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Distribution of Apple Scab Race Flora and Identification of Resistant Sources against *Venturia inaequalis* in Kashmir

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ABSTRACT

Apple scab caused by a fungus *Venturia inaequalis* cause enormous losses to growers both qualitatively and quantitatively. In order to manage the crop, farmers routinely spray 8-12 fungicides from pink bud till harvest. Hence, the best strategy to manage the disease is planting apple scab resistant cultivars. In the present study, four races viz., (0), (1), (2) and (1, 2) were reported from various commercial apple growing regions of Kashmir. Apple scab races were spatially distributed in all apple growing regions of Kashmir. Race 1 was most prevalent among the four races. Screening of 31 apple genotypes under controlled conditions revealed majority of commercial cultivars susceptible. Genotypes carrying *RVi3* to *RVi13* scab resistance genes along with the cultivars American Apirouge, H27, Shireen and Firdous (latter two contain *RVi6* scab resistance gene) were found resistant to all the four races present in Kashmir. Resistant genotypes reported in the present study can easily be exploited by breeders for management of apple scab resistance using marker assisted selection approach.

Key words: *Venturia inaequalis*, apple scab, pathogenic variability, race, resistance evaluation

INTRODUCTION

Apple scab caused by *Venturia inaequalis* is a catastrophic disease world over causing enormous loss to growers. It is the most important disease of apple in Kashmir, a North Western Himalayan state of India. Like other apple growing regions worldwide, scab is currently being managed by fungicidal sprays from pink bud to harvest in the State (Padder *et al.*, 2013). Public concerns over pesticide residue in food have generated a great deal of interest in reducing fungicide use on all food crops. Several strategies like disease predictors or forecasters (Fisher and Lillevik, 1977; Ellis *et al.*, 1984; Jones *et al.*, 1984) combined with ergosterol biosynthesis inhibiting fungicides have been employed in scab management with varying degree of success (Sutton, 1996). The latter strategy readily promotes selection of fungicidal resistant strains leading to failure of disease management in various apple growing regions. In spite of the fact, control of apple scab in most of countries heavily depends on foliar application of fungicides. Thus, the best strategy to manage disease is planting resistant cultivars, which is most effective, least expensive and easiest for farmers to adopt. Many scab resistant cultivars have been bred in various countries (Crosby *et al.*, 1992; MacHardy *et al.*, 2001) majority of which contain *Rvi6* (previously known as *Vf*) resistance gene. Development of these varieties had no market impact and hence did not resulted in displacement of major susceptible cultivars (MacHardy, 1996). Therefore, efforts to produce durable scab resistant cultivars with market acceptability should be given priority in breeding programmes. In order to

devise such programmes with success, apple genotypes have to be screened for scab resistance under *in vitro* condition with fungus races present in particular region. Although various commercial apple cultivars have been reported susceptible to scab under natural infection (Didelot *et al.*, 2007; Le Van *et al.*, 2011) however, work on elucidating resistance in the greenhouse conditions against individual races is diminutive. Hence, there is a need to search for scab resistant cultivars against different races for fungus.

In India, particularly Kashmir valley, there is little information on the susceptibility of apple cultivars to scab races, including the cultivars such as Lal Ambri, Gulshan, Shreen, Firdous, Akbar, Shalimar 1 and Shalimar 2 which have been bred in the state. Deployment of these cultivars spatially and temporally depends on understanding of the population biology, virulence spectrum, genomics and evolutionary process of *V. inaequalis* in a region. Elucidation of virulence structure showed four distinct races viz., (0), (1), (2) and (1, 2) in Kashmir. Population structure based on RAPD, ISSR and CAPS revealed low genetic differentiation in the different scab pathogen population's thereby indicating occurrence of frequent gene flow in the region (Padder *et al.*, 2011, 2013). This information is a vital component for deployment of resistant cultivars spatially, in organic farming and of any integrated pest and disease management program. Study under investigation was designed with the following objectives to (1) Assess the pathogenic variability in *V. inaequalis* with an aim to identify any shift in virulence pattern from the already reported races in the region (Padder *et al.*, 2013), (2) Distribute the apple scab race flora (from the present investigation and previously reported) spatially in commercial apple growing regions of Kashmir valley and (3) Evaluate apple genotypes against each individual race under controlled conditions with an intention to identify scab resistant cultivars and their distribution/deployment in time and space.

MATERIALS AND METHODS

Collection of new scab samples and distribution of race flora: Seventy one isolates characterized by Padder *et al.* (2013) revealed the existence of 4 races viz., (0), (1), (2) and (1, 2) within the 71 of isolates. These isolates were collected during the year 2009 and 2010. In order to know whether there has been any pathogen virulence shift, commercial apple growing areas (45 locations) were surveyed during 2014 in 10 districts viz., Anantnag, Bandipora, Badgam, Pulwama, Shopian, Kulgam, Kupwara, Ganderbal, Srinagar and Baramulla of Kashmir valley with majority of *V. inaequalis* isolates from Red Delicious cultivar. All the isolates were collected from the same locations surveyed previously. Mono-conidial isolations were done using single spore technique and each isolate was inoculated on an international differential set comprising 14 apple accessions (Patocchi *et al*., 2009). The inocula, produced as per Barbara *et al.* (2008), were each adjusted to 5×10^5 conidia per milliliter and the conidial suspensions applied till runoff with a manual atomizer. Three one-year-old potted trees were inoculated for each isolate and incubated in a Hi-Tech glasshouse (Rajdeep Agri Products, New Delhi, India) set at 20°C with 85% relative humidity. To ensure constant leaf wetness, the potted trees were placed in polythene covered structure and incubated for 48 h in dark at a constant temperature of 20°C; leaf wetness was maintained using a humidifier. Symptoms were assessed 14 days after inoculation. The potted trees were classified as per 0-4 scale of Chevalier *et al.* (1991), wherein trees in classes 0, 1, 2 and 3a were graded as resistant and trees showing class 3b and 4 were graded as susceptible.

In order to know the prevalence of particular race(s) spatially in different commercial apple growing areas, previous races characterized earlier by authors and the new races characterized in the present study (a total of 116 isolates) were distributed to various districts according to their location.

Resistance evaluation under glass house conditions: For resistance evaluation under controlled conditions, 31 genotypes of apple were inoculated with 4 races (single isolate). Inoculum from each isolate was produced as described by Barbara *et al.* (2008). The inoculum of each race was adjusted to 5×10^5 conidia per milliliter and the conidial suspensions applied till runoff with a manual atomizer. One-year-old potted trees were inoculated (three sets for each race), in a Hi-Tech glasshouse (Rajdeep Agri Products, New Delhi, India), set at 20°C with 85% relative humidity. To ensure constant leaf wetness, the potted trees were placed in polythene covered structure and incubated for 48 h in dark at a constant temperature of 20°C; leaf wetness was maintained using humidifier. Symptoms on potted trees were assessed 14 days after inoculation. The potted trees were classified according to the grading system of (Chevalier *et al.*, 1991) with 0-4 class scale: Trees with class 0, 1, 2 and 3a were graded as resistant and trees showing class 3b and 4 were graded as susceptible.

RESULTS AND DISCUSSION

Among the 45 *V. inaequalis* isolates inoculated on apple scab differentials, 32 (71.11%) were categorized into race (1). These isolates were able to breach the resistance gene *Rvi1* present in Golden Delicious in addition to Royal Gala. Seven isolates were able to overcome two scab resistance genes *Rvi1* and *Rvi2* present in Golden Delicious and TSR34T15, respectively and were designated as a complex race (1, 2). Three isolates each were grouped into race (0) and race (2) (Table 1). Emphases is being given to monitor the virulence alleles throughout the world under the umbrella of ''Monitoring of *Venturia inaequalis* virulences'' by Patocchi *et al.* (2009). Many researchers across the globe are monitoring the new virulence's in apple scab fungus and data is being validated and maintained (www.vinquest.ch).

Table 1: Discerning the 45 isolates of *Venturia inaequalis* into different races based on reaction on International differential set proposed by Patocchi *et al*. (2009)

Isolates	Reaction of apple differentials*														Race designation
	h (0)	h (1)	h (2)	h (3)	h (4)	h (5)	h (6)	h (7)	h (8)	h (9)	h (10)	h (11)	h (12)	h (13)	
V106, V132, V143	+	-	-	-	-	-	-	-	-	-	-	-	-	-	(0)
V101, V103, V105, V107, V109, V110, V111, V112, V113, V114, V115, V116, V118, V120, V119, V121, V122, V123, V124, V125, V126, V128, V130, V133, V135, V136, V137, V138, V139, V142, V144, V145	+	+	-	-	-	-	-	-	-	-	-	-	-	-	(1)
V102, V108, V134	+	-	+	-	-	-	-	-	-	-	-	-	-	-	(2)
V104, V117, V127, V129, V131, V140, V141	+	+	+	-	-	-	-	-	-	-	-	-	-	-	(1, 2)

H0: Royal gala, h (1): Golden Delicious, h (2): TSR34T15, h (3): Geneva, h (4): TSR33T239, h (5): 9-AR2T196, h (6): Priscilla, h (7): *M. x floribunda* 821, h (8): B45, h (9): K2, h (10): A723-6, h (11): *M. baccata jackii*, h (12): Hansen's baccata #2, h (13): Durello di Forlì , +: Susceptible, -: Resistant

Thus, our main objective was to identify any shift in the virulence spectrum. This shift in pathogenicity was not exhibited by the isolates collected during 2014. These isolates were collected after three years of our previous study (Padder *et al.,* 2013) which may be attributed to the less time lapse in survey or the absence of scab resistance genes (*Rvi3* to *Rvi13*) in the orchards of Kashmir valley. Additionally, *V. inaequalis* is described as a good model for host adaptation in plant pathogens (Giraud *et al.*, 2010) because mating only occurs between strains that are able to infect identical hosts, thus facilitating the maintenance of adaptations to a host when host ranges do not overlap (Guerin and Cam, 2004; Guerin *et al.*, 2007; Gladieux *et al.*, 2011). Moreover, population genetic studies have shown that apple resistance genes might induce specialization in *V. inaequalis* populations, thus favouring host-related adaptations (Guerin and Cam, 2004; Gladieux *et al.*, 2011). Since, majority of area under apple belts in Kashmir is under Red Delicious, Golden Delicious, Star Crimson and Royal Delicious cultivars, thus fungus has adopted and evolved to overcome the resistance specificities present in them. The race (1) was most wide spread (71.11% isolates belonged to this race) and the results are in conformity with our previous study and with many researchers especially from the Europe and the USA (Heaton *et al.*, 1991; Parisi *et al.*, 2004; Bus *et al.*, 2011).

The distribution pattern of races in different apple growing areas of Kashmir valley depicted in disease map (Fig. 1) revealed the presence of one or the other race of scab pathogen, *V. inaequalis*. Race (1) was most widespread and prevalent in most of the apple growing of area districts. Various locations in district Srinagar and Budgam contained all the four races whereas three out of four races were present in locations of Kupwara district. In addition to race (1), race (0) was also observed in areas of Shopian and Bandipora whereas, locations in district Baramulla, Kulgam and Anantnag only race (1) isolates were present. In India, Gupta (1990) speculated the presence of only 2 races of apple scab pathogen in various apple growing regions of Kashmir and Himachal Pradesh however, Padder *et al.* (2013) first time reported presence of 4 different races of apple scab from Kashmir valley and were reconfirmed in present study. So, presence of these races in Himachal Pradesh cannot be unnoticed and the occurrence of many more (both simple and complex races) cannot be ruled out from North Western Himalayan region because of its close proximity to the centre of origin of apple. Since, discerning pathogenic variability in *Venturia inaequalis* populations forms the back bone of any breeding strategy, timely monitoring of different virulence's should be given priority in India like the European DARE programme (Lespinasse, 1989; Patocchi *et al.*, 2009).

Screening of 31 apple genotypes under glass house conditions with 4 races of scab pathogen prevalent in the Kashmir valley revealed majority of commercially grown genotypes susceptible to one or the other race of pathogen (Table 2). Genotypes, which contain well characterized scab resistance genes were resistant to all the 4 races. Among the commercially grown apple cultivars American Apirouge, H27, Shireen and Firdous exhibited resistance to all the races, whereas Saharanpuri showed resistance towards 3 races viz., race (0), (1), (1, 2). Two cultivars Star Crimsion and Sunhari were resistant to race (0) whereas, White Dotted Red and Golden Delicious were resistant to two race (0), (1) and race (0, 2), respectively. Commercially grown apple cultivars viz., Ambri, Cox Orange Pippen, Gala, Gulshan, Lal Ambri, Red Delicious, Red Gold and Spartan were susceptible to all the 4 races of pathogen (Table 2). Most of the differentials and few cultivars exhibited pin point hypersensitivity reaction (type 1 symptoms) and chlorosis (type 2) on inoculation with 4 races. Apple scab resistance is

Fig. 1: Distribution of *Venturia inaequalis* race flora in different districts of Kashmir

Table 2: Resistant and susceptible apple genotypes to 4 races of *Venturia inaequalis* in Kashmir

Race and resistant	Susceptible
(0)	
Ambri x Maharaji (H27), American Apirouge, Benoni, Firdous, Gulshan, Saharanpuri, Shireen, Star Crimson, Sunheri, White Dotted Red, Golden Delicious, Geneva, TSR33T239, 9-AR2T196, Priscilla, *M. x floribunda*, B45, K2, A723-6, *M. baccata jackii*, Hansen's baccata #2, Durello di Forlì	Ambri, Cox's orange pippin, Gala, Lal Ambri, Red Delicious, Red Gold, Royal Gala, Spartan, TSR34T15
(1)	
Ambri x Maharaji (H27), American Apirouge, Benoni, Firdous, Gulshan, Saharanpuri, Shireen, Sunheri, White Dotted Red, , TSR34T15, Geneva, TSR33T239, 9-AR2T196, Priscilla, *M. x floribunda*, B45, K2, A723-6, *M. baccata jackii*, Hansen's baccata #2, Durello di Forlì	Ambri, Cox's orange pippin, Gala, Lal Ambri, Red Delicious, Red Gold, Spartan, Star Crimson, Golden Delicious, Royal Gala,
(2)	
Ambri x Maharaji (H27), American Apirouge, Firdous, Gulshan, Shireen, Sunheri, White Dotted Red, Geneva, TSR33T239, 9-AR2T196, Priscilla, *M. x floribunda*, B45, K2, A723-6, *M. baccata jackii*, Hansen's baccata #2, Durello di Forlì	Ambri, Benoni, Cox's orange pippin, Gala, Lal Ambri, Red Delicious, Red Gold, Saharanpuri, Spartan, Star Crimson, Royal Gala, Golden Delicious, TSR34T15
(1, 2)	
Ambri x Maharaji (H27), American Apirouge, Cox's orange pippin, Firdous, Gulshan, Saharanpuri, Shireen, Sunheri, White Dotted Red, Geneva, TSR33T239, 9-AR2T196, Priscilla, *M. x floribunda*, B45, K2, A723-6, *M. baccata jackii*, Hansen's baccata #2, Durello di Forlì	Ambri, Benoni, Gala, Lal Ambri, Red Delicious, Red Gold, Spartan, Star Crimson, Royal Gala, Golden Delicious, TSR34T15

the most preferred one among the various management practices in containing the scab disease particularly under the circumstances of organic agriculture and the fungicide resistant pathogen strains (Schnabel and Jones, 2001; Martinez-Bilbao *et al.*, 2012). Many resistant sources against apple scab has been reported from India and abroad, mainly based on evaluation of apple genotypes under natural conditions (Mercier *et al.*, 2000; Blazek *et al.*, 2003; Lefrancq *et al.*, 2004; Gelvonauskiene *et al.*, 2006; Brun *et al.*, 2008; Biggs *et al.*, 2010; Farooqui *et al.*, 2014). Such findings

may sometime be erroneous owing to disease escape and mixed infection by many pathotypes of pathogen. In order to remove such unambiguity from present investigation, genotypes were evaluated under artificial conditions with all the races present in the valley. A few promising genotypes like American Apirouge, H27, Shireen and Firdous were resistant to all the races. However their consumer unacceptability renders them unfit for spatial and temporal deployment. However, presence of *Rvi6* resistance gene in Shireen and Firdous can be exploited easily to bred scab resistance cultivars using the aid of marker assisted backcrossing. The other useful scab resistance genes present in the differentials (*Rvi3* to *Rvi13*) seems good candidates for durable scab management as the genes were not defeated by the pathogen and will be harder for the pathogen to defeat them in combination. These genes can be combined together provided comprehensive breeding strategy will be initiated and such strategies has already begun in many countries (Bus *et al*., 1999; Gleichauf *et al*., 2009; Flachowsky *et al*., 2011). A few cultivars showed resistance response to one or the other race of pathogen suggesting a good source of scab resistance in the collection. In such cultivars, pathogen germinated and penetrated the host (class 1 to 3a) but, further growth of the fungus was halted resulting in the hypersensitivity, suggesting that resistance in these cultivars is not due to host barriers but rather existence of effective defence mechanism which nevertheless needs to be elucidated by further studies at molecular level. Quantitative scab resistance in apple genotypes is well documented (Gessler *et al*., 2006; Jha *et al*., 2010; Bowen *et al*., 2011; Bus *et al*., 2011) and might be present in the cultivars like White Dotted Red, Saharanpuri and H27 as they have been reported moderately resistant under natural conditions (Farooqui and Dalal, 2003). Apple cultivar Golden Delicious exhibited susceptibility against race (1). Since, this cultivars carries *Rvi1* scab resistance gene which is easily breached by the race (1) and (1, 2).

Development of high quality disease resistant apple cultivars is the preferred long-term means of reducing fungicide use in apples, in the short term growers are restricted by the lack of suitable resistant cultivars. This study has provided information about the susceptibility of a range of apple cultivars to scab, which should be useful in planning integrated pest and disease management programs for Kashmir fruit growers. For example, this information can be used by growers when planning new plantings of apples. If growers consider that for their enterprise, it is important to minimise applications of fungicides, then they have a rationale for making a decision between cultivars. Growers of 'organic' fruit would also find this information helpful in planning new plantings. In addition, this information should be a useful guide to selecting the most susceptible cultivars to target the scouting activities in, thus saving time and money.

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