Thermo Scientific, Thermo Electron Corporation) and placed in 96 well thermal cycler. The PCR conditions include initial denaturation at 94 ºC for 5 minutes followed by 35 cycles at 94 ºC for 1 minute, at annealing temperature (Table 1) for 1 minute, 72 ºC for 60 seconds and a final extension at 72 ºC for 6 minutes.

**Table 1:** RAPD primers, their sequences and annealing temperature for PCR analysis of different isolates of *Stemphylium vesicarium*

| S. No. | Primer | Primer sequence (5' to 3') | Annealing temperature (ºC) |
|---|---|---|---|
| 1. | SV-A03 | AGTCAGCCAC | 34.30 |
| 2. | SV-A04 | AATCGGGCTG | 35.10 |
| 3. | SV-A05 | AGGGGTCTTG | 32.60 |
| 4. | SV-F01 | ACGGATCCTG | 33.30 |
| 5. | SV-F02 | GAGGATCCCT | 30.90 |
| 6. | SV-Y01 | GTGGCATCTC | 32.10 |
| 7. | SV-Y02 | CATCGCCGCA | 42.10 |
| 8. | SV-Y03 | ACAGCCTGCT | 37.40 |
| 9. | SV-Y04 | GGCTGCAATG | 34.40 |
| 10. | SV-Y05 | GGCTGCGACA | 41.20 |
| 11. | SV-Y06 | AAGGCTCACC | 34.10 |

### Visualization of PCR products

5µl of the 6X loading dye was added to 25µl of the amplified product so as to obtain a final concentration of the loading buffer in reaction samples to 1X. The PCR product was resolved on 1.2% agarose gelsprepared in 0.5X TAE buffer. Ethidium bromide was added at concentration of 0.5µg $µl^{-1}$. The samples was run at 5V/cm under UV light and photographed using Alfa Imager gel documentation system (Alfa Imager EC, Protein Simple, USA).

### Data Analysis

### Data generated by RAPD primers

Nine polymorphic and reproducible primers *viz.*, SV-F01, SV-F02, SV-Y02, SV-Y05, SV-A04, SV-Y03, SV-Y04, SV-A05, and SV-Y01 were used for RAPD profiling. The band pattern obtained from agarose gel electrophoresis was digitalized to a binary-matrix (0 and 1 for absence and presence of RAPD-bands, respectively). The data of all the primers were combined. DARwin software (version 6.0.12) [15, 16] using the Jaccard coefficient was used to generate dissimilarity matrix and dendrogram was thus constructed on the basis of unweighted pair group method of arithmetic average (UPGMA) clustering.

## Results and Discussion

### Polymorphism using Random amplified polymorphic DNA (RAPD) primers

Only nine primers that gave clear and polymorphic amplification patterns were used for genetic diversity analysis for fingerprinting of 36 isolates of *Stemphylium vesicarium*

obtained from different geographical regions (Plate 1). The number of scorable and polymorphic bands ranged from 7 to 160 and from 2 to 10, respectively (Table 2). Highest scored bands were 160 in SV-A04 with highest polymorphic band (10) showing 6.25 per cent polymorphism. The least scored bands were 7 in SV-F02 with least polymorphic bands (2) showing 28.57 per cent polymorphism. In total, 579 bands were scored, of which 54 were polymorphic, thereby showing 9.32 per cent polymorphism.

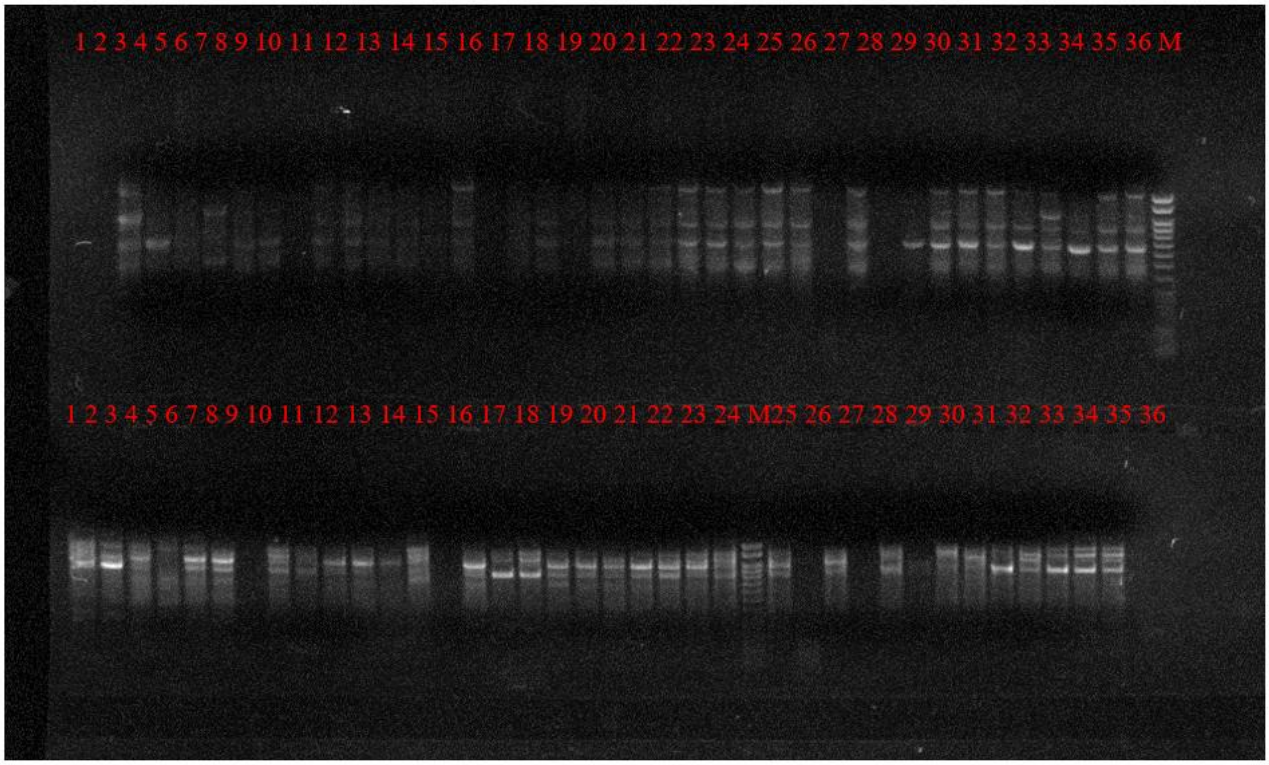


**Plate 1:** RAPD profile of 36 isolates of *S. vesicarium* using SV-Y02 and SV-Y05

**Table 2:** Polymorphism survey in 36 *Stemphylium vesicarium* isolates based on RAPD genotyping

| Primer | Scored bands | Polymorphic bands | Polymorphism (%) |
|---|---|---|---|
| SV-F01 | 71 | 07 | 09.85 |
| SV-F02 | 07 | 02 | 28.57 |
| SV-Y02 | 108 | 06 | 05.55 |
| SV-Y05 | 80 | 07 | 08.75 |
| SV-A04 | 160 | 10 | 06.25 |
| SV-Y03 | 47 | 07 | 14.89 |
| SV-Y04 | 41 | 04 | 09.75 |
| SV-A05 | 50 | 06 | 12.00 |
| SV-Y01 | 15 | 05 | 33.33 |
| Total | 579 | 54 | 9.32 |

**Dissimilarity coefficient**

Jaccard's pair-wise dissimilarity coefficient values were calculated among *S. vesicarium* isolates based on RAPD data. Genetic dissimilarity of the isolates varied from 1.00 to 0.14 (Table 3) with maximum of 1.00 between Sv-14; Sv-03 and Sv-14; Sv-04 and Sv-14; Sv-05 and Sv-14; Sv-06 and Sv-14; Sv-07 and Sv-14; Sv-08 and Sv-14; Sv-09 and Sv-14; Sv-10 and Sv-14; Sv-12 and Sv-14; Sv-13 and Sv-25; Sv-14 and Sv-26; Sv-14 and Sv-27; Sv-14 and Sv-28; Sv-14 and Sv-29; Sv-14 and Sv-30; Sv-14 and Sv-31; Sv-14 and Sv-32; Sv-14 and Sv-34; Sv-14 and Sv-26; Sv-04 and Sv-28; Sv-03 and Sv-28; Sv-04 and Sv-28; Sv-06 and Sv-27; Sv-26 and Sv-28; Sv-25 and Sv-28; Sv-27 and Sv-29; Sv-28 and Sv-30; Sv-28 and between Sv-07 with rest of all the isolates while as the minimum average genetic dissimilarity of 0.14 between Sv-19 and Sv-20 belonging to same geographical location indicating that these two isolates have the least resemblance among themselves as compared to all other isolates.

**Table 3:** Jaccard's dissimilarity matrix based on RAPD data between 36 isolates of *Stemphylium vesicarium*

| | Sv-1 | Sv-2 | Sv-3 | Sv-4 | Sv-5 | Sv-6 | Sv-7 | Sv-8 | Sv-9 | Sv-10 | Sv-11 | Sv-12 | Sv-13 | Sv-14 | Sv-15 | Sv-16 | Sv-17 | Sv-18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sv-1 | | | | | | | | | | | | | | | | | | |
| Sv-2 | 0.45 | | | | | | | | | | | | | | | | | |
| Sv-3 | 0.78 | 0.84 | | | | | | | | | | | | | | | | |
| Sv-4 | 0.90 | 0.87 | 0.65 | | | | | | | | | | | | | | | |
| Sv-5 | 0.55 | 0.73 | 0.63 | 0.77 | | | | | | | | | | | | | | |
| Sv-6 | 0.68 | 0.66 | 0.72 | 0.84 | 0.65 | | | | | | | | | | | | | |
| Sv-7 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | |
| Sv-8 | 0.65 | 0.63 | 0.58 | 0.80 | 0.67 | 0.66 | 1 | | | | | | | | | | | |
| Sv-9 | 0.55 | 0.75 | 0.77 | 0.87 | 0.58 | 0.54 | 1 | 0.69 | | | | | | | | | | |
| Sv-10 | 0.71 | 0.77 | 0.70 | 0.76 | 0.64 | 0.68 | 1 | 0.52 | 0.54 | | | | | | | | | |
| Sv-11 | 0.83 | 0.91 | 0.69 | 0.70 | 0.67 | 0.72 | 1 | 0.63 | 0.64 | 0.36 | | | | | | | | |
| Sv-12 | 0.91 | 0.93 | 0.90 | 0.95 | 0.87 | 0.71 | 1 | 0.88 | 0.73 | 0.76 | 0.75 | | | | | | | |
| Sv-13 | 0.65 | 0.7 | 0.69 | 0.76 | 0.62 | 0.52 | 1 | 0.57 | 0.52 | 0.52 | 0.69 | 0.82 | | | | | | |
| Sv-14 | 0.95 | 0.91 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | |
| Sv-15 | 0.77 | 0.85 | 0.81 | 0.88 | 0.65 | 0.76 | 1 | 0.88 | 0.66 | 0.88 | 0.83 | 0.8 | 0.88 | 0.92 | | | | |
| Sv-16 | 0.61 | 0.71 | 0.75 | 0.85 | 0.64 | 0.55 | 1 | 0.59 | 0.47 | 0.60 | 0.70 | 0.83 | 0.36 | 0.94 | 0.68 | | | |
| Sv-17 | 0.70 | 0.70 | 0.8 | 0.92 | 0.73 | 0.58 | 1 | 0.76 | 0.57 | 0.65 | 0.81 | 0.85 | 0.63 | 0.91 | 0.8 | 0.5 | | |
| Sv-18 | 0.68 | 0.73 | 0.72 | 0.78 | 0.66 | 0.70 | 1 | 0.56 | 0.57 | 0.3 | 0.5 | 0.78 | 0.5 | 0.88 | 0.80 | 0.45 | 0.55 | |
| Sv-19 | 0.66 | 0.80 | 0.7 | 0.8 | 0.6 | 0.73 | 1 | 0.65 | 0.55 | 0.56 | 0.6 | 0.86 | 0.70 | 0.9 | 0.5 | 0.5 | 0.76 | 0.41 |
| Sv-20 | 0.7 | 0.8 | 0.64 | 0.79 | 0.63 | 0.72 | 1 | 0.58 | 0.64 | 0.47 | 0.52 | 0.85 | 0.69 | 0.89 | 0.60 | 0.47 | 0.75 | 0.39 |
| Sv-21 | 0.58 | 0.75 | 0.65 | 0.82 | 0.56 | 0.62 | 1 | 0.55 | 0.40 | 0.51 | 0.60 | 0.84 | 0.55 | 0.91 | 0.57 | 0.33 | 0.6 | 0.38 |
| Sv-22 | 0.76 | 0.77 | 0.67 | 0.77 | 0.70 | 0.70 | 1 | 0.57 | 0.71 | 0.41 | 0.57 | 0.87 | 0.52 | 0.90 | 0.79 | 0.48 | 0.73 | 0.33 |
| Sv-23 | 0.64 | 0.73 | 0.63 | 0.81 | 0.62 | 0.65 | 1 | 0.45 | 0.58 | 0.48 | 0.57 | 0.87 | 0.52 | 0.90 | 0.75 | 0.27 | 0.62 | 0.33 |
| Sv-24 | 0.66 | 0.79 | 0.65 | 0.78 | 0.56 | 0.62 | 1 | 0.60 | 0.51 | 0.33 | 0.55 | 0.84 | 0.5 | 0.96 | 0.72 | 0.46 | 0.6 | 0.32 |
| Sv-25 | 0.71 | 0.77 | 0.6 | 0.76 | 0.59 | 0.68 | 1 | 0.52 | 0.65 | 0.31 | 0.36 | 0.76 | 0.65 | 1 | 0.79 | 0.60 | 0.71 | 0.45 |
| Sv-26 | 0.8 | 0.84 | 0.95 | 1 | 0.86 | 0.86 | 1 | 0.94 | 0.78 | 0.82 | 0.94 | 0.87 | 0.94 | 1 | 0.86 | 0.94 | 0.75 | 0.9 |
| Sv-27 | 0.68 | 0.73 | 0.81 | 0.84 | 0.70 | 0.7 | 1 | 0.66 | 0.54 | 0.61 | 0.66 | 0.8 | 0.52 | 1 | 0.7 | 0.38 | 0.73 | 0.59 |
| Sv-28 | 0.95 | 0.91 | 1 | 1 | 0.95 | 1 | 1 | 0.93 | 0.89 | 0.94 | 1 | 1 | 0.93 | 1 | 0.92 | 0.94 | 0.91 | 0.94 |
| Sv-29 | 0.83 | 0.82 | 0.6 | 0.81 | 0.73 | 0.68 | 1 | 0.59 | 0.70 | 0.60 | 0.65 | 0.83 | 0.70 | 1 | 0.79 | 0.66 | 0.71 | 0.58 |
| Sv-30 | 0.68 | 0.82 | 0.83 | 0.91 | 0.76 | 0.77 | 1 | 0.73 | 0.72 | 0.75 | 0.73 | 0.72 | 0.73 | 1 | 0.77 | 0.68 | 0.94 | 0.77 |
| Sv-31 | 0.65 | 0.75 | 0.64 | 0.79 | 0.6 | 0.54 | 1 | 0.45 | 0.48 | 0.47 | 0.52 | 0.85 | 0.45 | 1 | 0.81 | 0.33 | 0.63 | 0.39 |
| Sv-32 | 0.63 | 0.72 | 0.66 | 0.72 | 0.61 | 0.64 | 1 | 0.56 | 0.51 | 0.39 | 0.5 | 0.81 | 0.61 | 1 | 0.78 | 0.52 | 0.66 | 0.44 |
| Sv-33 | 0.65 | 0.75 | 0.73 | 0.83 | 0.71 | 0.54 | 1 | 0.69 | 0.53 | 0.65 | 0.74 | 0.8 | 0.52 | 0.95 | 0.72 | 0.33 | 0.5 | 0.52 |
| Sv-34 | 0.71 | 0.76 | 0.62 | 0.76 | 0.69 | 0.69 | 1 | 0.56 | 0.57 | 0.45 | 0.56 | 0.76 | 0.66 | 1 | 0.78 | 0.57 | 0.72 | 0.5 |
| Sv-35 | 0.62 | 0.66 | 0.8 | 0.86 | 0.7 | 0.57 | 1 | 0.72 | 0.5 | 0.56 | 0.72 | 0.77 | 0.60 | 0.94 | 0.75 | 0.42 | 0.52 | 0.54 |
| Sv-36 | 0.69 | 0.74 | 0.68 | 0.78 | 0.63 | 0.66 | 1 | 0.59 | 0.6 | 0.44 | 0.53 | 0.83 | 0.68 | 0.95 | 0.75 | 0.55 | 0.64 | 0.48 |

| | Sv-19 | Sv-20 | Sv-21 | Sv-22 | Sv-23 | Sv-24 | Sv-25 | Sv-26 | Sv-27 | Sv-28 | Sv-29 | Sv-30 | Sv-31 | Sv-32 | Sv-33 | Sv-34 | Sv-35 | Sv-36 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sv-20 | 0.14 | | | | | | | | | | | | | | | | | |
| Sv-21 | 0.24 | 0.28 | | | | | | | | | | | | | | | | |
| Sv-22 | 0.44 | 0.36 | 0.41 | | | | | | | | | | | | | | | |
| Sv-23 | 0.38 | 0.29 | 0.23 | 0.30 | | | | | | | | | | | | | | |
| Sv-24 | 0.42 | 0.40 | 0.34 | 0.29 | 0.35 | | | | | | | | | | | | | |
| Sv-25 | 0.5 | 0.40 | 0.51 | 0.48 | 0.48 | 0.46 | | | | | | | | | | | | |
| Sv-26 | 0.90 | 0.95 | 0.88 | 0.96 | 0.96 | 0.83 | 0.88 | | | | | | | | | | | |
| Sv-27 | 0.5 | 0.54 | 0.52 | 0.6 | 0.54 | 0.62 | 0.47 | 1 | | | | | | | | | | |
| Sv-28 | 0.95 | 0.95 | 0.91 | 0.95 | 0.95 | 0.96 | 1 | 0.8 | 1 | | | | | | | | | |
| Sv-29 | 0.61 | 0.6 | 0.57 | 0.53 | 0.53 | 0.51 | 0.54 | 0.94 | 0.68 | 1 | | | | | | | | |
| Sv-30 | 0.68 | 0.66 | 0.73 | 0.80 | 0.76 | 0.77 | 0.61 | 0.91 | 0.53 | 1 | 0.91 | | | | | | | |
| Sv-31 | 0.5 | 0.48 | 0.40 | 0.42 | 0.29 | 0.40 | 0.47 | 0.95 | 0.4 | 0.95 | 0.47 | 0.72 | | | | | | |
| Sv-32 | 0.48 | 0.46 | 0.5 | 0.51 | 0.46 | 0.44 | 0.31 | 0.86 | 0.45 | 0.95 | 0.62 | 0.57 | 0.33 | | | | | |
| Sv-33 | 0.60 | 0.59 | 0.46 | 0.58 | 0.48 | 0.51 | 0.65 | 0.90 | 0.54 | 0.95 | 0.65 | 0.66 | 0.41 | 0.46 | | | | |
| Sv-34 | 0.58 | 0.51 | 0.5 | 0.56 | 0.46 | 0.5 | 0.52 | 0.91 | 0.64 | 0.95 | 0.52 | 0.69 | 0.46 | 0.38 | 0.4 | | | |
| Sv-35 | 0.62 | 0.61 | 0.53 | 0.65 | 0.55 | 0.53 | 0.62 | 0.83 | 0.57 | 0.94 | 0.73 | 0.63 | 0.56 | 0.41 | 0.28 | 0.34 | | |
| Sv-36 | 0.56 | 0.5 | 0.48 | 0.54 | 0.44 | 0.43 | 0.5 | 0.87 | 0.66 | 0.95 | 0.60 | 0.72 | 0.5 | 0.37 | 0.38 | 0.16 | 0.26 | - |

**Cluster analysis**

Cluster analysis was conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based Arithmetic Average (UPGMA) and dendrogram generated (Fig.1). Dendrogram showed one independent lineages compromising of isolates (Sv-07) and one main clusters. Cluster I was subdivided into one independent lineage compromising of one isolate (Sv-14) and cluster Ia showing an average genetic diversity of approximately 2.4 per cent and genetic similarity of 97.6 per cent between them. Cluster Ia was subdivided into two clusters Iaa and cluster Iab. Cluster Ia compromised of two isolates (Sv-26 and Sv-28) and cluster Iab compromised of rest of the isolates showing an average genetic diversity of approximately 3.9 per cent and genetic similarity of 96.1 per cent between them.

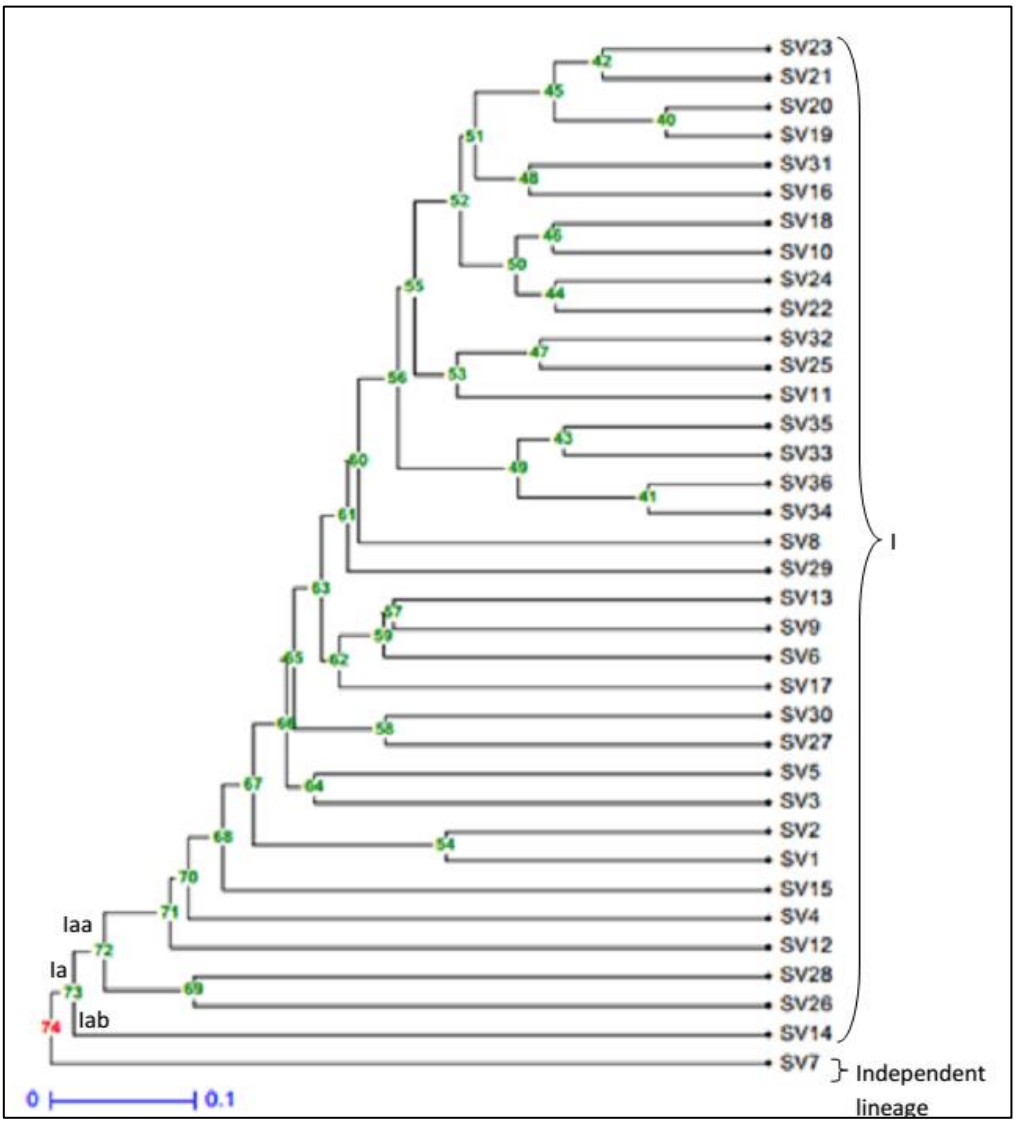


**Fig 1:** Dendrogram of 36 isolates of *Stemphylium vesicarium* generated by unweighted pair group method arthimetic mean (UPGMA) analysis of RAPD bands obtained with 09 primers.

The present study is the first report dealing with the study of genetic diversity using RAPD markers in *S. vesicarium* causing Stemphylium blight of onion in Kashmir. The study of genetic diversity using RAPD markers during the present study revealed moderate level of genetic diversity. These findings show that RAPD markers can be used in revealing genetic diversity of *S. vesicarium* causing Stemphylium blight of onion in Kashmir. RAPD markers have been already used for the study of genetic diversity in *S. vesicarium* in several earlier studies [17, 12].

The analysis of RAPD data revealed that there was no agreement of clustering of different fungal isolated from the same geographical region/district since isolates from different districts were clustered together. For instance, sub clusters Ia, Iaa and Ibb contained isolates from different districts. Our results are in agreement with the results of some earlier studies. For instance, 55 and 69% polymorphism was reported in *S. lycopersici* isolates using RAPD (Random amplified polymorphic DNA) and ISSR (inter simple sequence repeat) markers, respectively [18]. High genetic diversity was observed in *S. solani* isolates from cotton and tomato using RAPD markers [17]. It was reported that variation due to geographic region was observed for the tomato isolates but not for the cotton isolates. The perfect state of Stemphylium species belongs to *Pleospora* (Pers.er.Fr.) Rabenh is formed under prolonged cold conditions (4 $^{o}$C) prevalent in Kashmir valley. Formation/occurrence of perfect state on this fungus on onion has been already reported [19]. Therefore, substantial variation between different fungal isolates can be attributed to the phenomenon of sexual hybridisation. The high genetic diversity reported in some studies may be due to mutation, recombination (sexual or parasexual), migration (gene flow) and adaptation to diverse hosts

## Conclusion

Therefore, in conclusion, we can say that RAPD markers were found suitable for studying genetic diversity in this pathogen. RAPD data revealed a high level of genetic diversity within populations. High diversity indicated that *S. vesicarium* is quickly evolving fungus and has potential to overcome management strategies using fungicides and resistant varieties.

## References

1. Vohra SB, Rizman M, Khan JA. Medicinal uses of common Indian vegetables. Planta Medica 1974;23:381-393.
2. Hassan MHA, Hussein MAM. New Disease Report. Plant Pathology Department, Faculty of Agriculture, Assiut University, Egypt 2007, pp. 724.

3. Slimestad R, Fossen T, Vangenlm. Onions: A source of unique dietry flavonoides. Journal of Agriculture and Food Chemistry 2007;55:24-34.
4. Meah B, Khan AA. Checklist of vegetables and fruit diseases in Bangladesh. Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh 1987, pp. 22.
5. Daljeet S, Dhiman JS, Sidhu AS, Singh H. Current status of onions in India. Strategies for distance resistance breeding for sustained production. Onion News Letters for the Tropics 1992;4:43-44.
6. Tomaz I, Lima A. An important disease of onion caused by *Stemphylium vesicarium* {(Wallr.) Simmons} in Portugal. Horticultural Abstracts 1988;68:618.
7. Rather RA, Nisar MU, Raina ZK, Ahanger FA, Bhat NA, Chesti F, *et al*. Population Structure of *Sclerotinia sclerotiorum* (Lib.) de Bary Causing White Mold of Bean in Kashmir, India. Int. J Curr. Microbiol App Sci 2018;7(09):3795-3800.
8. Collignon AM, Van de Sype H, Favre JM. Geographical variation in random amplified polymorphic DNA and quantitative traits in Norway spruce. Canadian Journal of Forest Research 2002;32:266-282.
9. Pathak VN. Essentials of Plant Pathology, Prakash Publishers, Jaipur India 1972, 448p.
10. Hassan M, Yousuf V, Bhat ZA, Bhat NA, Shah TA, Khan MA, *et al.* Morpho-cultural and pathogenic variability among isolates of *Stemphylium vesicarium* (Wallr.) E. Simmons, causing Stemphylium blight in onion collected from different geographical regions of Kashmir valley. Indian Phytopathology 2020;73:469-481.
11. Basallotte-Ureba MJ, Prados-Ligero AM, Melero-Vara JM. Aetiology of leaf spot of garlic and onion caused by *Stemphylium vesicarium* in Spain. Plant Pathol 1999;48:139-145.
12. Williams JGK, Kubelik AR, Livak KJ, Rafalski JAA. Tinggey SV. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 1990;18:6531-6535.
13. Murray MG, Thomson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 1980;8:4321-4325
14. White TJ, Bruns T, Lee S, Tailor S. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In*:* PCR protocols. A guide to methods and applications. (Eds M. A. Gelf, D. H. Sninsky. J. J. White and T. J. San Diego) California, Academic Press, USA 1990, pp. 315-322.
15. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, *et al*. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988;239:487-91.
16. Perrier X, Flori A, Bonnot F. Genetic diversity of cultivated tropical plants pp. 43 - 76. In:Data analysis methods, (Eds. P.S. Hamon, M. Perrier, and J. C. Glaszmann). Enfield, Science Publishers, Montpellier, USA 2003.
17. Perrier X, Jacquemoud JP. DARwin software 2006. <http://Darwin.Cirad.fr/darwin>
18. Mehta YR. Genetic diversity among isolates of *Stemphylium solani* from cotton. Fitopathologia Brasileira 2001;26:703-709.
19. Nasehi A, Kadir JB, Nasr Esfahani M, Abed Ashtiani F, Wong MY, Rambe SK *et al.* Analysis of genetic and virulence variability of *Stemphylium lycopersici* associated with leaf spot of vegetable crops. European Journal of Plant Pathology 2014;140:261-273.
20. Bhat S. Studies on stemphylium blight [*Stemphylium vesicarium* (Wallr.) E. Simmons] of onion in Kashmir. M. Sc. thesis submitted to Sher-e-Kashmir University of Agricultural science and Technology of Kashmir, Shalimar, Srinagar 2007, 51.