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Morphological and cultural variation in different oilseed *Brassica* isolates of *Alternaria brassicae* from different geographical regions of India

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ABSTRACT

Variation in morphology and cultural characteristics among 13 representative Indian geographical isolates from 219 collections of *Alternaria brassicae*, the causal agent of Alternaria blight of rapeseed-mustard, was studied. All the isolates showed high level of variability *in vitro* in respect of conidia length, width, beak length and number of septa. Conidia of Nazirhat isolate (SS 04) were smallest in size with lowest number of septa. Substantial variation was found in mycelial growth, sporulation among these isolates in different nutrient media and artificial environmental condition, viz temperature, relative humidity, light, hydrogen ion concentration. Different temperature ranges (25–30°C; 15–35°C) were found optimum for different isolates for mycelial growth and sporulation, respectively. All the thirteen isolates grew best at 100% relative humidity. However, they sporulated the most at different % relative humidity (40–100%). This reflected the adaptation of the respective isolates to the ambient conditions in the different cropping areas, where the disease occurs in varied proportions in different years, which may have also induced the available cultural variability. All the isolates did not grow and sporulate abundantly on the same nutrient medium. However, on an average Asthana and Hawker's medium was good for all the cultures. Variation in optimum *p*H and light condition for mycelial growth, sporulation was also observed. Cluster analysis for data on cultural variability among thirteen *A. brassicae* isolates found a close relationship among isolates from Uttar Pradesh, Uttaranchal and Haryana but was distantly related to others.

Key words: *Alternaria brassicae*, *Brassica*, Cultural, Morphological, Variability

In India, rapeseed-mustard is an important group of edible oilseed crops, second only to groundnut and contributes around 26.1% of the total oilseed production. Out of 61.63 million tonnes of rapeseed-mustard seed produced over 31.02 million ha in the world, India produced 7.20 million tonnes from 6.19 million ha (FAO 2011). A wide gap exists between the potential yield and the yield realized at the farmers' field, which is largely because of number of biotic and abiotic stresses to which the rapeseed-mustard crop is exposed. Among the biotic stresses, Alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc. is one of the most common and destructive diseases of Indian mustard causing up to 47% yield loss (Chattopadhyay 2008). The disease has also been reported from all the continents of the world with no proven transferable source of resistance against the same reported till date in any of the hosts. Severity of Alternaria blight on oilseed Brassicas differ among seasons and regions as also between individual crops within a region. This may be due to existence of variability among isolates of *Alternaria* species. Many reports on the existence of morphological variability within the isolates of other *Alternaria* species have been reported by earlier workers (Varma *et al.* 2006). Variability in the morphological characteristics in *A. brassicae* isolates of different regions of India have been reported (Meena *et al.* 2005, Kaur *et al.* 2007, Singh *et al.* 2007).

Some researchers have worked on cultural variability in *Alternaria* species in respect of mycelial growth and sporulation on different temperature, relative humidity, hydrogen ion concentration (Ansari *et al.* 1989), media (Patni *et al*. 2005) and light (Ansari *et al*. 1989). Variability on the basis of morphology, sporulation, growth and other cultural characteristics also have been reported earlier (Kaur *et al.* 2007). Different temperatures were found optimum for growth and sporulation of *A. brassicae* in a range of 20–25°C (Singh *et al.* 2007) and 20–30°C (Meena *et al.* 2005), respectively. Mycelial growth, sporulation of *A. brassicae* is also affected

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by relative humidity and showed variation in its requirement (Meena *et al.* 2005).

However, detailed, holistic study on morphology and cultural variability among oilseeds *Brassica* isolates of *A. brassicae* from different geographical regions of India is not available, which is reported in this paper for the first time with isolates from across the rapeseed-mustard growing region of the country.

MATERIALS AND METHODS

Thirteen representative Indian geographical isolates from 219 collections of cultures of *Alternaria brassicae* of different geographical locations of India (Meena *et al.* 2005), viz Uttar Pradesh, Uttaranchal, Rajasthan, Haryana, Jammu and Kashmir, West Bengal and Asom during 28 Feb 2005 – 8 Mar 2006 (Table 1) were isolated from infected leaves or pods of *Brassica juncea* and *B. rapa* var. *toria* exhibiting typical symptoms of Alternaria leaf spot by *A. brassicae*. These selected spots were washed 3-4 times in sterilized distilled water and then surface sterilized by dipping in 4% NaOCl solution for 1 min, followed by washing with sterilized water 3-4 times. Surface sterilized leaf spot pieces were then aseptically transferred into 9 cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 23 ± 2°C for seven days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces on the medium were aseptically transferred into another Petri plate containing PDA medium, where it was grown for 15 days at 23 ± 2°C in the BOD incubator. On the basis of their conidiophore and conidial morphology as described by Simmons (2007), the pathogen was identified as *Alternaria brassicae* (Berk.) Sacc. and purified by single spore isolation method. The isolated fungal pathogen cultures were maintained on PDA slants at 4°C. The isolates have been submitted to the National Bureau for Agriculturally Important Microorganisms (ICAR), Mau Nath Bhanjan (Uttar Pradesh, India; NAIMCC F 02189-02201).

Table 1 Thirteen single-spored *Alternaria brassicae* isolates with passport data

Isolate no.	Place of collection	Host	Plant part
SS 01	Navgaon (Rajasthan); 27°1'N, 74°13'E	*Brassica juncea* (cv. RN 505)	Leaf
SS 02	Udheywalla (Jammu & Kashmir); 34°8'N 76°49'E	*B. juncea*	Leaf
SS 03	Mohanpur (West Bengal); 23°39'N, 88°24'E	*B. juncea* (cv. Varuna)	Leaf
SS 04	Nazirhat (Asom); 26°12'N, 92°56'E	*B. rapa* sub sp. *oleifera* (toria)	Pod
SS 05	Hatikhuti (Asom); 26°12'N, 92°56'E	*B. juncea*	Leaf
SS 06	Dausa (Rajasthan); 26°53'N, 76°20'E	*B. juncea*	Leaf
SS 07	Jaipur (Rajasthan); 26°54'N, 75°48'E	*B. juncea*	Leaf
SS 08	Mau (Uttar Pradesh); 25°15'N, 81°22'E	*B. juncea*	Leaf
SS 09	Pantnagar (Uttaranchal); 29°3'N, 79°31'E	*B. juncea* (acc. EC 399296)	Leaf
SS 10	Sachha Khera (Haryana); 29°3'N, 76°5'E	*B. juncea*	Leaf
SS 11	Samalakha (Haryana); 29°14'N, 77°0'E	*B. juncea*	Leaf
SS 12	Jagadhari (Haryana); 30°20'N, 76°52'E	*B. juncea*	Leaf
SS 13	Wazirpur (Haryana); 28°41'N, 77°10'E	*B. juncea*	Leaf

Ocular micrometer was calibrated and by use of micrometry (Meena *et al*. 2005), morphological variability among the 13 isolates of *A. brassicae* was studied in 2006. Cultural variability among the 13 single-spore cultures of *A. brassicae* was studied in *in vitro* condition at different temperature, relative humidity (RH), light duration, *p*H and in different nutrient media during 2006–09.

Cultural variability among 13 single-spore cultures of *A. brassicae* was studied at seven different temperatures (5, 10, 15, 20, 25, 30 and 35°C), five different RH conditions (20, 40, 60, 80 and 100%), six different *p*H (5, 6, 7, 8, 9 and 10). 5 mm mycelial disc of growing *A. brassicae* cultures was inoculated on PDA medium of *p*H 7.0. After inoculation, Petri plates were incubated at seven different temperatures, 100% relative humidity and 12 hr darkness - 12 hr light/day (1000 l×) for 28 days. Each treatment was replicated thrice. To study effect of RH on mycelial growth and sporulation, different humidity solutions were maintained by the method given by Goyal (2009). After inoculation, Petri plates were incubated at desired RH or *p*H and optimum temperature (25 or 30°C) according to cultures, and at 12 hr darkness - 12 hr light/day (1000 l×) for 28 days. Each treatment was replicated four times. To study the effect of culture media on mycelia growth and sporulation, six different culture media, viz Asthana and Hawker's, Brown's, Czapek Dox Agar, Elliot's, Glucose-Asparagine and Richard's media were prepared and *p*H was adjusted to 5.0, 6.0, 7.0, 8.0, 10.0 according to culture. Each treatment was replicated four times. To study effect of light on mycelia growth and sporulation, nine different light durations of 1000 l×/day (6 hr light- 18 hr darkness, 8 hr light-16 hr darkness, 10 hr light-14 hr darkness, 12 hr light- 12 hr darkness, 14 hr light-10 hr darkness, 16 hr light-8 hr darkness, 18 hr light-6 hr darkness, 24 hr light-0 hr darkness and 0 hr light-24 hr darkness) were tested in Asthana and Hawker's medium adjusted to *p*H 5.0, 6.0, 7.0, 8.0, 10.0 according to culture. Each treatment was replicated thrice. Radial growth of colony at different temperatures, RH, *p*H, different culture media, light durations was recorded at one-

day intervals till nine days after inoculation. Observations for sporulation were recorded 28 days after inoculation.

For clustering 13 isolates of *A. brassicae* based on observations for cultural variability, the data were grouped for mycelial growth of isolates (on 9th day) and their sporulation (on 28th day). Thus, separate dendrograms were produced based on data for mycelial growth of isolates and their sporulation. To produce these dendrograms, hierarchical method of clustering (Chatfield and Collins 1980, Johnson and Wichern 1988, Sharma 1996) was done using statistical software SAS Version 9.1.

RESULTS AND DISCUSSION

The 13 single-spore cultures of *A. brassicae* showed significant ($P < 0.05$) morphological variability in respect of conidia length, conidia width, beak length and number of septa (Table 2). Average conidial length, which varied from 31.2 to 51.8µm (range: 24.0–62.6 µm), was highest in Samalakha isolate (SS 11), i e 51.8 µm and lowest in Nazirhat isolate (SS 04), i e 31.2 µm. Average conidial width, which varied from 6.7 to 9.6 µm (range: 4.8–12.0 µm), was highest in Mohanpur (SS 03) and Sachha Khera (SS 10) isolates, i e 9.6 µm and lowest in Nazirhat (SS 4), Hatikhuti (SS 05) and Jaipur (SS 07) isolates, i e 6.7 µm. Average beak length, which varied from 8.2 to 20.6 µm (range: 4.8–33.6 µm), was highest in Pantnagar isolate (SS 09), i e 20.6 µm and lowest in Nazirhat isolate (SS 04), i e 8.2 µm. The average number of transverse septa, which varied from 4.0 to 7.2 (range: 3–8), was highest in Udheywalla (SS 02) and Samalakha (SS 11) isolates, i e 7.2 and lowest in Nazirhat isolate (SS 04), i e 4.0. The average number of longitudinal septa varied from 0 to 0.4 (range: 0–2). Finally it was revealed that the smallest size of conidia and lowest number of septa was in Nazirhat isolate (SS 04). Microscopic examination of conidia at 40X magnification revealed variability in conidia size and could be categorized into two groups, i e shorter (<41µm) and longer (>41µm) but not according to their geographical origin. The shorter group included isolates from Rajasthan and Asom while longer group included isolates from Jammu & Kashmir, West Bengal, Uttar Pradesh, Uttaranchal and Haryana states. These results are in agreement with earlier workers (Varma *et al*. 2006, Meena *et al.* 2005, Kaur *et al.* 2007, Singh *et al.* 2007), who observed morphological variability in different geographical isolates within an *Alternaria* species.

Mycelial growth and sporulation varied among the different isolates at different temperatures. Radial growth on 9th day of ten isolates [Navgaon (SS 01), Nazirhat (SS 04), Hatikhuti (SS 05), Dausa (SS 06), Jaipur (SS 07), Mau (SS 08), Pantnagar (SS 09), Samalakha (SS 10), Jagadhari (SS 12), Wazirpur (SS 13)] was higher at 25°C (range: 21.7–31.9 mm) and among them, the Jaipur (SS 07) isolate showed highest growth (31.9 mm) while the Pantnagar (SS 09) isolate showed least growth (21.7 mm). Radial mycelial growth of remaining three isolates [Udheywalla (SS 02), Mohanpur (SS 03), Sachha Khera (SS 11)] was higher at 30°C (range: 25.5–29.2 mm) and among these isolates Udheywalla (SS 02) isolate showed highest growth (29.2 mm) and Mohanpur (SS 03) isolate showed least growth (25.5 mm). Sporulation on 28th day of four isolates [Navgaon (SS 01), Hatikhuti (SS 05), Pantnagar (SS 09), Sachha Khera (SS 10)] was higher at 20°C (range: 0.5–32.5 × 10^5/ml) and among them Hatikhuti isolate (SS 05) sporulated the most (32.5 × 10^5/ml) and

Table 2 Morphological variation in conidia size and septation of *Alternaria brassicae* isolates obtained from different geographic locations of India

Isolate no.	Conidial length (µm)		Conidial breadth (µm)		Beak length (µm)		Septation (no.)			
							Transverse		Longitudinal	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
SS 01	39.8	36.0–48.0	7.2	4.8–9.6	11.5	7.2–24.0	4.8	4–5	0.2	0–1
SS 02	49.0	43.2–55.2	8.6	7.2–9.6	19.2	14.4–21.6	7.2	6–8	0.0	0–0
SS 03	45.6	43.2–50.4	9.6	7.2–12.0	13.0	9.6–14.4	6.6	5–8	0.4	0–1
SS 04	31.2	24.0–36.0	6.7	4.8–7.2	8.2	4.8–12.0	4.0	3–5	0.4	0–1
SS 05	40.8	36.0–48.0	6.7	4.8–7.2	15.4	9.6–24.0	5.8	4–8	0.0	0–0
SS 06	39.8	36.0–43.2	8.2	7.2–12.0	13.4	9.6–19.2	4.8	4–6	0.2	0–1
SS 07	36.0	32.2–40.8	6.7	4.8–7.2	13.0	9.6–21.6	5.0	4–6	0.0	0–0
SS 08	41.3	36.0–48.0	7.2	7.2–7.2	10.6	7.2–12.0	5.4	4–8	0.0	0–0
SS 09	50.9	38.4–62.4	8.2	7.2–12.0	20.6	14.4–31.2	6.6	6–7	0.4	0–1
SS 10	49.9	38.4–62.4	9.6	7.2–12.0	19.2	9.6–33.6	6.6	4–8	0.4	0–2
SS 11	51.8	50.4–55.2	8.6	7.2–12.0	18.2	14.4–24.0	7.2	7–8	0.4	0–2
SS 12	44.2	38.4–48.0	7.2	4.8–9.6	17.3	7.2–28.8	5.8	5–7	0.0	0–0
SS 13	43.7	36.0–48.0	7.7	4.8–11.6	16.8	7.2–24.0	6.6	5–7	0.2	0–0
LSD ($P < 0.05$)	2.7		1.2		5.2		1.5		0.2	

SS 01, Navgaon; SS 02, Udheywalla; SS 03, Mohanpur; SS 04, Nazirhat; SS 05, Hatikhuti; SS 06, Dausa; SS 07, Jaipur; SS 08, Mau; SS 09, Pantnagar; SS 10, Sachha Khera; SS 11, Samalakha; SS 12, Jagadhari; SS 13, Wazirpur

Pantnagar (SS 09) isolate sporulated the least (0.5×10^5/ml). Four other isolates [Nazirhat (SS 04), Mau (SS 08), Samalakha (SS 11), Wazirpur (SS 13)] had higher sporulation at 25°C (range: 0.6–10.6 $\times$ 10^5/ml) and among them, Wazirpur (SS 13) isolate sporulated the most (10.6×10^5/ml) and Nazirhat (SS 04) isolate sporulated the least (0.6×10^5/ml). Three other isolates [Udheywalla (SS 02), Jaipur (SS 07), Jagadhari (SS 12)] had higher sporulation at 15°C (range: 1.6–34.9 $\times$ 10^5/ml) and among them Udheywalla (SS 02) isolate sporulated the most (34.9×10^5/ml) and Jaipur (SS 07) isolate sporulated the least (1.6×10^5/ml). Mohanpur (SS 03) and Dausa (SS 06) isolates had higher sporulation at 35°C, i e 28.7×10^5 and 12.2×10^5, respectively per ml. Variability in respect of mycelial growth and sporulation of several *Alternaria* species at different temperatures has been reported earlier by many workers (Meena *et al.* 2005, Singh *et al*. 2007). In the present study, different temperatures were found optimum for mycelial growth and sporulation of different isolates of *A. brassicae*, which showed cultural variability among them. This temperature ranged from 25 to 30°C and 15 to 35°C for mycelia growth and sporulation, respectively. These findings are supported by Meena *et al.* (2005) and Singh *et al*. (2007), who also found variability among different *A. brassicae* isolates of different geographical origin for temperature requirement. Further, the higher temperature being favourable for West Bengal isolate could be related with climatic condition of the state, where temperature during rapeseed-mustard crop season is generally higher than other geographical regions, viz Asom, Haryana, Rajasthan, Uttar Pradesh and Uttaranchal.

Mycelial growth and sporulation varied among the different isolates at different RH. Radial growth on 9th day of all the thirteen isolates was highest at 100% RH (range: 15.0–25.6 mm). Among them, Jaipur isolate (SS 07) showed highest growth (25.6 mm) and Mau isolate (SS 08) showed least growth (15.0 mm). Hence, no variability was observed among them at that condition. However, isolates from Asom, Jammu and Kashmir, Rajasthan and West Bengal required relatively higher RH (80–100%) than isolates from Haryana (40–60%) for sporulation, while some isolates, viz Mau (Uttar Pradesh), Pantnagar (Uttaranchal) and Samalakha (Haryana) did not sporulate at any of the RH conditions. These results depicted variability among them. It was similar to finding of Meena *et al*. (2005). Sporulation on 28th day of four isolates [Udheywalla (SS 02), Mohanpur (SS 03), Hatikhuti (SS 05), Dausa (SS 06)] was highest at 100% RH (range: 0.5 -17.7 $\times$ 10^5/ml) and among them Hatikhuti (SS 05) sporulated the most (17.7×10^5/ml) and Mohanpur (SS-03) isolate sporulated the least (0.5×10^5/ml). Two other isolates, viz Navgaon (SS 01) and Jagadhari (SS 12) sporulated the most at 60% RH, i e 1.3×10^5 and 0.4×10^5/ml, respectively. Nazirhat (SS 04) and Jaipur (SS 07) isolates sporulated the most at 40% RH (0.3×10^5/ml) and 80% RH (0.8×10^5/ml), respectively. Two isolates from Sachha Khera (SS 10) and Wazirpur (SS 13) sporulated the most at both 40%, 60% RH, i e 0.05×10^5/ml and 03×10^5/ml, respectively. No sporulation was observed in Mau (SS 08), Pantnagar (SS 09) and Samalakha (SS 11) isolates. Keeping in view the fact that isolates of Mohanpur (West Bengal) and Dausa (Rajasthan) sporulated at 35°C and several isolates had increased fecundity under higher RH, it seems that as per recent changes towards warmer and humid winters, being in line with current projections for future climate change (Waugh *et al*. 2003), existence of such isolates could pose more danger to the oilseed Brassicas due to Alternaria blight in times to come. The immense variation available among only thirteen representative isolates of *A. brassicae* also indicates their ability to adapt to varied climatic situations.

Mycelial growth and sporulation varied among the different isolates at different *p*H. Radial growth on 9th day of five isolates [Navgaon (SS 01), Udheywalla (SS 02), Jaipur (SS 07), Sachha Khera (SS 10), Jagadhari (SS 12)] was higher at *p*H 7.0 (range: 23.6–31.4 mm). Among them, Navgaon (SS-01) isolate showed highest growth (31.4 mm) while Samalakha (SS-10) isolate grew the least (23.6 mm). Radial growth of five isolates [Mohanpur (SS 03), Nazirhat (SS-04), Hatikhuti (SS-05), Dausa (SS 06), Wazirpur (SS 13)] was higher at *p*H 8.0 (range: 24.7–29.4 mm). Among them, Nazirhat (SS-04) isolate showed highest growth (29.4 mm) while Mohanpur (SS 03) isolate had the least growth (24.7 mm). Radial growth of Mau (SS 08), Pantnagar (SS 09) and Samalakha (SS 11) isolates was higher at *p*H 5.0 (23.3 mm), 10.0 (25.2 mm) and 6.0 (33.5 mm), respectively. Sporulation on 28th day of six isolates [Nazirhat (SS 04), Hatikhuti (SS 05), Mau (SS 08), Pantnagar (SS 09), Samalakha (SS 11), Jagadhari (SS 12)] was higher at *p*H 7.0 (range: 0.03-63.5 $\times$ 10^5/ml). Among them, Hatikhuti (SS-05) isolate sporulated the most (63.5×10^5/ml) while the Pantnagar (SS-09) isolate sporulated the least (0.03×10^5/ml). Three other isolates, viz Navgaon (SS 01), Mohanpur (SS 03) and Jaipur (SS 07) isolates sporulated most at *p*H 10.0 (range: 0.6 -23.9 $\times$ 10^5/ml). Among them, Navgaon (SS 01) isolate sporulated the most (23.9×10^5/ml) and Jaipur (SS 07) isolate sporulated the least (0.6×10^5/ml). Udheywalla (SS 02) and Dausa (SS 06) isolates sporulated the most at *p*H 6.0 (16.3×10^5/ml) and *p*H 9.0 (8.9×10^5/ml), respectively. No sporulation was observed in Sachha Khera (SS 10) and Wazirpur (SS 13) isolates. Gupta *et al.* (1969) observed that mycelial growth and sporulation of *A. brassicae* isolate occurred on a wide range of *p*H, i e 3.0–9.0. However, Lapis and Ricaforte (1974) observed profuse mycelial growth of *A. brassicae* at *p*H 6.0–10.0 and abundant sporulation at *p*H 5.0–9.0. During the present investigation, it was also found that a wide range of *p*H, i e 6.0–10.0 supported high mycelial growth and sporulation of 13 *A. brassicae* isolates, respectively, while some isolates did not sporulate at any *p*H. However, optimum *p*H level was different for different isolates, which showed presence of variability among them.

Isolates from Jammu and Kashmir, Haryana and Rajasthan states grew well in neutral situation (*p*H 7), isolates from Asom, Uttaranchal and West Bengal in alkaline condition and isolate from Uttar Pradesh in acidic environment. However, requirements for sporulation were quite different. Isolates from Asom, Haryana, Uttar Pradesh and Uttaranchal states sporulated well on neutral *p*H while isolates from Rajasthan and West Bengal did so in alkaline environment with isolate from Jammu and Kashmir on acidic *p*H. Thus, the above scenario indicates that requirement of *p*H for different isolates were somewhat specific for mycelial growth and sporulation, which is not possible to generalize within a particular bracket. However, requirements of *p*H for different isolates of *A. brassicae* under the present study indicate that a chunk of them hailing from the major oilseed *Brassica* growing areas (except ones from Mohanpur and Hatikhuti) are favoured by neutral or acidic *p*H for their mycelial growth. A spore of *A. brassicae* after landing on the plant surface germinates, then extends by growing out and spreading its mycelia, penetrates epidermis to infect the plant and necrotises the tissue (Goyal 2009). So, to develop oilseed *Brassica* resistant to *A. brassicae*, the plant has to have an alkaline *p*H at its surface so as to disfavour growth of mycelia of the invading pathogen.

Mycelial growth and sporulation varied among the different isolates in different synthetic media. According to average of radial growth of different isolates on different media, Asthana and Hawker's medium was optimum for all the cultures. Radial growth on 9th day of nine isolates [Udheywalla (SS 02), Dausa (SS 06), Jaipur (SS 07), Mau (SS 08), Pantnagar (SS 09), Sachha Khera (SS 10), Samalakha (SS 11), Jagadhari (SS 12), Wazirpur (SS 13)] was higher on Asthana and Hawker's medium (range: 27.8–39.8 mm). Among them, Jaipur isolate (SS 07) showed highest growth (39.8 mm) while the Sachha Khera isolate (SS 10) grew the least (27.8 mm). Radial mycelial growth of two isolates, viz Navgaon (SS 01) and Hatikhuti isolate (SS 05) was highest on Elliot's medium, i e 27.8 mm and 34.4 mm, respectively. Radial growth of Mohanpur (SS 03) and Nazirhat (SS 04) isolates were highest on Glucose-Asparagine (31.1 mm) and Brown's (29.8 mm) media, respectively. Sporulation on 28th day of four isolates [Hatikhuti (SS 05), Dausa (SS 06), Jaipur (SS 07), Jagadhari (SS 12)] was higher on Asthana and Hawker's medium (range: 0.7–2.3 × 10^5/ml). Among them, Jaipur (SS 07) isolate sporulated the most (2.3 x 10^5/ml) while the Hatikhuti (SS 05) isolate sporulated the least (0.7 × 10^5/ml). Two other isolates, viz Navgaon (SS 01) and Mau (SS 08) sporulated most on Czapek Dox Agar medium i.e 2.5 × 10^5 and 0.08 × 10^5/ml, respectively. Two other isolates from Mohanpur (SS-03) and Sachha Khera (SS 10) sporulated the most on Elliot's medium, i e 0.2 × 10^5 and 0.03 × 10^5/ml, respectively. Nazirhat (SS 04) isolate sporulated the most on both Asthana and Hawker's and Glucose-Asparagine media, i e 0.2 × 10^5/ml. No sporulation was observed in Udheywalla (SS 02), Pantnagar (SS 09), Samalakha (SS 11) and Wazirpur (SS 13) isolates. *Alternaria brassicae* did not grow and sporulate abundantly on the same nutrient medium (Lapis and Ricaforte 1974). In the present study, the thirteen isolates of *A. brassicae* also did not show their best mycelial growth and sporulation on the same medium, which supported earlier findings (Ansari *et al*. 1988).

Mycelial growth and sporulation varied among the different isolates in light of 1000 l× for different durations/ day. Radial growth of five isolates [Navgaon (SS 01), Udheywalla (SS 02), Nazirhat (SS 04), Jaipur (SS 07), Pantnagar (SS 09)] was higher with 14 hr light - 10 hr darkness duration (range: 27.6–34.0 mm). Among them, Jaipur (SS 07) isolate showed highest growth (34.0 mm) while Nazirhat (SS 04) isolate grew the least (27.6 mm). Radial growth of three isolates from Dausa (SS 06), Jagadhari (SS 12) and Wazirpur (SS-13) was highest with 12 hr light - 12 hr darkness duration (range: 29.9–32.6 mm). Among these isolates, the one from Dausa (SS 06) showed highest growth (29.9 mm) while that from Wazirpur (SS 13) grew the least (32.6 mm). Radial growth of Mohanpur (SS 03) and Samalakha (SS 11) isolates was highest at 18 hr light - 06 hr darkness, i e 33.1 and 31.1 mm, respectively. Radial growth of Hatikhuti (SS 05), Mau (SS 08) and Sachha Khera (SS 10) isolates was highest with 10 hr light - 14 hr darkness (30.2 mm), with 08 hr light - 16 hr darkness (26.3 mm) and with 16 h light - 08 h darkness (29.5 mm), respectively. Sporulation of four isolates [Navgaon (SS 01), Dausa (SS 06), Jaipur (SS 07), Jagadhari (SS 12)] was higher with 14 hr light - 10 hr darkness duration (range: 1.0–2.7 × 10^5/ml). Among them, Dausa (SS 06) isolate sporulated the most (2.7 × 10^5/ml) while Navgaon (SS-01) isolate sporulated the least (1.0 × 10^5/ml). Two other isolates, viz Udheywalla (SS 02) and Mohanpur (SS 03) sporulated the most with 16 h light - 8 h darkness duration ie 1.7 × 10^5 and 1.3 × 10^5/ml, respectively. Hatikhuti (SS 05) isolate sporulated the most with 12 hr light-12 hr darkness duration (1.2 × 10^5/ml). However, Nazirhat (SS 04) isolate sporulated the most with both 12 hr light - 12 hr darkness and 14 hr light - 10 hr darkness duration (0.1 × 10^5/ ml). No sporulation was observed in Mau (SS 08), Pantnagar (SS 09), Sachha Khera (SS 10), Samalakha (SS 11) and Wazirpur (SS 13) isolates. Many workers have studied the influence of light and dark condition on mycelial growth and sporulation of *A. brassicae* (Ansari *et al.* 1989). Isolates from Haryana and West Bengal states required longer light duration/day for excellent mycelial growth while isolate from Uttar Pradesh required only 8 hr of light for the purpose. Sporulation of isolates from Jammu and Kashmir and West Bengal was best at 16 hr light duration/ day; of isolates from Rajasthan at 14 hr and of isolate from Asom at 12 hr light duration. However, isolates from Uttar Pradesh, Uttaranchal and Haryana did not sporulate under any light condition. Observations here indicate that isolates of *A. brassicae* studied now require longer light durations for

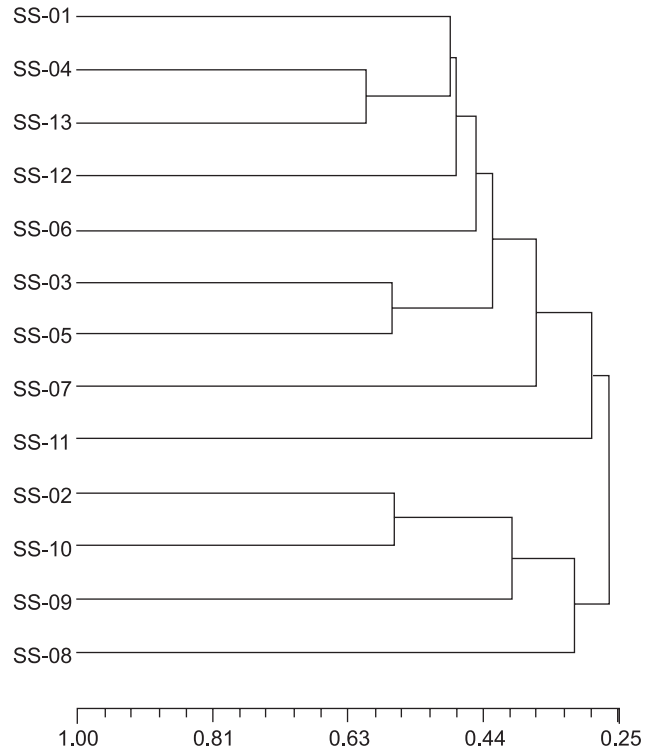

Fig 1 Dendrogram showing cultural variability in mycelial growth of 13 *Alternaria brassicae* isolates at different temperature, per cent relative humidity, hydrogen ion concentration (*p*H), nutrient media and light duration/day

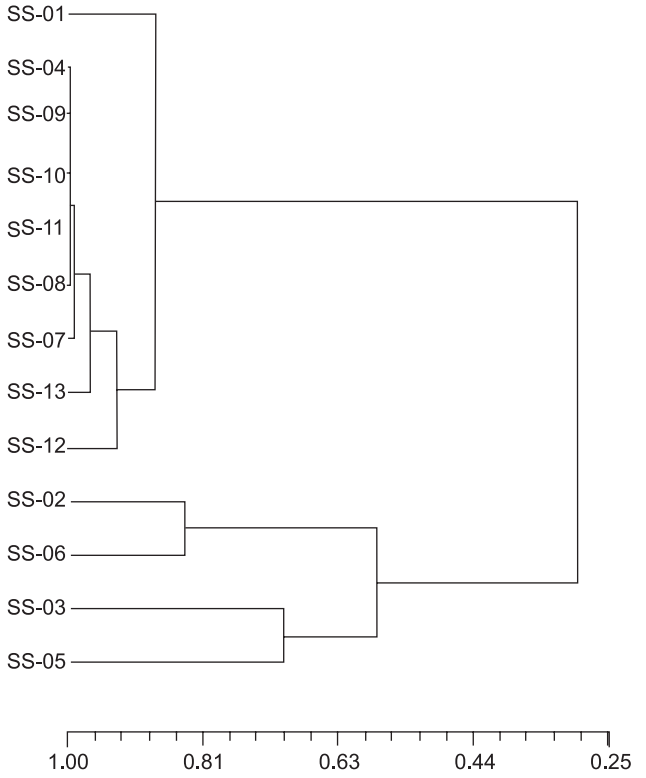

Fig 2 Dendrogram showing cultural variability in sporulation of 13 *Alternaria brassicae* isolates at different temperature, per cent relative humidity, hydrogen ion concentration (*p*H), nutrient media and light duration/day

growth and sporulation although they vary in requirements for the duration. These match with findings of some of the earlier workers (Lapis and Ricaforte 1974) but differ from findings of some others (Mukadam and Deshpande 1979).

A dendrogram (Fig 1) was constructed based on data for mycelial growth of *A. brassicae* isolates on the 9th day at different temperatures, relative humidity, *p*H, nutrient media and light durations from the similarity coefficient by using Unweighted Pair Group Method with Average Means (UPGMA). This dendrogram identified two major clusters with 70% variability. One cluster (group I) comprised nine isolates from Navgaon (SS 01), Mohanpur (SS 03), Nazirhat (SS 04), Hatikhuti (SS 05), Dausa (SS 06), Jaipur (SS 07), Samalakha (SS 11), Jagadhari (SS 12) and Wazirpur (SS 13) while another cluster (group II) comprised of remaining four isolates from Udheywalla (SS 02), Mau (SS 08), Pantnagar (SS 09) and Sachha Khera (SS 10). Group I was further sub-clustered into two, of which first sub-cluster (group IA) had only one isolate from Samalakha (SS 11) and the second cluster (group IB) included eight other isolates from Navgaon (SS 01), Mohanpur (SS 03), Nazirhat (SS 04), Hatikhuti (SS 05), Dausa (SS 06), Jaipur (SS 07), Jagadhari (SS 12) and Wazirpur (SS 13). Group IB was again sub-divided into two clusters, i e group IBa and group IBb. Group IBa comprised of only isolate Jaipur (SS 07) and group IBb had seven isolates [Navgaon (SS 01), Mohanpur (SS 03), Nazirhat (SS 04), Hatikhuti (SS 05), Dausa (SS 06), Jagadhari (SS 12), Wazirpur (SS 13)]. Three isolates from Navgaon (SS 01), Dausa (SS 06) and Jagadhari (SS 12) further formed separate individual clusters while isolates from Nazirhat (SS 04) and Wazirpur (SS 13) formed a cluster with 40% variability. Mohanpur (SS 03) and Hatikhuti (SS 05) isolates formed another cluster with 45% variability. Two isolates of group II, Mau (SS 08), Pantnagar (SS 09) did not share any cluster while Udheywalla (SS 02) and Sachha Khera (SS 10) isolates formed a cluster with 45% variability.

A dendrogram (Fig 2) was also constructed based on data on the 28th day for sporulation of *A. brassicae* isolates in the same way as for mycelia growth. This dendrogram identified two major clusters with 45% variability. One cluster (group I) comprised of nine isolates from Navgaon (SS 01), Nazirhat (SS 04), Jaipur (SS 07),), Mau (SS 08), Pantnagar (SS 09), Sachha Khera (SS 10), Samalakha (SS 11), Jagadhari (SS 12) and Wazirpur (SS 13) while another cluster (group II) comprised the remaining four isolates from Udheywalla (SS 02), Mohanpur (SS 03), Hatikhuti (SS 05) and Dausa (SS 06). Group I was further sub-clustered into two, group IA composed of only one isolate Navgaon (SS 01) and group IB comprised of eight isolates from Nazirhat (SS 04), Jaipur (SS 07), Mau (SS 08), Pantnagar (SS 09), Sachha Khera (SS

10), Samalakha (SS 11), Jagadhari (SS 12) and Wazirpur (SS 13) with a similarity coefficient of 90%. In group IB, six isolates from Nazirhat (SS 04), Jaipur (SS 07), Mau (SS 08), Pantnagar (SS 09), Sachha Khera (SS 10) and Samalakha (SS 11) exhibited close relationship with only 3% variability while isolates from Jagadhari (SS 12) and Wazirpur (SS 13) further formed separate individual clusters. In group II, isolates of Udheywalla (SS 02) and Dausa (SS 06) formed a cluster with a 18% variability while isolates Mohanpur (SS 03) and Hatikhuti (SS 05) formed a cluster with 35% variability.

Cluster analysis also revealed cultural variability among thirteen *A. brassicae* isolates and found a close relationship among Mau (SS 08), Pantnagar (SS 09) and Sachha Khera (SS 10) isolates in respect of mycelial growth and sporulation at different cultural conditions. Cluster analysis of sporulation data of thirteen isolates demonstrated close relationship among three Haryana isolates, viz Samalakha (SS 11), Jagadhari (SS 12) and Wazirpur (SS 13) and this was supported by RAPD analysis (Goyal 2009).

This study provides information about favourable cultural conditions *in vitro* when such situations become available under cropping environment, which could result in severe disease infection or development of Alternaria blight due to isolates existing in different geographical regions of India. This also reflected the adaptation of the respective isolates to the ambient conditions in the different cropping areas, where the disease occurs in varied proportions in different years, which may have also induced the available cultural variability. Taking a holistic look at the performance of different isolates under varied cultural situations as also the more favourable ones for the isolates, it seems that most of the conditions favouring an isolate are met at Uttaranchal and West Bengal (Chattopadhyay *et al*. 2005), which is the possible reason for higher Alternaria blight severity in those states.

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