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Screening of malus genotypes against marssonina leaf blotch

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Abstract
Apple (*Malus* × *domestica* Borkh.) is attacked by several diseases causing economic damage and amongst them Marssonina blotch caused by *Marssonina coronaria* (Ell. & J. J. Davis) J. J. Davis is one of the most widespread diseases of apple and has posed a serious threat to this crop in India and abroad. Marssonina blotch was reported to cause direct losses by inciting severe defoliation in apple plants thereby affecting the fruit size, colour, quality and quantity, besides affecting the tree vigour and the fruit bearing capacity in the following years. The present study was conducted during the year 2014-15 in the Division of plant pathology SKUAST-K. The germplasm were procured from Division of Fruit Sciences SKUAST-K. The evaluation test was performed under artificial conditions in well-equipped polyhouse of Division of Plant Pathology SKUAST-K. To ensure the presence of any latent infection prior to inoculation the potted plants were constantly observed for two weeks. The experiment was carried out in CRD manner with two replications. The potted one year old apple seedlings were sprayed with the freshly prepared spore suspension 5×10^4 conidia ml^{-1} with the help of atomiser. Seventeen cultivars were evaluated to determine their resistance against the disease. It was observed that none of the cultivar exhibited resistance against the disease. However, nine cultivars were categorized as susceptible, seven cultivars were categorized as highly susceptible and only one cultivar Granny Smith showed the moderately susceptible reaction against the disease.

Keywords: *Marssonina coronaria*, apple blotch, germplasm screening, artificial inoculation

Introduction
Apple (*Malus* × *domestica* Borkh.) is the most important fruit crop of the world and has been under cultivation since time immemorial. Apple tree owes its origin in South Eastern Europe and Tien Shan Mountains of Kazakistan in Asia (Gastier, 2000) [2] where vast forests of wild apple trees exist even today. Marssonina blotch is nowadays wide spread foliar disease of Malus *spp.* and is emerging as the most destructive disease wherever apple is grown and causes premature defoliation. Marssonina blotch of apple was first reported from Japan by Miyake (1907) [7]. Later, its occurrence was reported from Canada, Korea, Romania, China and Tiawan (Leite *et al*. 1986) [5]; Jia, (1994) [3]. In India, the disease was first reported from H.P by Sharma and Gautam (1997) [10] and later on, from A.P, Sikkim and J&K (Sharma, 2000) [9].The fungus mainly infects leaves but can also infect twigs and fruits (Sharma *et al*. 2004) [11]; Sagong *et al*. (2011) [8]. Appearance of fruit spots on the produce makes it unsaleable in the market leading to direct economic loss to the growers (Sharma *et al*. 2011) [13]. The associated economic losses to apple production would be even more severe if not for the continued reliance on fungicides (Verma and Sharma, 2003) [16]; Zhao *et al*. (2009) [19]; Thakur and Sharma (2010) [14]; Yin *et al*. (2013) [18]. The most effective and economical way to control a disease is to plant resistant cultivars. However, limited information is available about resistance and susceptibility of apple genotypes to Marssonina blotch (Sharma *et al*. 2011) and in-situ results are confounded by variations in field conditions. Therefore, successful evaluation for resistant apple germplasm requires a controlled growing environment and artificial inoculation. We evaluated 17 cultivars of apple and assessments were conducted in vitro by inoculating the leaves with the conidial suspension. Our objective was to obtain the data that can be used while carrying out resistance breeding of apple against Marssonina blotch.

Materials and Methods
Seventeen cultivars were screened for degree of resistance or susceptibility against disease. The germplasm were procured from Division of Fruit Sciences SKUAST-K. The evaluation test was performed under artificial conditions in well equipped polyhouse of Division of Plant Pathology SKUAST-K. To ensure the presence of any latent infection prior to inoculation the

potted plants were constantly observed for two weeks.

Preparation of inoculum: To prepare the inoculum, the fungus was grown on potato dextrose agar medium for 30 days. After incubation, fungal colonies from one petriplate were scraped and grounded with mortar and pestle in 2 ml of sterile water to make culture suspension after filtering the suspension through muslin cloth followed the procedure of Yin *et al.* (2013). The concentration of conidia in the suspension was determined with the help of haemocytometer and adjusted to 5×10^4 conidia ml^{-1} of water.

Inoculation procedure: The experiment was carried out in CRD manner with two replications. The potted one year old apple seedlings were sprayed with the freshly prepared spore suspension 5×10^4 conidia ml^{-1} with the help of atomiser.

Observations recorded: Disease incidence and severity of Marssonina leaf blotch were recorded after 45 days post inoculation. The disease incidence and disease severity was recorded as per formulae:

Per cent disease incidence was calculated by using the following formulae:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total Number of leaves assessed}} \times 100$$

The disease intensity was recorded using 0-5 scale of Yin *et al*. (2013) as given below:

Table 1

Category	Numerical value	Description rating
I	0	No evidence of disease on leaf
II	1	1-10% leaf area infected
III	2	11-30% leaf area infected
IV	3	31-50% leaf area infected
V	4	> 50% leaf area infected
VI	5	Leaf fall

Per cent disease intensity (PDI) was calculated as per the following formula:

$$\text{Per cent disease intensity} = \frac{\Sigma\,(n \times v)}{N \times G} \times 100$$

Where,

Σ = Summation
n = Number of diseased leaves
v = Numerical value of the category
N = Total number of leaves examined, and
G = Highest grade value

Disease reaction: Disease reaction of the cultivars were categorised on the basis of an established scale (McKinny, 1923) [6].

Table 2

Category	Disease Index (%)	Reaction
I	0-5.0	Resistant (R)
II	5.1-10.0	Moderately Resistant (MR)
III	10.1-25.0	Moderately Susceptible (MS)
IV	25.1-50.0	Susceptible (S)
V	More than 50.0	Highly Susceptible (HS)

Latent period: Latent period was taken as time in number of days from inoculation to appearance of first lesion on each cultivar. The mature leave whorls were examined for the appearance of lesions everyday starting from 24 h after inoculation up to appearance of lesions in every cultivar.

Virulence indexing: The numerical values of disease reaction, disease severity and latent period were used to calculate the virulence index with the following formula:

Virulence Index (VI) = PDI × LP^{-1}

Where,

PDI= Percent Disease Index

LP= Latent period

Results and Discussion

It is evident from Table 3 that none of the screened cultivars were found resistant against the pathogen of Marssonina blotch disease. Out of the seventeen cultivars only one cultivar Granny Smith showed moderate resistance against the pathogen with an incidence and intensity of 65.30 and 22.85 per cent respectively. However, the cultivar Benoni showed lowest incidence of 60.05 per cent followed by Granny Smith (65.30%), Lal-cider (67.0%) and White dotted red (70.0%) respectively. Whereas, the the lowest intensity were recorded in case of Granny Smith (22.85%), White dotted red (29.60%), Benoni (31.10%) and in Lal-cider (37.9%). However, on the basis of disease reaction scale seven cultivars were categorized highly susceptible (HS) including Shalimar-1, King of Pippin, Golden Delicious, Vance Delicious, Star Krimson, Golden Delicious Reindeers and Star King Delicious showed the disease severity more than 50 per cent and only one cultivar Granny Smith was found moderately susceptible (MS) which showed less than 25 per cent disease severity after 45 days of post inoculation. The susceptible reaction of the cultivars including Red Delicious, Red Gold, Vance Delicious and Golden Delicious were in conformity with the studies carried out by Sharma and Gautam (1997), Yan *et al*. (2012) [17], Kumar and Sharma (2014) [4]. However, our evaluation indicated that 'Granny Smith' was moderately susceptible, is in conformity with the findings of Bala *et al*. (2001) [1], Yin *et al*. (2013) and Kumar and Sharma (2014) who found that this cultivar exhibited intermediate/moderate reaction. However, Verma and Sharma (1999) [15], Sharma (2003) [12] and Sharma *et al*. (2011) reported this cultivar to be resistant against the disease. The Difference in inoculation method is a potential cause of discrepancy (Yin *et al*., 2013). The results of Sharma *et al.* (2011) and Yan *et al*. (2012) were recorded under natural epiphytotic conditions, whilst artificial inoculation was used in this study and different results of the resistance levels of cultivar/cultivars between these studies may be due to differences in the growth status of experimental material or in the environment.

Table 3: Screening of apple genotypes against *Marsssonina coronaria* (Ell. & J. J. Davis) J. J. Davis under controlled conditions.

Genotypes	Disease incidence (%)	Disease intensity (%)	Disease reaction*	Latent period (days)	Virulence index	Lesion size range (mm)
Oregon Spur	90.9	45.09	S	14	3.2	0.5-15
Red Delicious	94.4	40.55	S	13	3.1	0.5-15
Shalimar-1	100.0	70.50	HS	12	5.8	0.5-10
Shalimar-2	81.57	42.63	S	15	2.8	0.5-15
King of pippin	100.0	78.12	HS	12	6.5	0.5-12
Golden Delicious	94.59	64.50	HS	11	5.8	0.5-15
Vance Delicious	100.0	72.55	HS	11	6.5	0.5-15
Granny Smith	65.30	22.85	MS	17	1.3	1-6
Star Krimson	100.0	58.07	HS	12	4.8	1-10
Benoni	60.05	31.10	S	14	2.2	0.5-13
Red chief	92.70	49.45	S	12	4.1	0.5-15
Red Gold	83.33	48.14	S	15	3.2	0.5-10
White dotted Red	70.0	29.60	S	17	1.7	1-5
Golden Delicious Reindeers	100.0	71.00	HS	12	5.9	1-5
Sunhari	96.0	47.18	S	14	3.3	0.5-8
Lal-cider	67.0	37.0	S	17	2.1	1-8
Star King Delicious	92.18	79.37	HS	12	6.6	0.5-5

*S-Susceptible; MS-Moderately susceptible; HS-Highly susceptible

Conclusion

The prominent apple cultivars showed varied response to pathogen [*Marssonina coronaria* (Ell. & J. J. Davis) J. J. Davis]. Out of seventeen cultivars evaluated nine cultivars were categorized as susceptible (S), seven cultivars were found highly susceptible (HS) and only one cultivar Granny Smith exhibited moderately susceptible (MS) reaction. Lowest disease incidence (60.05%) was recorded on Benoni and lowest disease intensity (22.85%) was found on Granny Smith. The highest disease incidence (100%) was recorded on Shalimar-1, King of Pippin, Vance Delicious, Star Krimson and Golden Delicious Reindeers. However, highest disease intensity (79.37%) was recorded on Star King Delicious.

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