



RESEARCH ARTICLE

Identification and quantification of resistance against *Ascochyta* blight complex in garden pea (*Pisum sativum* L.)

Sandeep Jain*, Anjali, Rajinder Kumar Dhali¹ and Ritu Rani

Abstract

One hundred elite pea genotypes were evaluated under natural as well as artificial epiphytotic conditions for three consecutive seasons to determine their relative resistance to the three predominant concurrently infecting pathogens associated with blight complex, *Didymella pinodes*, *D. pinodella* and *Ascochyta pisi*. Disease reaction under natural epiphytotic conditions varied with percent disease incidence and severity ranging from 38.45 to 89.45% and 11.59 to 55.14%, respectively, among test genotypes. Among the test genotypes, wide variation in the susceptibility index (Sx), area under disease progress curve (AUDPC), and relative area under disease progress curve (RAUDPC), apparent rate of infection (r), disease incidence, disease severity, percent stem girdling (%HTM) and incubation period (IP50) was recorded under artificial epiphytotic field conditions. Field trials conducted both under natural and artificial epiphytotic conditions, five genotypes, namely, Eddy, PS-24, Arya Veer, CHPMR-2 and PS-19, showed consistent resistance with susceptibility index (Sx) values less than 2 and r between 0.06 to 0.08. CHPMR-2 showed a maximum incubation period of 7.46 days and a minimum percent stem girdling of 7.03%. A positive correlation between AUDPC and stem girdling, as well as disease severity, whereas AUDPC was negatively correlated with IP50, was recorded. It is the first report in Punjab that evaluated disease resistance under simultaneous stress caused by three different pathogens that cause pea blight. The identified resistant sources have the potential to provide impetus to pea disease resistance breeding.

Keywords: *Pisum sativum*, *Didymella*, AUDPC, susceptibility index, apparent rate of infection.

Introduction

Peas (*Pisum sativum* L.), also called garden peas, are among the earliest domesticated crops in the subtropical and temperate regions. India, with an area of 573000 ha and a production of 5823000 MT of green peas, is the second largest producer after China (Anonymous 2021). In Punjab, the pea is the second most widespread vegetable crop after potatoes, with an area of 43.89000 ha and a production of 467.01000 tons (Anonymous 2021). The pea crop is attacked by various economically important fungal diseases such as powdery mildew (*Erysiphe polygoni*), downy mildew (*Peronospora viciae*), rust (*Uromyces fabae*), wilt, root rot and collar rot (*Fusarium oxysporum* and *Rhizoctonia solani*) (Pratap and Kumar 2011). Pea blight also known as *Ascochyta* blight, is a disease complex involving three fungal species belonging to phylum Ascomycota: *Ascochyta pinodes* (Teleomorph: *Didymella pinodes*, formerly known as *Mycosphaerella pinodes*), *Phoma medicaginis* var. *pinodella*, formerly known as *Ascochyta pinodella* (Jones 1927) and *Ascochyta pisi* Lib. (Teleomorph: *Didymella pisi*) and *Ascochyta* (*Phoma*) *koolunga* (Davidson et al. 2009; Liu et al. 2013). Globally, the blight complex poses a serious threat

to the legume and pea-exporting industries (Rubiales and Fondevilla 2012; Khan et al. 2013).

Ascochyta blight, foot rot, leaf and pod spot, and black stem are the diverse symptoms associated with the blight complex. *Ascochyta pinodes* can infect plants at the seedling stage and affect all aerial parts of the plant, resulting in leaf spots, blackening at the base of the stem and foot rot in

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seedlings, stem lesions, seed shrinkage and dark brown discoloration of seeds. *Phoma medicaginis* var *pinodella* and *Ascochyta pinodes* both cause foot rot (Hare and Walker 1944). However, *Phoma pinodella* causes more severe foot rot but less damage to aerial parts. In contrast, *Ascochyta pisi* causes small, circular, tan lesions with dark edges on the leaf, pod and stem (Chilver et al. 2009). Several species have been identified in recent times as being associated with the *Ascochyta* blight complex. However, *Didymella pinodes* has been found to be by far the most damaging and prominent, causing yield losses of up to 70% (Tivoli and Banniza 2007).

The development of resistant pea varieties appears to be the best long-term strategy for controlling *Ascochyta* blight. *Pisum sativum* has received limited research attention on *Ascochyta* complex with respect to resistance despite extensive screening due to the involvement of multiple species of pathogens. As of now, there are no cultivars with effective field resistance to *Ascochyta* blight available in India (Khan et al. 2013). Hence the present study was conducted to determine the level of resistance in elite pea genotypes under the stress caused by three concurrently occurring pathogen species, as well as to identify the resistance donors.

Materials and methods

Plant material and experimental site

The experiment was conducted during the *Rabi* season (from the months of October to March) in 2017-18, 2018-19 and 2019-20 with one hundred pea genotypes used for screening for their relative response to the disease complex under natural and artificial epiphytotic conditions. The studies were conducted at the Experimental farm of the Department of Plant Pathology (30.8987411 Lat and 75.7955394 Long), Punjab Agricultural University, Ludhiana, Punjab, India, with an altitude of 247 m above mean sea level. Located in the Trans-Gangetic plains of India and in the central plains region of Punjab state, this area is classified as agro-climatic zone 6. It has a sub-tropical and semi-arid climate with summer temperatures between 26 and 48°C, a winter temperature between 5 and 22°C, and average annual rainfall between 70 and 125 cm. The soil in the experimental area was loamy sand (79.8% sand, 12.2% silt, 7.8% clay), pH 7.5, 179 kg/h nitrogen, 0.42% organic carbon, 20 kg/h phosphorus, and 144 kg/h potassium.

The genotypes were classified according to their maturity group, i.e., early maturity requires 57 to 65 days till first picking, mid maturity takes 80 to 90 days and late maturity takes more than 90 days until first picking (Table 1.). The plant canopy was divided into three equal parts (upper, middle and lower canopy) in order to measure the disease severity and periodic disease progress was recorded.

Screening under natural epiphytotic conditions

A field trial for screening under natural epiphytotic conditions was carried out with three replications and 20 plants per replication in a randomized block design. The seed of each pea genotype was planted in rows 30 cm apart with a spacing of 7 cm between plants using the standard recommended package of pea growing practices (Anonymous 2021).

Under artificial epiphytotic conditions, the test genotypes were screened in the following winter seasons, i.e., November-March 2018-19 and 2019-20. Mass-multiplication of the pea blight pathogens (*Didymella pinodes* OK605313; *Didymella pinodella* OK605316; *Ascochyta pisi* MH857263.1) was conducted on thrice autoclaved barley seeds in conical flasks of 500 mL (Dhingra and Sinclair 1995) and was used for artificial inoculations in the field. The spray inoculation technique was used to artificially inoculate each entry at 5 weeks of age using the most virulent isolates for each of the three pathogens. The conidial suspension (1×10^6 spores/mL) was prepared by scraping 20-day-old culture plates with a hairbrush and mixing equal amounts of spore suspensions from each pathogen (1:1:1).

Disease assessment

The assessment of disease was performed in terms of disease incidence and severity by adopting a rating scale of 0 to 6 (Schoeny et al. 2010) where grade 0: no symptoms, 1: few flecks, 2: numerous flecks, 3: coalescing necrotic lesions covering <25% of the organ area, 4: 25 to 50% of the organ area necrotic, 5: 50 to 75% of the organ area necrotic, 6: >75% of the organ area necrotic.

The scoring for disease severity was conducted at a weekly interval (7, 14, 21, 28, 35, 42, 49 and 56 days after inoculation) and the PDI (percent disease index) was calculated using the formula given by Wheeler (1969):

$$PDI = 100 \left[\frac{\sum SNR}{N} \right] \left[\frac{1}{MDR} \right]^{-1}$$

Where $\sum SNR$ is the sum of all the numerical ratings, N is the number of observations and MDR is the maximum disease rating based on 0–6 scale.

Based upon the calculated PDI values, the test genotypes were categorized into six categories namely highly resistant (0–5%), resistant (5.1–15%), moderately resistant (15.1–25%), moderately susceptible (25.1–35%), susceptible (35.1–45%) and highly susceptible (>45.1%) under natural epiphytotic conditions.

Area under disease progress curve (AUDPC) values were calculated for each genotype using the mid-point method (Campbell and Madden 1990), indicating the importance of measuring disease progress in germplasm using the formula (Shaner and Finney 1977) given below.

$$AUDPC = \sum_{i=1}^n \left[\frac{(Y_{i+1} + Y_i)}{2} \right] [X_{i+1} - X_i]$$

Table 1. Detail of pea genotypes, including sources and maturity groups

S. No.	Genotype	Maturity group*	Source of genotypes	S. No.	Genotype	Maturity group	Source of genotypes
1	C-400	Mid	New Zealand	51	Airtel	Mid	PAU, Ludhiana
2	Pb-87	Mid	PAU (Pusa-2 x Morassis-55)	52	Arka ajit	Mid	IHR, Bangalore
3	Buddy	Mid	United Kingdom	53	Darl-104	Early	IIVR, Varanasi
4	NS-2	Mid	Namdhari Seeds, Pvt Ltd	54	DGP-207	Mid	Durgapur
5	CHP-2	Mid	Rahuri	55	2016/PMVAR-8	Mid	IIVR, Varanasi
6	CHP-1	Mid	Rahuri	56	KS-20	Mid	Kalayanpur
7	GP-2	Mid	Century seeds	57	AC-Tomour	Mid	USA
8	Jagat Pura	Mid	PAU, Ludhiana	58	PS-11	Mid	Karnal
9	Seena	Mid	United Kingdom	59	Bilaspur Lincoln	Mid	Bilaspur, Himachal Pradesh
10	JP-179	Mid	JNKVV, Jabalpur	60	Arkel	Mid	IARI, New Delhi
11	MA-7	Early	PAU, Ludhiana(MA-6 x AP-3)	61	Winner	Early	United Kingdom
12	DGP-207	Mid	Durgapur	62	PMR-62	Mid	Pantnagar
13	Larex	Mid	United Kingdom	63	Bliss	Mid	United Kingdom
14	NS-1202(W)	Early	Namdhari Seeds Pvt. Ltd, India	64	Legacy	Mid	United Kingdom
15	Eddy	Mid	United Kingdom	65	AP-3	Early	CSAUAT, Kanpur (AP-1 x Arkel)
16	VP-434	Mid	Almora	66	2012/PMVAR-5	Mid	IIVR, Varanasi
17	PMR-19	Mid	Pantnagar	67	E-1	Mid	PAU, Ludhiana
18	Nirali	Mid	Agro Seeds Pvt Ltd, India	68	Mithi Phali	Mid	PAU, Ludhiana
19	2014/PMVAR-1	Mid	IIVR, Varanasi	69	VL-7	Early	VPKAS, Almora
20	VRP-6	Early	IIVR, Varanasi	70	2012/PEVAR-1	Early	IIVR, Varanasi
21	PB-89	Mid	PAU, Ludhiana	71	PM-69	Early	PAU, Ludhiana
22	PM-65	Early	GBPAUT, Pantnagar	72	PEW-9	Mid	USA
23	2011/PEVAR-1(W)	Early	IIVR, Varanasi	73	2012/PEVAR-3	Early	IIVR, Varanasi
24	GP-1	Mid	Century Seeds, India	74	LPF-48	Mid	PAU, Ludhiana
25	GP-3	Mid	Century Seeds, India	75	Little Marvel	Early	England
26	PSM-3	Early	GBPAUT, Pantnagar	76	JM-5	Mid	JNKVV, Jabalpur
27	GS-10	Mid	Golden Seeds Pvt Ltd. India	77	JP-19	Mid	JNKVV, Jabalpur
28	Marina	Mid	Suttind Seed Company, India	78	IC-36	Early	Austria
29	NDVP-104	Mid	Faizabad	79	2016/PMVAR-7	Mid	IIVR, Varanasi
30	PS-24	Mid	Karnal	80	2010/PMVAR-1	Mid	IIVR, Varanasi
31	NS-Afila	Mid	PAU, Ludhiana	81	Ambassador	Mid	United Kindgom
32	2014/PMVAR-2	Mid	IIVR, Varanasi	82	2008/PMVAR-5	Mid	IIVR, Varanasi
33	NDVP-8	Mid	Faizabad	83	2014/PMVAR-6	Mid	IIVR, Varanasi

34	C-308	Mid	New Zealand	84	2016/PMVAR-5	Mid	IIVR, Varanasi
35	Arya Veer	Mid	KS Seeds Pvt Ltd, India	85	2014/PMVAR-4	Mid	IIVR, Varanasi
36	Vasundhra	Mid	Tycoon Seeds, India	86	2014/PMVAR-5	Mid	IIVR, Varanasi
37	Cascatia	Mid	USA	87	2014/PEVAR-3	Early	IIVR, Varanasi
38	Easy Peasy	Mid	USA	88	2014/PEVAR-7	Early	IIVR, Varanasi
39	2011/PEVAR-2	Early	IIVR, Varanasi	89	2010/PMVAR-3	Mid	IIVR, Varanasi
40	Tiger	Mid	New Zealand	90	2016/PEVAR-8	Early	IIVR, Varanasi
41	Heildi	Mid	United Kingdom	91	2016/PMVAR-1	Mid	IIVR, Varanasi
42	Electra	Mid	United Kingdom	92	2016/PEVAR-2	Early	IIVR, Varanasi
43	NS-Non-Afila	Mid	PAU, Ludhiana	93	2011/PMVAR-5	Mid	IIVR, Varanasi
44	Espirit	Med	United Kingdom	94	2015/PEVAR-5	Early	IIVR, Varanasi
45	CHPMR-2	Mid	Ranchi	95	2015/PEVAR-1	Early	IIVR, Varanasi
46	IC-36	Early	Austria	96	2016/PEVAR-7	Early	IIVR, Varanasi
47	VRP-7	Mid	IIVR, Varanasi	97	2017/PMVAR-6	Mid	IIVR, Varanasi
48	UN-53-6-W	Mid	IIHR, Bangalore	98	2017/PMVAR-5	Mid	IIVR, Varanasi
49	VRP-22	Mid	IIVR, Varanasi	99	2015/PEVAR-6	Early	IIVR, Varanasi
50	PS-19	Mid	Karnal	100	Kinnauri	Mid	Solan,HP

*Maturity group; Early =57–65 days till first picking; Mid = 80–90 days till first picking and Late = >90 days till first picking

where, Y_i = severity at the i^{th} observation; X_i = time (days) at the i^{th} observation; n = total number of observations.

RAUDPC=AUDPC/Maximum area of the graph

RaRAUDPC = RAUDPC of individual test genotype/
RAUDPC of standard genotype

The apparent rate of infection (r) is a measure of the speed at which an epidemic develops. ' r ' was calculated from the blight complex disease severity recorded at an interval of seven days to 56 days using Vander Plank's (1963) formula:

$$r = [2.3/t_2 - t_1] [\log_{10}(x_2/1-x_2) - \log_{10}(x_1/1-x_1)]$$

Where, r is the apparent infection rate in the non-logarithmic phase, x_1 is the disease index at time t_1 , x_2 is the disease index at subsequent week time t_2 .

The susceptibility index (S_x) values were calculated using the equation given by Yuen and Forbes (2009) to calculate the relative level of resistance among the test genotypes, equation as follows:

$$S_x = S_y (D_x/D_y)$$

Where S_y and D_y represent the assigned susceptibility scale values and observed disease progress values (AUDPC or RaRAUDPC) for the standard genotype (PB-89) and S_x and D_x represent the calculated susceptibility scale values and observed disease progress value for the genotype

in question (individual test genotype). The commercial cultivar, *i.e.*, PB-89, was used as a reference cultivar and the susceptibility scale value was formulated to test the susceptibility level among pea cultivars against the pea blight complex.

On the basis of susceptibility index (S_x) values, the reaction of test genotypes was categorized as highly resistant (0–1.0), resistant (1.1–2.0), moderately resistant (2.1–3.0), moderately susceptible (3.1–4.0), susceptible (4.1–5.0) and highly susceptible (>5.1).

The genotypes were examined daily for the incubation period (IP50) (days) (when 50% of inoculated plants exhibited disease lesions) until 10 days after inoculation. In order to calculate the percentage of the stem girdled by lesions induced by the blight complex pathogen on pea cultivars, the length of the stem girdled by lesions was divided by the length of the main stem of the cultivar at 49 DAI (Days After Inoculation) using the following formula:

$$\%HTM = (HTM/HT) \times 100$$

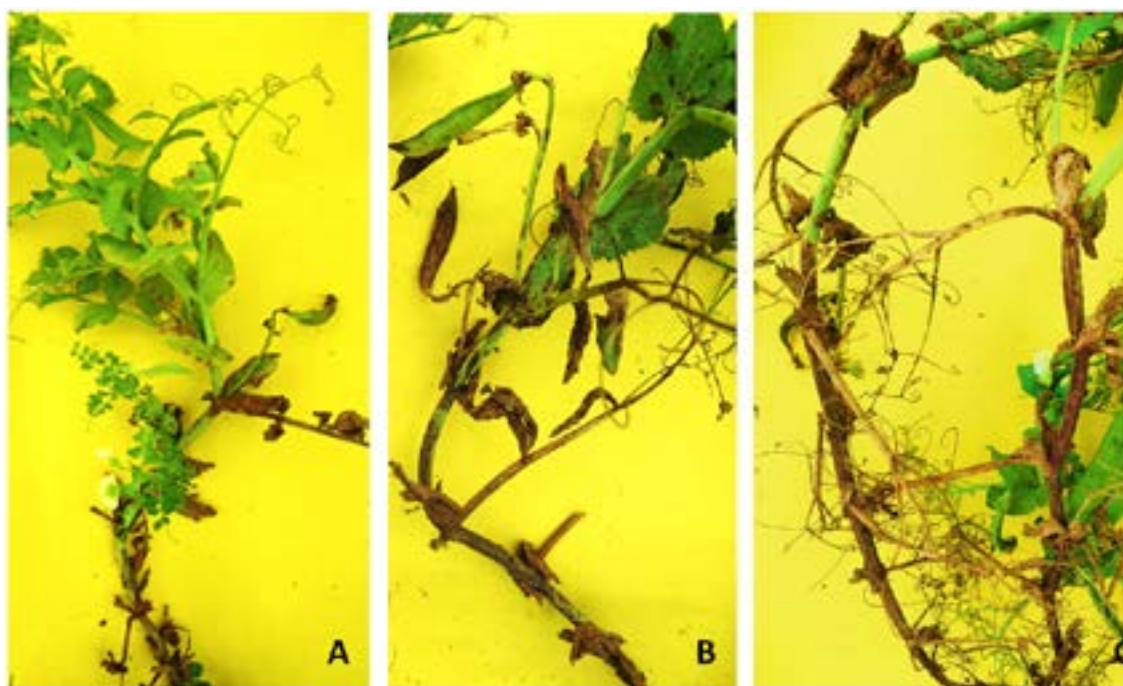
Where %HTM = percentage of the stem girdled by lesions; HTM = length of the stem girdled by lesions; HT = total length of the stem

Statistical analysis

Statistical analysis of data was done through analysis of variance with the help of Statistical Package for the Social Sciences (SPSS) version 20.0, and a 95% confidence interval

Table 2. Disease reaction of pea genotypes under natural field conditions during the year 2017-18

S. No	Genotypes	Number of genotypes	Disease severity scale (%)	Host reaction
1	-	Nil	0-5	HR
2	Eddy, PS-24, Arya Veer, CHPMR-2, PS-19,	5	5.1-15	R
3	CHP-2, CHP-1, GP-2, Jagat Pura, JP-179, Larex, PMR-19, 2014/PMVAR-1, Marina, NDVP-104, NDVP-8, C-308, Heildi, Espirit, VRP-22, Arkaajit, KS-20, AC-Tomour, PS-11, Bilaspur Lincoln, Mithi Phali, LPF-48, Little Marvel, JP-19, 2008/PMVAR-5, 2014/PMVAR-6, 2014/PMVAR-4, 2010/PMVAR-3, Kinnauri	29	15.1-25	MR
4	Seena, Nirali, PM-65, 2011/PEVAR-1(W), GP-1, GP-3, GS-10, Vasundhra, Cascatia, Easy Peasy, Electra, NS-Non-Afila, IC-36, Arkel, PMR-62, Legacy, AP-3, E-1, PEW-9, JM-5, IC-36, 2016/PMVAR-7, 2010/PMVAR-1, Ambassador, 2016/PMVAR-5, 2014/PMVAR-5, 2014/PEVAR-3, 2016/PMVAR-1, 2016/PEVAR-7, 2017/PMVAR-5	30	25.1-35	MS
5	C-400, Pb-87, Buddy, MA-7, DGP-207, NS-1202(W), VP-434, VRP-6, Pb-89, PSM-3, NS-Afila, 2014/PMVAR-2, Tiger, VRP-7, UN-53-6-W, Airtel, Darl-104, 2016/PMVAR-8, Bliss, 2012/PMVAR-5, PM-69, 2015/PEVAR-5, 2017/PMVAR-6	23	35.1-45	S
6	NS-2, 2011/PEVAR-2, DGP-207, Winner, VL-7, 2012/PEVAR-1, 2012/PEVAR-3, 2014/PEVAR-7, 2016/PEVAR-8, 2016/PEVAR-2, 2011/PMVAR-5, 2015/PEVAR-1, 2015/PEVAR-6	13	>45.1	HS

**Fig. 1.** Reaction of pea germplasm against *Ascochyta* blight complex. A. Reaction of resistant cultivar CHPMR-2. B. Reaction of commercial cultivar PB-89. C. Reaction of susceptible cultivar C-400

for each parameter was generated. The correlation analysis at 5% significance between the parameters best explaining the disease severity was also performed with SPSS version 20.0.

Results

Screening under natural epiphytotic conditions

The field screening of test genotypes under natural conditions (Table 2) revealed that five genotypes, namely

Eddy, PS-24, Arya Veer, CHPMR-2 and PS-19, were found to be resistant (R) with <15% disease severity and <50% disease incidence, while, 24 genotypes, exhibited susceptible (S) reaction with disease severity 35.1 to 45% and the remaining 12 genotypes were categorized as highly susceptible (HS) with disease severity of >45% and disease incidence, >75%.

Screening under artificial inoculation conditions

Monitoring of disease parameters of the genotypes at weekly intervals up to 56 days post-inoculation was carried

out under artificial epiphytotic field conditions (Fig. 1, Tables 3 and 4). The genotypes Eddy, PS-24, Arya Veer, CHPMR-2, and PS-19 had a susceptibility index (Sx) value of less than two over both seasons, indicating that they were resistant (R) to the disease complex (Fig. 1a). During the year 2018-19, 29 genotypes viz: CHP-2, GP-2, Jagat Pura, JP-179, Larex, PMR-19, 2014/PMVAR-1, NDVP-104, 2014/PMVAR-2, NDVP-8, C-308, Vasundhara, Heildi, Electra, VRP-22, Arka Ajit, KS-20, AC-Tomour, PS-11, Bilaspur Lincoln, LPF-48, Little Marvel, JP-19, 2008/PMVAR-5, 2014/PMVAR-6, 2014/PMVAR-4, 2010/PMVAR-3, 2016/PEVAR-7 and Kinnauri were found to be moderately resistant (MR) with AUDPC, RaRAUDPC, the apparent rate of infection and susceptibility index value ranging from 840.70 to 1244.40, 0.35 to 0.51, 0.05 to 0.10 and 2.1 to 3.0, respectively. Eighteen genotypes viz: CHP-1, Nirali, GP-1, GP-3, GS-10, Marina, Cascatia, Easy Peasy, NS-Non-Afila, Espirit, IC-36, Darl-104, PMR-62, Legacy, Mithi Phali, JM-5, 2016/PMVAR-5 and 2014/PMVAR-5 were found to be moderately susceptible (MS) with AUDPC, RaRAUDPC, the apparent rate of infection and susceptibility index value ranging from 11.89.10 to 1422.80, 0.09 to 0.67, 0.08 to 0.12 and 3.1 to 4.0. However, thirteen genotypes, namely, Seena, 2011/PEVAR-1(W), Tiger, VRP-7, UN-53-6-W, Arkel, AP-3, E-1, IC-36, 2016/PMVAR-7, Ambassador, 2014/PEVAR-3, 2016/PMVAR-1 showed susceptible (S) reaction with AUDPC, RaRAUDPC, the apparent rate of infection and susceptibility index value ranging from 1076.00 to 1552.60, 0.37 to 0.82, 0.06 to 0.12 and 4.1 to 5.0 while the remaining 35 genotypes were recorded as highly susceptible (HS) with susceptibility index value >5.1 (Fig.1b and 1c). Resistant genotypes, namely Eddy, PS-24, Arya Veer, CHPMR-2 and PS-19, showed the lowest apparent rate of infection and RaRAUDPC varying from 0.06 to 0.07 and 0.23 to 0.34 with AUDPC and susceptibility index value ranging from 504.96 to 1029.10 and 1.3 to 2.0, respectively.

Likewise, twenty-two genotypes viz: JP-179, C-308, 2010/PMVAR-3, LPF-8, JP-19, 2016/PEVAR-7, Kinnauri, PMR-19, NDVP-8, Heildi, Electra, Bilaspur Lincoln, PS-11, VRP-22, Larex, Jagat Pura, GP-2, CHP-2, Little Marvel, 2008/PMVAR-5, 2014/PMVAR-6 and 2014/PMVAR-4 were reported to be moderately resistant (MR) during the year 2019-20, with AUDPC, RaRAUDPC, the apparent rate of infection and susceptibility index value ranging from 606.52 to 837.97, 0.36 to 0.50, 0.08 to 0.12 and 2.1 to 3.0, respectively and twenty-six genotypes viz: CHP-1, GP-1, Vasundhara, Legacy, JM-5, 2016/PMVAR-5, 2014/PMVAR-5, GS-10, Buddy, Nirali, GP-3, Marina, 2014/PMVAR-2, Arkaajit, Darl-104, Mithi Phali, 2016/PMVAR-1, 2014/PMVAR-1, NDVP-104, Easy Peasy, Cascatia, NS-Non-Afila, IC-36, KS-20, AC-Tomour and PMR-62 were found to be moderately susceptible (MS) with AUDPC, RaRAUDPC, the apparent rate of infection and susceptibility index value ranging from 879.14 to 1105.81, 0.54 to 0.67, 0.09 to 0.14 and 3.1 to 4.0, respectively. Furthermore, 23 genotypes, namely: VRP-6, PSM-3, Tiger, Espirit, Arkel, 2012/PMVAR-5, E-1, PM-69,

PEW-9, 2010/PMVAR-1, Ambassador, 2015/PEVAR-5, 2014/PEVAR-3, 2017/PMVAR-6, PM-65, 2011/PEVAR-1(W), VRP-7, UN-53-6-W, Airtel, 2017/PMVAR-5, Seena, AP-3 and 2016/PMVAR-7 showed susceptible (S) reactions with AUDPC, RaRAUDPC, apparent rate of infection and susceptibility index value ranging from 1131.61 to 1375.05, 0.30 to 0.83, 0.05 to 0.15 and 4.1 to 5.0 while, the remaining 23 genotypes were recorded as highly susceptible (HS) with susceptibility index value >5.1. Resistant cultivars, namely Eddy, PS-24, Arya Veer, CHPMR-2 and PS-19, showed the lowest apparent rate of infection and RaRAUDPC varying from 0.06 to 0.08 and 0.20 to 0.34 with AUDPC and susceptibility index value ranging from 342.40 to 569.37 and 1.2 to 2.0, respectively.

The symptoms of the disease syndrome started to appear first on lower leaves of susceptible genotypes and the minimum incubation period (4.40 days) was noticed in 2015/PEVAR-1 which was on par with genotypes 2015/PEVAR-6, 2016/PEVAR-8, VL-7, 2012/PEVAR-1, 2014/PEVAR-7, 2016/PEVAR-2, 2015/PEVAR-5, 2016/PEVAR-7, 2012/PEVAR-3, Winner, 2014/PEVAR-3, 2010/PMVAR-3, 2011/PMVAR-5, Kinnauri, 2017/PMVAR-5, Mithi Phali and 2017/PMVAR-6. The maximum incubation period of 7.80 days was recorded in genotype CHPMR-2 during the year 2018-19. Similarly, during the year 2019-20, parallel data were obtained showing a minimum incubation length of 4.66 days in cultivar 2015/PEVAR-1 and a maximum incubation period of 8.03 days in resistant cultivar CHPMR-2.

Stem infections began at the soil line and extended upwards; lesions often coalesced to girdle the stem. The percentage of stem girdling measured at 49 days after inoculation in the year 2018-19 indicated the highest percentage of stem girdling recorded in genotypes, namely, 2015/PEVAR-1 (68.95%) accompanied by 2016/PEVAR-8 (68.82%), 2015/PEVAR-6 (68.15%), 2011/PMVAR-5 (67.55%), 2017/PMVAR-6 (67.52%) and 2016/PEVAR-2 (64.97%). However, the minimum percentage of stem girdling was observed in genotypes CHPMR-2 (7.03 %), which was at par with CHP-1 (8.14%), IC-36 (8.40%), PS-24 (9.59%), PS-11 (10.88%), Arya Veer (11.37%), VRP-6 (12.42%), KS-20 (13.52%), Heildi (14.09%), C-308 (14.12%), Jagat Pura (14.36%), PEW-9 (14.59%), UN-53-6-W (14.79%) with girdling percentage 15.13%, respectively. Likewise, in the subsequent year, 2019-20, the highest percentage of stem girdling was recorded in genotypes 2016/PEVAR-2 (63.11%) while minimum stem girdling was recorded in genotype CHPMR-2 (6.18%).

Correlation studies

The correlation analysis conducted for the years 2018-19 and 2019-20 revealed statistically significant relationships between various parameters such as an area under disease progress curve (AUDPC), the incubation period (IP50), percentage of stem girdling (%HTM) and disease severity (%) as presented in Table 5. AUDPC exhibited a significant positive correlation with %HTM and disease severity (%),

Table 3. Evaluation of pea genotypes under artificial epiphytotic conditions during the year 2018-19

S. No	Varieties	AUDPC	RaAUDPC	Apparent Rate(r)	Disease incidence(%)	Disease severity (%)	IP 50	%HTM	Susceptibility index (Sx)	Host reaction
1	C-400, Pb-87, Buddy, NS-2, MA-7, DGP-207, NS-1202(W), VP-434, VRP-6, Pb-89, PM-65, PSM-3, NS-Afila, 2011/PEVAR-2, Airtel, DGP-207, 2016/PMVAR-8, Winner, Bliss, 2012/PMVAR-5, VL-7, 2012/PEVAR-1, PM-69, PEW-9, 2012/PEVAR-3, 2010/PMVAR-1, 2014/PEVAR-7, 2016/PEVAR-8, 2016/PEVAR-2, 2011/PMVAR-5, 2015/PEVAR-5, 2015/PEVAR-1, 2017/PMVAR-6, 2017/PMVAR-5, 2015/PEVAR-6	1400.00 – 2444.10 ^(XXVI-XLVII)	0.37–2.39	0.06–0.21	83.12 – 94.91 ^(XLI-XXV)	45.00–86.11 ^(XVII-XLIII)	4.40–6.70 ^(I-XXIII)	12.42–68.95 ^(IX-XIII)	5.13-14.34	HS
2	Seena, 2011/PEVAR-1(W), Tiger, VRP-7, UN-53-6-W, Arkel, AP-3, E-1, IC-36, 2016/PMVAR-7, Ambassador, 2014/PEVAR-3, 2016/PMVAR-1	1076.00 – 1552.60 ^(VI-XXXVII)	0.37–0.82	0.06–0.12	82.71 – 87.99 ^(IX-XX)	31.11–53.70 ^(IV-XXXVIII)	4.60–5.63 ^(I-XXIII)	14.79–42.79 ^(IX-XI)	4.12-5.09	S
3	CHP-1, Nirali, GP-1, GP-3, GS-10, Marina, Cascatia, Easy Peasy, NS-Non-Afila, Espirit, IC-36, Darl-104, PMR-62, Legacy, Mithi Phali, JM-5, 2016/PMVAR-5, 2014/PMVAR-5	1189.10 – 1422.80 ^(XIII-XXIII)	0.09–0.67	0.08–0.12	69.58 – 86.66 ^(III-XVI)	37.59–47.22 ^(XII-XXXV)	4.53–6.13 ^(I-XXXII)	8.40–41.18 ^(IX-XL)	3.16-4.07	MS
4	CHP-2, GP-2, Jagat Pura, JP-179, Larex, PMR-19, 2014/PMVAR-1, NDVP-104, 2014/PMVAR-2, NDVP-8, C-308, Vasundhra, Heildi, Electra, VRP-22, Arkaajit, KS-20, AC-Tomour, PS-11, Bilaspur Lincoln, LPF-48, Little Marvel, JP-19, 2008/PMVAR-5, 2014/PMVAR-6, 2014/PMVAR-4, 2010/PMVAR-3, 2016/PEVAR-7, Kinnauri	840.70 – 1244.40 ^(II-XXII)	0.35–0.51	0.05–0.10	54.54 – 72.32 ^(III-XIV)	27.22–38.52 ^(III-XXII)	4.63–7.13 ^(I-XXX)	8.15–38.29 ^(IX-XL)	2.12-3.06	MR
5	Eddy, PS-24, Arya Veer, CHPMR-2, PS-19	504.96–1029.10 ^(I-IX)	0.23–0.34	0.06–0.07	40.11 – 53.21 ^(I-II)	16.29–30.56 ^(I-VIII)	5.63–7.80 ^(I-XXV)	7.03–24.46 ^(I-XXVI)	1.38-2.08	R

AUDPC=Area under disease progress curve; RaAUDPC = Relative area relative area under disease progress curve with respect to susceptible check; %HTM=Percentage of stem girdling; Superscripts depict that data followed by same roman number(s) are not significantly different (p = 0.05) according to Duncan's multiple range test

Table 4. Evaluation of pea genotypes under artificial epiphytotic conditions during the year 2019-20

S. No	Varieties	AUDPC	RaAUDPC	Apparent Rate(r)	Disease incidence(%)	Disease severity (%)	IP 50	%HTM	Susceptibility index (Sx)	Host reaction
1	C-400,NS-2,MA-7,DGP-207,NS-1202(W),VP-434,Pb-89,NS-Afla, 2011/PEVAR-2,DGP-207,2016/PMVAR-8,Winner,Bliss,VL-7,2012/PEVAR-1,2012/PEVAR-3,2014/PEVAR-7,2016/PEVAR-8,2016/PEVAR-2,2011/PMVAR-5,2015/PEVAR-1,2015/PEVAR-6,Pb-87	1449.04 – 3420.69 ^(III-XV)	0.88 – 2.15	0.10 – 0.18	81.12 – 90.99 ^(XIII-XXXVII)	56.42 – 94.88 ^(XXV-XXXVII)	4.66 – 7.20 ^(I-XXV)	37.47 – 63.11 ^(XXXI-XXXVIII)	5.34 – 12.96	HS
2	VRP-6,PSM-3, Tiger, Spirit,Arkel, 2012/PMVAR-5,E-1,PM-69,PEW-9, 2010/PMVAR-1,Ambassador, 2015/PEVAR-5,2014/PEVAR-3,2017/PMVAR-6,PM-65,2011/PEVAR-1(W),VRP-7,UN-53-6-W,Airtel,2017/PMVAR-5,Seena, AP-3, 2016/PMVAR-7	1131.61 – 1375.05 ^(II-X)	0.30 – 0.83	0.05 – 0.15	72.98 – 77.92 ^(XIX)	46.16 – 54.33 ^(XV-XXX)	4.86 – 7.13 ^(I-XXV)	10.34 – 60.76 ^(I-XXXIII)	4.17 – 5.07	S
3	CHP-1, GP-1, Vasundhara, Legacy, JM-5, 2016/PMVAR-5,2014/PMVAR-5, GS-10, Buddy,Nirali,GP-3,Marina,2014/PMVAR-2,Arkaajit,Darl-104,MithiPhali,2016/PMVAR-1, 2014/PMVAR-1, NDVP-104, Easy Peasy, Cascatia, NS-Non-Afla, IC-36, KS-20, AC-Tomour, PMR-62	879.14 – 1105.81 ^(II-X)	0.54 – 0.67	0.09 – 0.14	62.23 – 70.07 ^(V-XVI)	36.11 – 44.93 ^(VI-XXIII)	5.13 – 7.26 ^(II-XXV)	23.68 – 40.32 ^(VII-XXXVI)	3.25 – 4.07	MS
4	JP-179,C-308,2010/PMVAR-3, LPF-48, JP-19, 2016/PEVAR-7,Kinnauri, PMR-19, NDVP-8,Heildi, Electra, Bilaspur Lincoln, PS-11,VRP-22,Larex,Jagat Pura,GP-2,CHP-2, Little Marvel, 2008/PMVAR-5,2014/PMVAR-6, 2014/PMVAR-4	606.52- 837.97 ^(I-VII)	0.36-0.50	0.08-0.12	50.54-58.02 ^(II-XI)	29.09-34.85 ^(IV-XI)	4.93 - 7.36 ^(I-XXV)	18.24-26.42 ^(VI-XXVIII)	2.22-3.09	MR
5	Eddy, PS-24, Arya Veer, CHPMR-2, PS-19	342.40 – 569.37 ^(I-IV)	0.20 – 0.34	0.06 – 0.08	35.19 – 44.14 ^(I-IV)	13.67 – 24.44 ^(I-V)	5.80 – 8.03 ^(XII-XXVI)	6.18 – 24.95 ^(I-XXVI)	1.26 – 2.09	R

AUDPC=Area under disease progress curve; RaAUDPC = Relative area relative area under disease progress curve with respect to susceptible check; %HTM=Percentage of stem girdling; Superscripts depict that data followed by same roman number(s) are not significantly different (p = 0.05) according to Duncan's multiple range test

indicating that the two variables are complementary to each other. However, AUDPC showed a significant negative correlation with the incubation period (IP50). Additionally, disease severity (DS%) displayed a significant positive correlation with percentage stem girdling (%HTM), while a negative correlation with the incubation length (IP50) as demonstrated in Table 6. Furthermore, AUDPC depicted a significant relationship with %HTM ($r = 0.75^{**}$) and DS ($r = 0.98^{**}$) and %HTM displayed a significant positive correlation with DS ($r = 0.74^{**}$), indicating a series of positive associations. On the other hand, AUDPC ($r = -0.65^{**}$), %HTM ($r = -0.60^{**}$) and DS ($r = -0.59^{**}$) were significantly negatively correlated with IP50 value, suggesting that genotypes with lower IP50 value exhibit a higher percentage of blight disease.

Discussion

The *Ascochyta* blight complex disease poses a significant and ongoing threat to global pea (*Pisum sativum* L.) production. Developing a comprehensive understanding of the resistance mechanisms employed by peas on confronted with *Ascochyta* blight pathogens is crucial for the advancement of effective breeding strategies. However, the presence of diverse pathogen species associated with disease complexes presents a challenge to breeders' capability to breed for resistance. Previous studies by Khan et.al (2013); Setti et al. (2011); Tran et al. (2015) have reported limited sources of resistance in field peas, with no major resistance gene identified. This indicates that resistance to the complex disease is polygenic and lacks pathotype specificity, further complicating the unraveling of associated mechanisms (Joshi et al. 2022). The inheritance studies have shown that only the local cultivar, Kinnauri, impart resistance to *Ascochyta* blight (Rastogi and Saini 1984; Mohan et al.

2013; Amin et al. 2010). Our findings also support the reaction of Kinnauri as moderately resistance to complex pathogen species.

In our study, we evaluated a diverse set of one hundred pea genotypes and observed significant diversity in their susceptibility to the disease. All the genotypes exhibited significant differences in resistance levels when exposed to both artificial as well as natural epiphytotic conditions. Among the genotypes tested, only five, namely Eddy, PS-24, Arya Veer, CHPMR-2 and PS-19, demonstrated resistance under natural epiphytotic conditions, exhibiting disease severity levels of less than 15% and incidence below 50%. A similar resistance trend was observed in these five genotypes under artificial inoculation conditions, as evidenced by a susceptibility index (Sx) of less than two over two consecutive years. Data was also calculated on the basis of additional parameters such as AUDPC, RaAUDPC, Disease severity, %HTM, and apparent rate of infection (r). However, none of the genotypes exhibited a high level of resistance against the *Ascochyta* blight complex disease. Likewise under both natural and artificial epiphytotic conditions, the genotypes including JP-179, C-308, 2010/PMVAR-3, LPF-48, JP-19, Kinnauri, PMR-19, NDVP-8, Heildi, Bilaspur Lincoln, PS-11, VRP-22, Larex, Jagat Pura, GP-2, CHP-2, Little Marvel, 2008/PMVAR-5, 2014/PMVAR-6 and 2014/PMVAR-4 exhibited MR reaction. Nevertheless, under natural conditions, some of the genotypes, namely CHP-1, 2014/PMVAR-1, Marina, Espirit and Mithiphali, showed MR reaction and MS reaction under artificial inoculation conditions. This intriguing observation can be attributed to artificial inoculations combined with predisposing factors such as prolonged leaf wetness. Significant correlations were observed among all the parameters, including the area under the disease progress curve (AUDPC), the incubation

Table 5. Analysis of variance for *Ascochyta* blight complex resistance in pea genotype

Source		Types III Sum of Squares	df	Mean square	F value	p-value
Genotypes	AUDPC	560.17	99	565743.84	174.94*	.000
	IP 50	172.86	99	1.75	36.00*	.000
	%HTM	58204.22	99	587.92	34.18*	.000
	Disease Severity	85324.86	99	861.86	861.86*	.000

Alpha = 0.05 * = $p < 0.05$ (Significant)

Table 6. Correlation coefficient among AUDPC, IP 50, %HTM and disease severity(%)

		AUDPC	IP 50	%HTM	Disease Severity
AUDPC	Pearson Correlation	1			
IP50	Pearson Correlation	-.654**	1		
%HTM	Pearson Correlation	.755**	-.604**	1	
Disease severity	Pearson Correlation	.989**	-.597**	.742**	1

** Correlation is significant at the 0.01 level (2-tailed)

period (IP50), the percentage of stem girdling (%HTM) and disease severity (%), indicating that all the parameters are complementary to each other in assessing disease intensity. Whereas, a significant negative correlation was found between IP50 and all other parameters, suggesting that the short incubation period (IP50) results in a fast expansion of lesions related to the quick emergence of pycnidia. These findings are consistent with the research by Dutt et al. (2020).

The present study supports the findings of previous reports by various researchers (Kraft et al. 1998; Chasti et al. 2022), who observed a lack of resistance against *Ascochyta* blight pathogens in different regions worldwide. Francis et al. (2000) conducted field screening of approximately 500 lines and found that around 40 lines displayed partial resistance to *Ascochyta* blight disease. Warkentin et al. (2000) studied the relationship between components of partial resistance and yield reduction in 335 field pea lines originating from more than 30 countries against *Mycosphaerella* blight. Out of 335 *Pisum* lines, none of them possessed a high degree of resistance against *M. pinodes*. However, seven lines (Baccara, Carneval, Danto, Majoret, Miko, PI273605, and Yellow head) showed less area under AUDPC scores and cultivar Radley showed a partial resistant reaction. Liu et al. (2016) assessed the resistance level of 23 pea cultivars against *A. pinodes* and found that all the tested cultivars were susceptible to the fungus. Similarly, Assen (2016) evaluated 49 genotypes and identified 16 genotypes (32.65%) with moderate resistance and 33 genotypes (67.35%) as susceptible to *Ascochyta* blight disease. Joshi et al. (2022) evaluated 16 genotypes and observed that breeding lines 11HP-302-12HO-1 and 10HP249-11HO-7 displayed partial resistance to *Phoma pinodella* and *Didymella pinodes*. Additionally, Chasti et al. (2022) reported that out of 63 evaluated germplasm lines, 40 were susceptible, 12 moderately susceptible and 8 highly susceptible. Furthermore, Tadesse et al. (2021) assessed 11 genotypes and found that two genotypes (EH 012022-1 and EH 012020-7) were moderately resistant, 3 genotypes (Burkitu, Adi and EH 012019-1) were susceptible and the remaining seven genotypes were highly susceptible to *Ascochyta* blight disease.

As a consequence, it is important to note that breeding programs cannot remain focused on resistance against specific pathogen species while ignoring other pathogens within the disease complex. Targeting host resistance against a particular species or subset of the pathogen complex may lead to a shift in the population towards pathogen species that are least challenged by host resistance. Therefore, the most effective approach to managing the disease caused by the *Ascochyta* blight complex involves deploying host plant resistance accommodated with dynamic pathogen populations across different locations and over time, considering the wider diversity of pathogens within the complex.

This study represents the first assessment of disease resistance in the Punjab region, considering the co-existence of three distinct pathogens (*A. pisi*, *A. pinodes* and *D. pinodella*) causing pea blight. This poses a significant challenge, as no complete resistance has been reported to date, making blight complex disease the most devastating. The resistant genotypes identified in this study can be utilized in breeding programs to develop resistant varieties/hybrids and reduce reliance on chemical fungicides.

Authors' contribution

Conceptualization of research (SJ); Designing of the experiments (SJ); Contribution of experimental materials (SJ, PKD); Execution of field/lab experiments and data collection (A, RR, SJ); Analysis of data and interpretation (SJ, A); Preparation of manuscript (A, SJ).

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