Indian Phytopathology (2020) 73:469–481
https://doi.org/10.1007/s42360-020-00253-8

RESEARCH ARTICLE

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

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Received: 21 July 2019 / Revised: 15 April 2020 / Accepted: 21 May 2020 / Published online: 31 May 2020

Abstract

Stemphylium blight is the most destructive disease of onion crop and poses a grave threat to the very existence of its cultivation in Kashmir. Thirty six (36) isolates of *Stemphylium vesicarium* (Wallr.) E. Simmons were collected from different locations and characterized for cultural, morphological and pathogenic variations**.** Isolates produced velvety, cottony or fullfy colonies of different colours like whitish, light to dark grey, olivaceous with greenish tinge and brownish with filliform, entire and undulate margins. Significant variation in colony diameter and sporulation was observed among isolates. Mean hyphal width ranged from 3.11 to 5.48 µm. Conidiophore length varied from 20.07 to 92.56 µm. Similarly, mean conidiophore breadth varied from 2.84 to 7.58 µm. and were either light brown, light brown to brown, brown and dark brown in colour. Conidial colour of isolates varied from light brown, brown, light brown to brown and dark brown and are ovoid, ovoid to oblong and oblong in shape. Transverse septation varied from 0 to 6 and longitudinal from 0 to 5. Average ascus size of isolates varies from 103.74–204.24 × 23.00–33.11 µm. Average ascospore size varied from 12.08–41.96 × 10.06–17.38 µm among the isolates. Transverse and longitudinal septation varied from 3 to 7 and longitudinal from 0 to 6. Ascospores varied in colour from light brown, to dark brown. In shape the ascospores of different isolates were oblong with rounded base and conical apex, oblong with both ends rounded and ellipsoidal. Isolates exhibited variations in incubation period, number, size and colour of the lesions.

Keywords Onion · Stemphylium blight · *Stemphylium vesicarium* · Variability · Pathogenicity

The article was originally published electronically on the publisher's internet portal (currently SpringerLink) on 31 May 2020 with open access.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s42360-020-00253-8) contains supplementary material, which is available to authorized users.

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Introduction

Onion (*Allium cepa* L.) is one of the most important and familiar crop throughout the world that belongs to the family Alliaceae. It is used as a common favourite spice, salad and vegetable in many countries of Asia. Onion has manifold uses as spice and condiment for flavoring a number of foods and medicines (Vohra et al. 1974; Hassan and Hussein 2007). Onion bulbs are rich source of minerals like phosphorus, calcium and carbohydrates besides being rich in proteins and vitamin C. Onion contains chemical compounds with potential anti-inflammatory, anti-cholesterol and anti-cancer properties with well identified fungicidal and insecticidal properties (Slimestad et al. 2007; Mishra et al. 2014). Out of 15 important vegetables and spice crops listed by FAO, onion stands second in terms of annual world production (FAOSTAT 2015).

Onion is grown worldwide over an area of 3991.51 thousand hectares, with a total production of 76,377.21 thousand MT of which fifty per cent is grown in Asia. After China, India ranks second in production accounting for 26.8 percent of world area and 19.9% of onion production. The area and production of onion in India is about 11.81 metric hectares and 189.24 metric tonnes of bulb, respectively (National Horticulture Board 2015). In Jammu and Kashmir onion is cultivated commercially on an area of 950 hectares with a total production of 24.250 MT and productivity 25.40 tonnes per hectare (Anonymous 2014).

Onion suffers from many diseases caused by fungi, bacteria, viruses, nematodes and abiotic factors (Meah and Khan 1987). Among the fungal diseases stemphylium blight caused by *Stemphylium vesicarium* (Wallr.) E. Simmons, a Dematiaceous hyphomycetes with its perfect state as *Pleospora allii* (Rabenh.) Ces. & de Not. (Simmons 1969) is the most serious and devastating, limiting the quality and quantity of both bulb and seed (Daljeet et al. 1992).

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. It results in severe damage, more particularly to the onion seed crop by affecting leaves and seed stalk and results in losses of about 80–85% on the crop (Tomaz and Lima 1988).

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. In order to breed the varieties with durable resistance to the Stemphylium blight of onion, there is need for identification of source of resistance against the range of virulence present in the pathogen population. Study of population structure of the pathogen is the basic step to devise management strategies against a disease (Rather et al. 2018). Variability studies are important to document the changes occurring in the population and individuals with variability in morphological, cultural and pathogenic characteristics. Under natural epiphytotics, *S. vesicarium* has been found to express a wide range of variability in disease symptoms expression depending upon the onion cultivars, environmental conditions, etc., as observed by various workers (Hosen et al. 2009; Arzanlou et al. 2012; Nisha 2013). Keeping the above facts in view, study regarding variability among isolates of *Stemphylium vesicarium* was undertaken.

Materials and methods

Collection of diseased samples

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.

Isolation

The isolation of the pathogen from the diseased samples was carried out by tissue bit transfer method. The diseased leaf area along with some healthy portion was cut into small bits with a sharp sterilized blade. These bits were surface sterilized in 0.01% mercuric chloride ($HgCl_2$) for 30 s followed by three washings in sterilized distilled water to remove the traces of mercuric chloride (Pathak 1972). After blotting dry with sterilized filter paper, these bits were transferred to sterilized potato dextrose agar (PDA) medium in sterilized petriplates. Three such bits were placed in each petriplates and incubated for seven days at 24 ± 1 °C.

Purification and maintenance of the pathogen isolates

The pure culture of the fungal isolates was made through single spore isolation technique. The conidia from one week old culture were harvested by flooding with 10 ml of sterilized distilled water, serially diluted and streaked on water agar in petriplates. After 24 h of incubation, the germinating spores along with the agar disc were lifted with the help of sterilized scalpel, transferred to PDA medium in petriplates aseptically and incubated at (24 ± 1 °C) to maintain the growth. The pure cultures so obtained was maintained at 5 °C in the refrigerator for further studies.

Pathogenicity test

The pathogenicity of the isolates of causal fungus was established by proving the Koch's postulates on potted onion plants as per the method adopted by Basallote-Ureba et al. (1999). The isolates of *S. vesicarium*, were multiplied on PDA for 10 days in biological oxygen demand (BOD) incubator. After 10 Days of Incubation (DoI), spore suspension was prepared by flooding culture plates with sterilized distilled water. Scraping with a sterilized razor blade, straining through a double layer of sterile cheese cloth into a 150 ml flask, and adjusting the spore concentration with haemocytometer to 5×10^4 conidia per milliliter. The prepared conidial suspensions were used for pathogenicity test.

Onion seedlings were planted in pots containing sterilized potting mixture. The sixty days old onion plants were inoculated with the conidial suspension of 5×10^4 spores ml^{-1} of

causal pathogen by spraying thoroughly with glass atomiser. After inoculation, plants were maintained under day/night temperatures of 22–26 °C/18–20 °C and wetness was maintained on plants for 72 h by covering them with clear polyethylene bags sprayed inside with sterile distilled water. An uninoculated onion plant maintained under similar conditions served as check. After 72 h, polyethylene covers were removed and plants were moved to a greenhouse for three weeks. Disease development was assessed up to three weeks after inoculation.

Cultural variability

All the thirty six pathogenic isolates were raised in petriplates on PDA. Mycelial discs (5 mm) of 7 day old cultures were aseptically transferred to the center of the fresh PDA plates and incubated at 25 ± 1 °C for 7 days. Three replications were maintained for each isolate in completely randomized design. Cultural characteristics viz., colony type, colour, type of margins, colony diameter were recorded after 7 DoI, while sporulation and pigmentation were recorded after 10 DoI. The sporulation was studied, by thoroughly homogenizing a 10 mm mycelial disc in 3 ml of sterilized distilled water. The spore suspension thus obtained was used for counting the number of spores with the help of heamocytometer. Reverse side of cultural plate of each fungal isolate was observed to record colour on underside of plate.

Morphological variability

Ten days old culture of all the thirty six isolates grown on PDA were studied for any morphological variations. Temporary mounts were studied under compound microscope for morphological characteristics viz., shape, colour, size and septation of mycelium, conidiophore and conidia of all the thirty six isolates grown on potato dextrose agar. Further, the ascomata (pseudothecia) formed on the culture plates were studied for the size of the asci and size, colour and shape of the ascospores.

Measurements were taken with the help of software MagVision. Fifty recordings per replication were made for the purpose.

Data analysis of diversity of isolates on the basis of cultural and morphological characters

All the data of cultural and morphological variability was digitalized into two discrete character matrix (0 and 1 for absence and presence of a particular character, respectively). The binary data of tables pertaining to colony characters, pigmentation, colour of hyphae, colour of conidiophores, colour and shape of conidia and ascospores was generated on the basis of presence and absence of a particular character. The binary data of the tables pertaining to hypal width, conidiophore size and septation, conidial size and septation, ascospore size and septation were generated on the basis of critical difference (CD). The data of all the characters was combined. Characters that could be scored univocally for presence and absence were included in analysis. Binary matrices were analyzed by DARwin software (version 6.0.12) using the Jaccard coefficient to construct dendrogram using SHAN clustered programme, selecting the unweighted pair group method of arithmetic average (UPGMA) of DARwin software (version 6.0.12) (Perrier et al. 2003; Perrier and Jacquemoud 2006).

Pathological variability

Pathological variability of *S. vesicarium* isolates was studied on susceptible onion (cv. Yellow Globe) seedlings grown in pots. Onion seedlings of susceptible cultivar were separately inoculated with each test isolate. The method used for inoculation and incubation of inoculated seedlings was same as pathogenicity test described above. Observations were made on incubation period, lesion number, lesion size and colour. The size of the lesion was recorded 15 days after inoculation by taking average of two perpendicular measurement. The experiment was laid in CRD with three replications. Based on these observations, the test isolates were categorized into different pathogenic groups such as highly virulent, virulent, moderately virulent and mildly virulent on the basis of lesion size as per the scale generated given below:

Lesion size	Pathogenic group
< 1.50 mm	Mildly virulent
1.51–2.00 mm	Moderately virulent
2.01–2.50 mm	Virulent
> 2.51 mm	Highly virulent

Results and discussion

Cultural variability

Significant variation was observed among *S. vesicarium* isolates which differed with respect to cultural characteristics viz., colony type, colour, margin, colony diameter, sporulation and pigmentation. Isolates on PDA showed distinct variation in colony characters (Fig. 1). Colonies were either velvety, cottony or fullfy in mycelial growth with colour ranging from whitish, light to dark grey, olivaceous with greenish tinge to brownish in colour. Margins of the colonies were filliform, entire or undulate with whitish colour. (Table 1, Fig. 1).

Table 1 Colony characters of *Stemphylium vesicarium* isolates on onion from Kashmir

Isolate	Colony type	Colour	Margin	Growth after 7 days of incubation (mm)	Sporulation (number of conidia/mm^2)
Sv-01	Cottony, appressed centre with concentric zones	Whitish	Filliform, whitish	54.33	35.35
Sv-02	Cottony, non appressed	Whitish with light grey centre	Filliform, whitish	59.51	23.88
Sv-03	Velvety, with cottony appressed centre	Light grey with whitish centre	Filliform, whitish	52.71	23.88
Sv-04	Cottony, appressed with blackish ring	Greenish grey	Entire, whitish	69.39	71.65
Sv-05	Cottony with appressed centre	Dirty white	Entire, whitish	62.59	71.65
Sv-06	Cottony, non appressed	Light grey to whitish	Filliform, whitish	71.62	53.58
Sv-07	Velvety, non appressed	Light grey	Undulate, whitish	53.92	52.54
Sv-08	Velvety with cottony appressed centre	Light grey to whitish	Filliform, whitish rim	54.55	23.88
Sv-09	Velvety with fullfy appressed centre	Whitish	Filliform, whitish filliform	69.55	23.88
Sv-10	Cottony, appressed with concentric rings	Concentric zones of white and greenish grey regions	Filliform, with brownish margins	55.44	53.58
Sv-11	Velvety, appressed centre	Concentric zones of dark and light grey	Filliform, whitish	65.59	23.58
Sv-12	Velvety, with appressed cottony centre	Brownish grey with whitish centre	Filliform, whitish	61.33	23.58
Sv-13	Cottony with fullfy centre	Light grey with whitish centre	Filliform, whitish	84.48	23.58
Sv-14	Velvety, with appressed centre	Dark grey	Filliform, whitish	58.41	23.58
Sv-15	Velvety, non appressed	Light grey	Entire, whitish	64.74	23.58
Sv-16	Velvety, non appressed	Light grey	Filliform, whitish	59.59	35.53
Sv-17	Cottony, appressed centre	Light grey to whitish	Filliform, whitish	50.34	23.88
Sv-18	Cottony, appressed centre	Dark grey to brownish	Filliform, whitish	52.63	23.88
Sv-19	Cottony, non appressed	Light grey	Filliform, whitish	67.58	23.88
Sv-20	Velvety, non appressed	Light grey	Filliform, whitish	52.66	23.88
Sv-21	Velvety, appressed centre	Light grey	Entire, whitish	64.77	23.88
Sv-22	Fullfy, appressed	Greenish with light grey centre	Undulate, whitish	62.58	71.65
Sv-23	Velvety, non appressed	Dark grey to brownish	Filliform, whitish	58.59	23.88
Sv-24	Velvety, appressed centre with concentric rings	Olivaceous green with light grey centre	Filliform, whitish	62.89	71.65
Sv-25	Cottony, appressed centre	Light grey	Filliform, whitish	82.38	23.88
Sv-26	Cottony, appressed	Light grey to whitish	Filliform, whitish	52.56	23.88
Sv-27	Fullfy, appressed	Light grey to whitish	Undulate, whitish	43.59	23.88
Sv-28	Velvety with cottony appressed central growth	Brownish with light grey centre	Filliform, whitish	59.35	23.88
Sv-29	Cottony, appressed centre	Light grey	Filliform whitish	52.65	23.88
Sv-30	Velvety, appressed centre	Dark grey	Filliform, whitish	52.64	23.88
Sv-31	Velvety, appressed centre	Browish to light grey	Filliform, whitish	59.58	23.88
Sv-32	Cottony, appressed centre	Dark grey	Filliform, Whitish	74.66	23.88
Sv-33	Cottony, appressed	Greenish grey	Filliform, Whitish	64.66	35.35
Sv-34	Fullfy, non appressed	Light grey	Filliform, whitish	55.66	52.54
Sv-35	Velvety with cottony centre, appressed centre	Light grey	Filliform, whitish	51.44	35.35
Sv-36	Cottony, appressed	Light grey	Filliform, whitish	44.49	35.35
$CD_{(p \leq 0.05)}$				00.81	0.01

Table 2 Variability in the conidiophores of *Stemphylium vesicarium* isolates on onion from Kashmir

Isolate	Conidiophore		Septation (No.)	Colour
	Length (µm)[a]	Breadth (µm)[a]		
Sv-01	56.77	4.46	1–5	Brown
Sv-02	79.76	4.23	2–7	Light brown
Sv-03	48.14	4.24	2–8	Brown
Sv-04	81.51	3.06	2–8	Brown
Sv-05	59.71	4.12	2–6	Brown
Sv-06	56.44	4.64	0–6	Light brown
Sv-07	60.44	5.10	2–5	Brown
Sv-08	48.10	5.20	2–7	Brown
Sv-09	59.11	4.80	1–4	Brown
Sv-10	60.84	5.19	1–8	Light brown to brown
Sv-11	46.56	4.47	1–6	Brown
Sv-12	62.17	4.30	1–7	Brown
Sv-13	67.76	4.87	1–6	Light brown
Sv-14	52.66	4.28	1–3	Brown
Sv-15	39.75	7.30	0–2	Light brown
Sv-16	27.65	2.86	0–2	Light brown
Sv-17	20.56	5.33	0–2	Dark brown
Sv-18	48.98	4.09	1–3	Light brown
Sv-19	36.16	5.40	1–3	Light brown
Sv-20	26.12	4.76	1–6	Light brown
Sv-21	67.04	4.06	2–6	Brown
Sv-22	27.69	7.60	0–2	Brown
Sv-23	90.08	4.79	2–6	Light brown
Sv-24	66.40	5.48	1–3	Light brown
Sv-25	20.07	4.98	0–2	Light brown
Sv-26	64.25	6.02	0–2	Light brown
Sv-27	52.12	3.77	1–4	Light brown
Sv-28	60.28	4.00	2–8	Light brown
Sv-29	71.24	4.76	3–8	Light brown
Sv-30	59.84	4.18	2–6	Light brown
Sv-31	92.56	4.30	3–7	Light brown
Sv-32	46.80	3.02	1–4	Brown
Sv-33	79.95	4.30	1–6	Light brown
Sv-34	52.80	4.50	2–6	Light brown to brown
Sv-35	49.98	4.43	1–7	Light brown
Sv-36	54.84	4.87	2–4	Light brown
$CD_{(p \leq 0.05)}$	0.316	0.009		

[a]Average of 50 observation

The data presented in Table 1 revealed that all the isolates of the pathogen exhibit variations in colony diameter after incubation period of 7 days. Isolate Sv-13 with mean colony diameter 84.48 mm was fastest growing while least colony diameter was observed in isolate Sv-27 (43.59 mm). Data presented in Table 1 revealed that some of the isolates exhibit significant variation with respect to sporulation but most of the isolates did not show any significant variation in sporulation with highest mean sporulation of (71.65 conidia mm^{-2}) in isolate Sv-04, Sv-05, Sv-22 and Sv-24, while as majority of the isolates exhibited least sporulation of 23.88 conidia mm^{-2}. The data on pigmentation [Supplementary Table 1 (S1)] revealed that isolates pigmented the media with different colours, mostly grey to brown with some

Table 3 Variability in the conidial size and septation of *Stemphylium vesicarium* isolates on onion from Kashmir

Isolate	Conidial size		Conidial septation	
	Length (µm)[a]	Breadth (µm)[a]	Transverse (No.)	Longitudinal (No.)
Sv-01	21.98	16.83	0–6	1–3
Sv-02	22.90	18.00	1–4	0–3
Sv-03	23.67	12.60	2–5	1–4
Sv-04	25.45	11.41	1–6	0–3
Sv-05	17.39	13.23	2–3	0–3
Sv-06	29.07	16.03	0–3	0–4
Sv-07	29.98	20.05	0–2	0–3
Sv-08	27.70	20.31	0–3	0–3
Sv-09	19.85	13.72	1–2	0–1
Sv-10	25.22	16.23	1–6	1–5
Sv-11	15.80	13.26	1–3	0–3
Sv-12	21.69	15.04	1–3	0–3
Sv-13	24.87	17.89	0–3	0–4
Sv-14	28.29	18.74	0–3	0–4
Sv-15	39.75	07.26	0–3	0–3
Sv-16	30.03	19.09	1–3	1–4
Sv-17	32.74	21.28	0–3	0–3
Sv-18	21.09	15.88	2–4	0–2
Sv-19	21.58	16.42	0–2	0–2
Sv-20	23.98	15.98	1–3	0–1
Sv-21	21.53	17.31	0–2	0–2
Sv-22	25.87	22.04	0–2	0–1
Sv-23	26.54	18.54	0–4	0–4
Sv-24	33.13	20.88	0–1	0–1
Sv-25	25.51	18.23	0–3	0–2
Sv-26	26.12	20.49	0–2	0–3
Sv-27	25.43	16.96	2–3	1–2
Sv-28	29.13	15.30	2–4	0–3
Sv-29	27.23	18.05	1–3	0–4
Sv-30	19.22	13.33	1–3	0–3
Sv-31	31.44	15.99	1–4	0–4
Sv-32	23.19	15.13	1–3	1–3
Sv-33	16.63	14.20	1–3	0–2
Sv-34	18.68	12.56	0–4	0–4
Sv-35	20.30	10.23	1–4	0–4
Sv-36	18.57	13.27	0–3	0–4
$CD_{(p \leq 0.05)}$	00.31	00.03		

[a]Average of 50 observation

variations which were clearly visible from the underside of the plate. The extent of difference significantly establishes that considerable variability exists in natural population of *S. vesicarium*. Several previous workers have also reported cultural variability among the isolates of *Stemphylium vesicarium* (Pei et al. 2010; Arzanlou et al. 2012; Nisha, 2013).

Morphological variability

Conspicuous variations were observed among isolates with respect to their morphological characters like hypal width and colour; condiophore size, septation and colour; and conidial size, septation, colour and shape.

The data on hyphal dimensions and colour (Table S1) revealed that mean hyphal width ranged from 3.11 to 5.48 µm with maximum hypal width in isolate Sv-24 and minimum in isolate Sv-13. Fifteen isolates had hyphae of light brown colour, seven had brown, eleven were hyaline and three isolates had light brown to brown hyphae. Observation recorded in Table 2 elucidated significant variation in conidiophore size, septation and colour with conidiophore length varying from 20.07 to 92.56 µm with maximum length of 92.56 µm in isolate Sv-31 and minimum length of 20.07 µm in Sv-25 isolate. Similarly, conidiophore breadth varied from 2.86 to 7.60 µm amongst the isolates with highest conidiophore breadth in isolate Sv-22 (7.60 µm) and the least in isolate Sv-16 (2.86 µm). Further, conidiophore septation among isolates was found to vary from 0 to 8. Light brown coloured conidiophores were observed in twenty isolates while light brown to brown in two isolates. Brown coloured conidiophores were observed in thirteen isolates, while only one isolate had dark brown coloured conidiophores.

The data presented in Table 3 revealed marked variation among isolates with respect to their morphological characters viz., conidial size and septation. The average maximum (39.75 µm) and minimum (15.80 µm) conidial length were recorded in isolate Sv-15 and Sv-11, respectively while as, average maximum (22.04 µm) and minimum (7.26 µm) conidial breadth were recorded in isolate Sv-22 and Sv-15, respectively. The conidial septation, both transverse and longitudinal, varied significantly among the isolates. Transverse septation varied from 0 to 6 and longitudinal from 0 to 5.

The data presented in Table 4 showed that distinct variations were found in colour and shape of conidia. Light brown colour was observed in eight isolates amounting to 22.22%, fourteen isolates were brown amounting to 38.89% of the isolates. Dark brown conidia were observed in nine isolates (25%), whereas only five isolates (13.88%) were light brown to brown. Distinct variations were observed in shape of conidia varying from ovoid, ovoid to oblong and oblong. Shape of the conidia of nine isolates (10%) was light brown. Seventeen isolates (47.22%) possessed

Table 4 Variability in the conidial colour and shape of *Stemphylium vesicarium* isolates on onion from Kashmir

Shape of conidia	Isolate		Colour of conidia	Isolate	
	Name	No		Name	No
Ovoid	Sv-02, Sv-09, Sv-13, Sv-19, Sv-21, Sv-26, Sv-30, Sv-34 and Sv-36	09	Light brown	Sv-10, Sv-15, Sv-18. Sv-19, Sv- 23, Sv-25, Sv-27 and Sv-31	08
Ovoid to oblong	(Sv-04, Sv-06, Sv-07, Sv-08, Sv-10, Sv-11, Sv-12, Sv-15, Sv-17, Sv-18, Sv-22, Sv-23, Sv-24, Sv-29, Sv-31, Sv-33 and Sv-35)	17	Brown	Sv-01, Sv-02, Sv-04, Sv-05, Sv-06, Sv-07, Sv-08, Sv-12, Sv-13, Sv-14, Sv-20, Sv-28, Sv-34 and Sv-36	14
Oblong	Sv-01, Sv-03, Sv-05, Sv-14, Sv-16, Sv-20, Sv-25, Sv-27, Sv-28 and Sv-32	10	Dark brown	Sv-03. Sv-09, Sv-11, Sv-17, Sv-26, Sv-29, Sv-30, Sv-32, and Sv-33	09
			Light brown to brown	Sv-16, Sv-21, Sv-22, Sv-24 and Sv-35	05

brown colour, whereas remaining 27.78% of the isolates were oblong in shape. Our findings are comparable with that of several workers who reported wide variability in *S. vesicarium* [Hassan et al. (2006); Pei et al. (2010); Arzanlou et al. (2012); Mc Kenzie (2013); Poursafar et al. (2016); Woudenberg et al. (2017)]. Similar observations were also made by Nisha (2013) who found a range of variability in the spore dimensions, colour, and shape of 24 isolates of *S. vesicarium.*

Morphological variations in perfect state (*Pleospora allii*) of *Stemphylium vesicarium* isolates

Significant variations were observed among the 36 isolates with respect to their morphological characters of perfect state produced in vitro on culture plates viz., ascus size, ascospore size, septation, colour and shape. The data presented in Table 5 revealed that average ascus size of isolates varies from 103.74–204.24 × 23.00–33.11 µm. The average maximum (204.24 µm) and minimum (103.72 µm) ascus length was recorded in isolates Sv-21 and Sv-16, respectively, while as average maximum (33.11 µm) and minimum (23.00 µm) ascus breadth was recorded in isolates Sv-06 and Sv-21, respectively. Further, average ascospore size varied from 12.08–41.96 × 10.06–17.38 µm. The average maximum (41.96 µm) and minimum (12.08 µm) ascospore length was observed in isolate Sv-26 and Sv-27, respectively, whereas, average maximum (17.38 µm) and minimum (10.06 µm) ascospore breadth were recorded in isolate Sv-26 and Sv-07, respectively. Isolates varied significantly in their transverse and longitudinal septation with transverse septation ranging from 3 to 7 and longitudinal from 0 to 6. An insight into the data presented in Table 6 revealed significant variations in colour and shape of ascospores. Ascospores of eleven isolates (36.55%) were light brown, of four isolates (11.11%) dark brown, one isolate (2.77%) brown to dark brown while light brown to brown and brown coloured ascospores composed ten isolates (27.77%) each. Distinct variations were observed in shape of ascospores varying from ellipsoidal and oblong with rounded base and conical apex or with both ends rounded. Out of thirty six isolates, the ascospores of 41.67% were oblong with rounded base and conical apex, of 8.33% were oblong with rounded base and conical apex to oblong with both ends rounded. Ellipsoidal ascospores were observed in ten isolates (27.78%) and only eight isolates (22.22%) were ellipsoidal to oblong with rounded base and conical apex. Our observations are comparable to the findings of Hassan et al. (2006); Mc Kenzie (2013) and Poursafar et al. (2016) who observed considerable morphological variability in perfect state of *S. vesicarium.*

Diversity analysis of *S. vesicarium* isolates on the basis of cultural and morphological characters

Jaccard's pair-wise dissimilarity coefficient (Table S2) values were calculated among *S. vesicarium* isolates based on cultural and morphological data. Dissimilarity matrix obtained from cultural and morphological characters data of the isolates varied from 0.45 to 0.84 with minimum between Sv-17 and Sv-15 belonging to same geographical location and maximum of 0.84 between majority of the isolates indicating that these isolates have the least resemblance among themselves. Cluster analysis was conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based Arithmetic Average (UPGMA) and dendrogram generated (Fig. 2). Dendrogram showed 8 independent lineages compromising of isolates (Sv-09, Sv-11, Sv-13, Sv-16, Sv-22, Sv-28, Sv-33, Sv-35) and 5 main clusters. Cluster I was subdivided into cluster Ia compromising of two isolates (Sv-10 and Sv-08) and one isolate Sv-12 formed independent lineage showing a average diversity of

Table 5 Variability in the ascus size, ascospore size and septation of perfect state (*Pleospora allii)* of *S. vesicarium* isolates

Isolate	Ascus size		Ascospore		Ascospore septation	
	Length (µm)[a]	Breadth (µm)[a]	Length (µm)[a]	Breadth (µm)[a]	Longitudinal range	Transverse range
Sv-01	158.99	26.29	31.75	16.29	05–07	00–04
Sv-02	178.99	30.52	29.24	12.24	03–06	01–06
Sv-03	186.13	27.90	25.25	17.18	05–07	00–04
Sv-04	186.20	27.72	32.56	11.42	03–06	00–06
Sv-05	107.98	27.10	35.81	10.31	05–07	02–04
Sv-06	164.79	33.11	34.30	10.22	05–07	00–06
Sv-07	172.21	27.38	33.53	10.06	03–07	01–03
Sv-08	155.17	33.05	32.05	14.65	03–06	01–03
Sv-09	170.16	28.51	29.07	13.09	03–07	00–06
Sv-10	130.03	24.51	33.09	13.43	03–07	01–02
Sv-11	137.00	24.06	36.35	14.11	03–07	00–06
Sv-12	157.29	30.91	27.39	12.62	05–07	02–03
Sv-13	148.21	28.57	33.36	12.73	03–06	01–02
Sv-14	188.30	29.08	36.93	15.22	05–07	00–04
Sv-15	187.80	25.55	36.34	13.47	03–07	00–04
Sv-16	103.72	28.05	36.30	16.28	05–07	02–04
Sv-17	163.06	28.32	35.07	13.63	05–07	00–04
Sv-18	190.00	25.23	25.00	16.67	03–07	00–03
Sv-19	173.05	26.05	41.15	15.93	05–07	00–04
Sv-20	159.76	23.34	23.90	11.30	03–04	00–02
Sv-21	204.24	23.00	36.64	14.55	05–06	00–04
Sv-22	180.67	29.99	33.36	15.41	03–07	00–04
Sv-23	180.25	26.92	25.34	11.33	03–05	00–02
Sv-24	175.14	29.42	31.08	12.55	03–06	01–03
Sv-25	140.12	27.04	35.70	14.07	05–07	00–06
Sv-26	185.14	32.09	41.96	17.38	03–06	01–06
Sv-27	190.12	28.19	12.08	12.37	03–06	00–06
Sv-28	170.07	24.23	30.21	11.02	05–06	01–04
Sv-29	140.03	26.30	34.03	15.16	03–06	00–05
Sv-30	181.76	30.06	32.10	15.37	05–06	00–03
Sv-31	176.68	26.19	33.15	13.12	04–06	01–04
Sv-32	145.70	25.35	31.90	11.51	03–07	00–03
Sv-33	176.23	30.08	28.91	11.19	05–06	00–05
Sv-34	179.99	24.25	28.39	17.35	03–05	00–04
Sv-35	189.67	26.59	31.14	11.41	03–07	01–05
Sv-36	152.96	25.42	37.43	13.48	04–07	01–05
$CD_{(p \leq 0.05)}$		00.65	00.87	00.39		

[a]Average of 50 observations

approximately 5.1 per cent and similarity of 94.9 per cent between them. Cluster II was subdivided into two clusters IIa and cluster IIb compromising of isolates (Sv-4 and Sv-7), (Sv-19, Sv-21 and Sv-29) respectively, showing an average diversity of approximately 3.9% and similarity of 96.1% between them. Cluster III was subdivided into cluster IIIa compromising of two isolates (Sv-36 and Sv-34) and one isolate Sv-32 formed independent lineage showing average diversity of approximately 1.9% and similarity of 98.1% between them. Cluster IV was subdivided into cluster IVa compromising of six isolates (Sv-23, Sv-01, Sv-17, Sv-15, Sv-26 and Sv-02) and one isolate Sv-24 formed independent lineage showing an average diversity of approximately 5.9% and similarity of 94.1% between them. Cluster V was

Table 6 Variability in the ascospore colour morphology and shape of perfect state (*Pleospora allii)* of *S. vesicarium* isolates

Colour of ascospore	Isolate name	No	Shape of ascospore	Isolate name	No
Light brown	Sv-04, Sv-07, Sv-08, Sv-09, Sv-10, Sv-13, Sv-16, Sv-19, Sv-20, Sv-21 and Sv-29	11	Oblong with rounded base and conical apex	Sv-01, Sv-03, Sv-05, Sv-06, Sv-08, Sv-09, Sv-12, Sv-14, Sv-18, Sv-20, Sv-22, Sv-25, Sv-30, Sv-31 and Sv-07	15
Light brown to brown	Sv-01, Sv-02, Sv-11, Sv-14, Sv-15, Sv-17, Sv-22, Sv-23, Sv-26 and Sv-36)	10	Oblong with rounded base and conical apex to oblong with both ends rounded	Sv-13, Sv-16 and Sv-26	03
Brown	Sv-03, Sv-06, Sv-18, Sv-25, Sv- 27, Sv-30, Sv-31, Sv-32, Sv-33, and Sv-35)	10	Ellipsoidal	Sv-04, Sv-10, Sv-11, Sv-15, Sv-17, Sv-19, Sv-21, Sv-29, Sv-33 and Sv-35	10
Dark brown	Sv-05, Sv-24, Sv-28 and Sv-34),	04	Ellipsoidal to oblong with rounded base and conical apex	Sv-02, Sv-23, Sv-24, Sv-27, Sv-28, Sv-32, Sv-34 and Sv-36	08
Dark brown colour	Sv-12	01			

subdivided into two clusters Va and cluster Vb compromising of isolates (Sv-18, Sv-06, Sv-25, Sv-05, Sv-30, Sv-31 and Sv-27) and (Sv-03, Sv-14 and Sv-20), respectively, showing an average diversity of approximately 2.1 per cent and similarity of 97.9% between them. The variation in isolates can be attributed to differences in collection sites. In addition it is well known that phenotype of an individual is influenced by the environment also in addition to individual's genotype. Therefore part of variation/diversity shown by fungal isolates can be due to role of environment. The perfect state of Stemphylium species belongs to Pleospora (Rabenh) Ces & de Not. is formed under prolonged cold conditions (4 °C) prevalent in Kashmir valley. Formation/occurrence of perfect state on this fungus in onion has been already reported (Bhat 2007). Therefore, substantial variation between different fungal isolates can be attributed to the phenomenon of sexual hybridization. There was no agreement of clustering of different fungal isolated from the same geographical region/district since isolates from different districts were clustered together. Brierley (1920) suggested that variations in fungi imperfecti may be due to mutation or by spilitting of an orginally impure genetic constitution or of gamatic or somatic segregation from heterozygotes.

Pathogenic variability

The present study revealed the prevalence of high pathogenic variability among the isolates. All the isolates were successful in producing disease lesions with variation in their incubation period, number, size and colour of lesions. Incubation period varied from 6 to 9 days in different isolates for the development of symptoms. Whitish colour was observed in 17 isolates, while as yellowish colour was observed in fourteen isolates. Brown coloured lesions were observed in only five isolates (Table 7). Based on lesion size isolates were grouped into four groups as group I (< 1.50 mm), group II (1.50–2.00 mm), group III (2.01–2.50 mm) and group IV (> 2.50 mm). Group I accommodated 25% of isolates (Sv-02, Sv- 05, Sv-18, Sv-20, Sv-21, Sv-26, Sv-30, Sv-32, Sv-34) and were considered mildly virulent. Group II accommodated 27.78% isolates (Sv-06, Sv-07, Sv-08, Sv-09, Sv-10, Sv-11, Sv-12, Sv-17, Sv-19, Sv-36) and were considered moderately virulent. Group III comprised of 25% isolates (Sv-03, Sv-13, Sv-14, Sv-15, Sv-22, Sv-23, Sv-27, Sv-28, Sv-31) and were considered virulent. Group IV accommodated 22.22% isolates (Sv-01, Sv-04, Sv-16, Sv-24, Sv- 25, Sv-29, Sv-33, Sv-35) and were considered highly

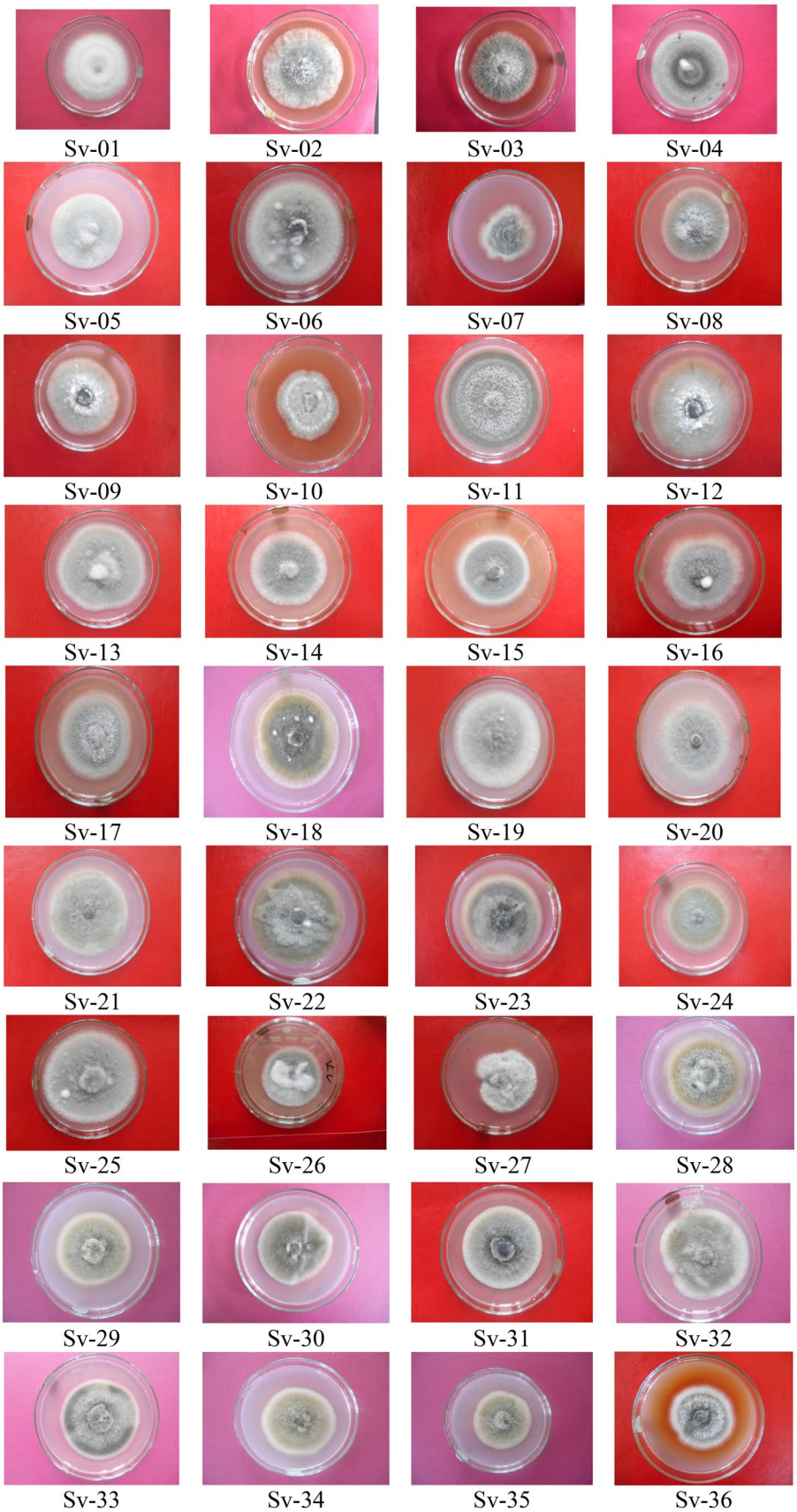

Fig. 1 Cultural variability of 36 *Stemphylium vesicarium* isolates causing Stemphylium blight of onion in Kashmir

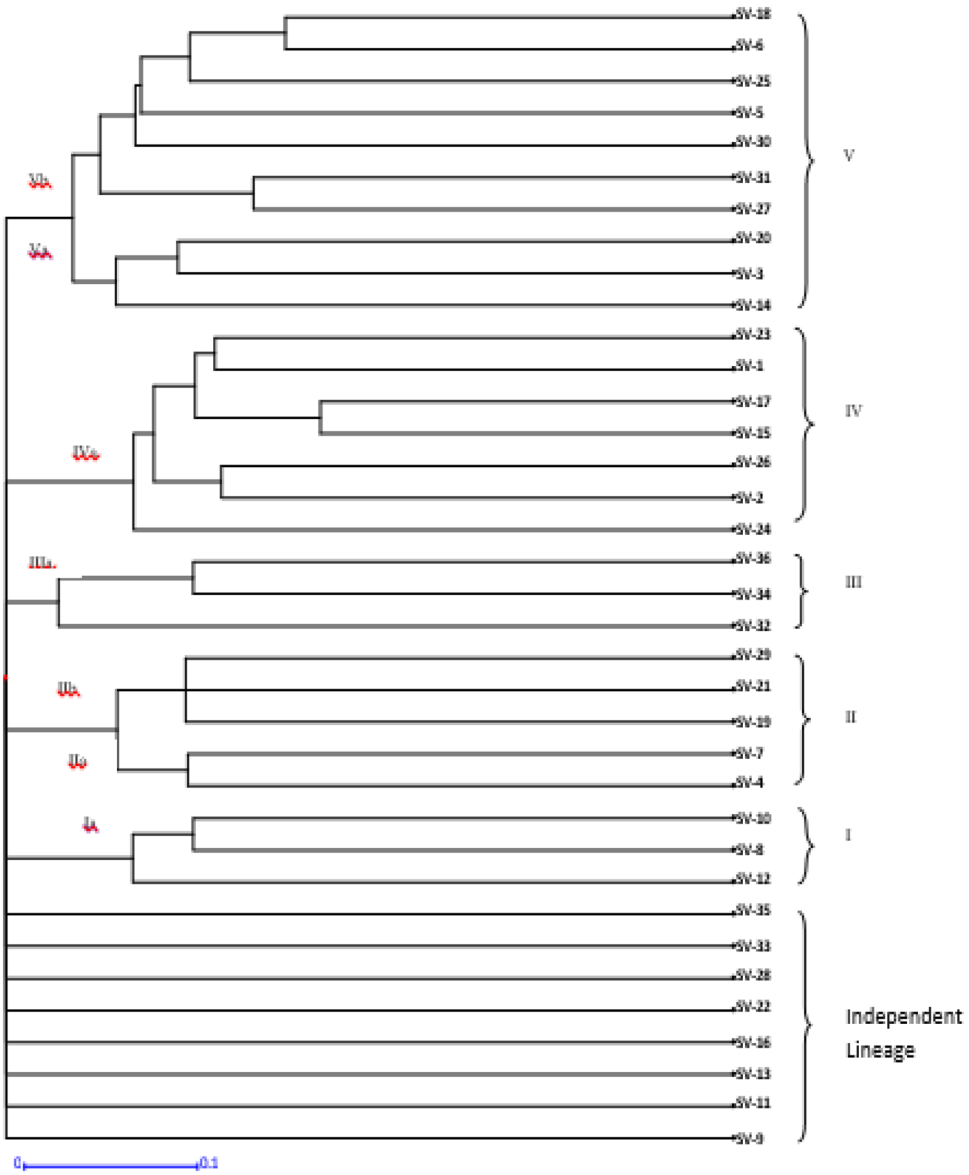

Fig. 2 Dendrogram of 36 isolates of *Stemphylium vesicarium* generated by unweighted pair group method arthimetic mean (UPGMA) analysis of cultural, morphological characteristics

virulent. Similar findings regarding virulence variability in *S. vesicarium* isolates have been reported by Llorente et al. (2012) who found that isolates of *S. vesicarium* could be divided into four virulence groups depending on the lesion lengths caused by artificial inoculations. Basallote-Ureba et al. (1999) also observed variation in incubation period, lesion colour and lesion size of 79 isolates *S. vesicarium* under artificial inoculation.

Table 7 Pathogenic variability in *Stemphylium vesicarium* isolates

Isolate	Pathogenic variability				
	Incubation period (days)	No. of lesions[a]	Size of lesion (mm)[a]		
			Range	Mean	Colour
Sv-01	06	17.59	2.5–4.0	02.59	Yellow
Sv-02	06	13.48	01–02	01.33	White
Sv-03	06	18.55	01–4.5	02.40	White
Sv-04	06	19.59	02–3.5	02.80	Yellow
Sv-05	07	17.62	01–02	01.10	White
Sv-06	08	18.72	01–02	01.60	White
Sv-07	08	23.55	01–03	01.80	White
Sv-08	09	11.48	01–02	01.56	Yellow
Sv-09	09	33.49	01–03	01.60	Yellow
Sv-10	08	15.35	01–03	01.80	Whitish
Sv-11	09	19.33	01–03	01.80	Brown
Sv-12	09	19.33	01–03	01.62	Yellow
Sv-13	09	12.35	02–03	02.01	Brown
Sv-14	09	26.50	02–03	02.30	White
Sv-15	09	15.49	01–03	02.20	White
Sv-16	08	11.00	02–03	02.99	Yellow
Sv-17	09	18.33	01–03	02.00	Yellow
Sv-18	09	17.33	01–02	01.15	Yellow
Sv-19	09	07.50	01–02	01.98	Brown
Sv-20	09	08.50	01–02	01.20	Yellow
Sv-21	09	14.16	01–02	01.10	Yellow
Sv-22	07	16.16	02–03	02.30	White
Sv-23	09	18.16	01–03	02.30	Yellow
Sv-24	08	17.33	02–03	02.60	Yellow
Sv-25	09	16.33	01–03	02.91	White
Sv-26	09	11.00	01–02	01.10	White
Sv-27	09	19.00	02–03	02.20	White
Sv-28	09	13.16	01–03	02.12	Yellow
Sv-29	09	15.20	02–03	02.60	White
Sv-30	09	13.16	01–02	01.49	Brown
Sv-31	09	14.00	01–03	02.25	White
Sv-32	09	21.00	01–02	01.12	Yellow
Sv-33	09	20.16	01–03	02.60	White
Sv-34	08	13.08	01–02	01.25	Brown
Sv-35	07	17.00	01–03	02.90	White
Sv-36	06	22.00	01–04	02.00	White
$CD_{(p \leq 0.05)}$		01.59			

[a]After 15 days of inoculation

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