AATCC Review

04 September 2024: Received
07 October 2024: Revised
11 November 2024: Accepted
28 November 2024: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Analysis of Biochemical Changes in Genotypes of Pea Against Erysiphe polygoni DC Causing Powdery Mildew

Dinesh Kumar Meena[1]*, Jitendra Singh[1], Pankaj Kumar Sharma[1], Waghamare Minal Bhujangrao[2], Meenu Kumari Meena[1], Kavita Kansotia[1]

[1]Department of Plant Pathology, SKN College of Agriculture, SKNAU, Jobner, Jaipur, Rajasthan, 303329, India
[2]Uttar Banga Krishi Vishwavidyalaya, Pundibari, Cooch Behar, West Bengal, 736165, India

ABSTRACT

Pea (Pisumsativum L) is a valuable crop all over the world, is also known as "Matar". It belongs to family Leguminosae and the family Papilionaceae. It's affected by various Fungal, Bacterial and Viral diseases. Powdery mildew of peas is one of the major diseases of pea incited by Erysiph epolygoni DC. Major symptoms of powdery mildew disease are the presence of white floury patches appearing on the leaves as well as stems, tendrils, pods and in severe conditions its cover whole plant parts except the root region and finally plant becomes the older and later stage of the crop whole plant become comparatively greyish brown and the infected parts convey dull appearance. In extreme conditions, infected pods and leaves fall down from the plant. An experiment was conducted at farm of SKNCOA, Jobner during the Rabi season 2021. Plants respond to external stimuli by altering metabolic pathways, resulting in a cascade of different interrelated changes in biochemical processes. The incidence of powdery mildew in pea induces changes in phenol, peroxidase and reducing sugar. Powdery mildew resistant and susceptible pea genotypes respond differentially to these traits. Powdery mildew resistant genotypes had comparatively less concentration of reducing sugar and high concentration of phenol and peroxidase before and after infection. All the traits being significantly correlated with disease severity can be an important component of an effective powdery mildew screening procedure in pea.

Keywords: *Phenol, Peroxidase, Reducing sugar, Powdery mildew, Pea, Biochmicals, Genotypes*

INTRODUCTION

The vegetable pea fits to the family (Fabaceae) *Leguminosae*. "*Pisum sativum* L." subspecies "*Hortense*" initiated from common field pea. *Pisum sativum* subspecies "*arvense*" is considered to be native to Ethiopia, the Mediterranean and Central Asia. It has chromosome number 2n=14.

Pea was originated from South western Asia, possibly in North western India, Pakistan or adjacent areas of the former USSR and Afghanistan.

Frost can harm the plants during the flowering stage. High humidity and gloomy weather condition result into feast of fungal diseases like damping-off and powdery mildew. Right temperature for growth is 13-18^{0}C.

Pulses constitute an important ingredient of the vegetarian diet in India and play a significant role in Indian farming. They are providing quality food to teeming million and restoring soil fertility through biological nitrogen fixation.India is the largest producer and consumer of pulses in the world.Legumes are unique crops in the world because they contain higher amount of protein, carbohydrates and other nutrients. Pea is an important vegetable crop in India. It's cool season crop and generally grown as *rabi* season crop.

Nutrient value in pea, generally pea seeds contain 17 to 22 g carbohydrates, 20 to 50 g starch, 14 to 26 g dietary fiber, 6.2 to 6.5 g protein, 0.4 g fat, 1.0 g ash per 100 g with 9 to 10 mg calcium, 3 to 5 mg sodium, 97 to 99 mg potassium per advance techniques like pulse electric field or ultra-sonication have shown remarkable impact on the efficiency by improving nutritional quality and techno- functional properties of pea and its protein [5] [7].

The crop is very much valuable in crop rotation [1]. It is an important legume crop next to soybean, groundnut and beans[2]. Field pea contain 5 to 20 per cent less of the trypsin inhibitors than soybeans and it allow directly fed to livestock without having to go through the extrusion heating process. In india pulse productivity low because its grown in marginal lands and low rainfall areas, and poor management practices using by farmers.

Powdery mildew disease occurs worldwide in the countries viz., India, Brazil, Philippines, South Australia, Sri Lanka, Bangladesh Taiwan, Thailand, Tropical Africa, France, USA, Pakistan, China, Russia, Canada and many other regions of the world it considered major disease.

In India, powdery mildew (*Erysiphepolygoni* DC) was reported to occur and cause heavy qualitative and quantitative loss in pea, urdbean, mungbean, methi and many other important pulse crops grown in the states of Rajasthan, Maharashtra, Karnataka, Andhra Pradesh, Orissa, Madhya Pradesh and Tamilnadu [3].

Powdery mildew is the major disease of pea throughout the world and it is the air borne disease worldwide distribution in severe form it reduces 24-27% pod weight, 21-30% pod number and up to 70% reduction in total yield loss [8].

The yield losses were reported to the tune of 50 to 90% when the intensity was high at pod formation stage [10].

Yield loss of 10-65% due to the disease has been reported [11].

Corresponding Author: **Dinesh Kumar Meena*

DOI: https://doi.org/10.21276/AATCCReview.2024.12.04.444

© 2024 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

© *2024 AATCC Review.* All Rights Reserved.

MATERIALS AND METHODS

Powdery mildew infected and healthy samples of pea leaves were collected in the morning from the experimental site at field number 7 plant pathology, SKNCOA, Jobner, of Pant P-498, IPF -20-17, IPF 20-21, HFP 1607, HFP 9907, RFP 2010-1, HUPT 1709, Pant P-497, RFPG-181, Aman, HFP 1702, RFPG 180, Pant P 42, VL- 72, Pant P-501, IPF 20-11, Local check germplasms for the analysis of biochemical.

Bio-chemical studies

The following experiments were conducted in order to study the effect of *Erysiphepolygoni*, on some biochemical constituents (Total phenol, Peroxidase content and Reducing sugar) of pea varieties/germplasms of Pant P-498, IPF -20-17, IPF 20-21, HFP 1607, HFP 9907, RFP 2010-1, HUPT 1709, Pant P-497, RFPG-181, Aman, HFP 1702, RFPG 180, Pant P 42, VL- 72, Pant P-501, IPF 20-11, Local check in healthy and diseased leaves.

These varieties were grown in plots of 2 x 1.2 m^2 keeping row to row and plant-to-plant distance of 45 x 10 cm field with three replications. Healthy leaves from above varieties were collected 45 - 50 days after sowing (DAS) before appearance disease and infected leaves were collected 75 - 80 days after sowing (DAS), after appearance disease for biochemical analysis. For the quantitative estimation of primary metabolites following different protocols were used.

Estimation of Peroxidase content in leaves

The peroxidase activity in the leaf sample was assayed by the method of Kumar and Khan [4].

Reagents

1. 0.1 M phosphate buffer (pH 7.0): Prepared by mixing 47.8 ml of 0.2 M NaH_2PO_4.2H2O solution and 76.3 ml 0.2 M Na_2HPO_4.2H2O. The pH was adjusted to 7.0 and the final volume was made to 250 ml.
2. Pyrogallol reagent (0.01 M): Prepared by dissolving 0.126 g of pyrogallol in 100 ml of distilled water.
3. Hydrogen peroxide solution (0.005 M): 103 µl of 30 % (v/v) hydrogen peroxide was pipetted in a 100 ml volumetric flask and the volume was made with distilled water. From this stock solution (1 M), 0.5 ml was pipetted in 100 ml volumetric flask and the volume was made with distilled water. This solution had the concentration of 0.005 M. the solution was prepared freshly at the time of experiment.

Procedure

A known quantity (0.25 g) of pea leaf samples was macerated separately with 6 ml of 0.1 M phosphate buffer in pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 x g at 4^0C for 30 min. 1 ml supernatant was diluted to 10 ml with distilled water and was used as the enzyme source.

The assay mixture of peroxidase contained 3.6 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml of 0.005 M hydrogen peroxide, 1 ml of 0.01 M pyrogallol and 1 ml of diluted enzyme extract. The absorbance was read at 420 nm on a Spectronic-20 Spectrophotometer for every 30 sec. up to 3 min and the reaction was stopped by adding 2.5 N H_2SO_4 exactly after 3 min.

One unit of peroxidase activity was determined as an increase in O.D. by 0.001. The enzyme activity was calculated for one g of sample and expressed as unit's min-1mg-1 soluble protein.

The inoculum of Erysiphepolygoni causing powdery mildew of pea was collected very early in the morning (8.00 AM) from the infected leaves showing characteristic disease symptoms.

The powdery mildew colonies from upper surface of the infected leaves were picked up gently with the help of cotton pad, camel's hair brush in sterilized buffer solution (pH 7.0) in double distilled water. This suspension was shaken to disperse the conidia and adjusted to give a final concentration of 5000 conidia ml^{-1} to enhance the germination of conidia for 24 hours.

Total phenol

The content of total phenol present in the leaves was estimated as per method suggested by Malik and Singh (1980)[6]. Weighed 0.5 g of fresh leaves without midrib and grind with mortar and pestle in 10 ml of 80 per cent ethanol. Thus, homogenate or extract obtained was centrifuged at 8000 rpm for 10 minutes. Extraction was repeated four times with 5 ml of 80 per cent ethanol each time and supernatants were collected into same beaker. Volume of the extract was made to 50 ml with 80 per cent ethanol. One ml of supernatant was taken and evaporated to dryness in water bath. One ml of Millipore water in each test tube and 0.5 ml of Folin&Ciocalteu reagent (1:1 with water) was added and kept for three min. After this, 2 ml of 20 per cent Na_2CO_3 was added and mixed thoroughly. The tubes were placed in boiling water for exactly one minute and cooled in ice water. The absorbance was read at 650 nm against a reagent blank [6].

The amount of phenols present in the sample was calculated as:

Phenol (mg/g) = Sample O.D. × Standard O.D. × Dilution factor

Where O.D. = Optical density.

Reducing sugar

Reducing sugar content was determined by Nelson Somogyi's method [9].

1. Reagent 'A': This contained 2.5g anhydrous sodium carbonate, 2.5 g Rochelle salt, 2.0 g sodium bicarbonate and 20g anhydrous sodium sulphate in a final volume of 100 ml distilled water.

2. Reagent 'B': Exactly 15g of copper sulphate was dissolved in distilled water containing 1-2 drops of concentrated sulphuric acid and the volume was made to 100 ml.

3. Alkaline copper tartrate reagent: It was a mixture of 25 parts of reagent 'A' and 1 part of reagent 'B' (v/v). a freshly mixed reagent was used for analysis.

4. Arsenomolybdate reagent: Prepared by dissolving 25g of ammonium molybdate in 450ml distilled water. To this 25ml of concentrated sulphuric acid was added. 3g of sodium arsenate was dissolved in 25 ml distilled water separately and poured in to above solution. The final volume was made to 500ml with distilled water. This reagent was incubated at 37^0C for 24-48 hr and then used for colour development.

5. Standard D-glucose solution: 100mg of D-glucose was dissolved in 100ml distilled water.

6. Working standard D- glucose solution: 10ml of the stock solution was pipette out into a 100ml volumetric flask and diluted to 100ml with distilled water. This working solution contained 100µg ml-1 of glucose.

Extraction

0.25 g of pea leaf samples weighed and macerated in mortar and pestle in 10 ml of 80% alcohol and centrifuged at 10000 x g for 15 min.

© *2024 AATCC Review.* All Rights Reserved.

Supernatant thus obtained was used as an enzyme extract for estimation of reducing sugar.

Colour development

One ml of enzyme extract was pipette in a test tube and 1 ml of alkaline copper tartrate reagent was added to it. The contents were mixed and heated for 10 minutes in boiling water bath. After cooling, 1 ml of arsenomolybdate reagent was added and the contents were diluted to 8 ml by adding 5 ml distilled water and the intensity of the colour was read at 520 nm.

RESULTS AND DISCUSSION

Peroxidase

Peroxidase activity (Table1) in leaves of healthy and infected *(Erysiphepolygoni)*plants of pea genotype was estimated at 45 (before disease appearance in the plant) and 75 DAS (after disease appearance in plants), respectively. The peroxidase activity was increased in infected plants as compared to healthy plants. Peroxidase activity was increased maximum (80.67%) in diseased plants of Pant P- 498 genotype a followed by IPF 20-17 (78.6%) and after it HFP 9907 B with 74.6% increase noted.

It was increased minimum in Pant P-42 (12.5%), IPF 20-11 (35.38%), Local check (39.7%) and Pant P 501 (50%), VL-72 (60.49%), respectively.

It is clear (Table 1) that genotype Pant P-498, IPF 20-17, HFP 9907 B, showed the lowest disease intensity 9.75, 11.86, 20.89 as these genotypes have the highest amount of peroxidase (2.38, 2.15 and 1.89), in the healthy leaves and infected leaves (4.30, 3.84 and 3.30), respectively. Whereas genotypes like Pant P-42, IPF 20-11, Local check and Pant P 501, VL-72 have low levels of increased peroxidase activity and showed maximum disease intensity (61.87, 60.12, 59.31 and 58.85, 55.30% disease intensity), respectively, were reported.

Total phenol

The phenol content (Table 1) in leaves of healthy and infected *(Erysiphepolygoni)* plant of pea genotypes were estimated at 45 (before disease appearance) and 75DAS (after disease appearance in the plant), respectively. The total phenol content was increased maximum (79.29%) in infected plant of Pant P-498 genotypes followed by IPF 20-17 (78.9%) and it was increased minimum in Pant P-42 (40.35%), IPF 20-11 (45.07%), Local check (49.77%) and Pant P 501 (59.017%), respectively.

It is cleared (Table 1) that genotypes Pant P-498, IPF 20-17 and HFP 9907 B showed was lowest disease intensity of 9.75, 11.86 and 20.89 as these genotypes have highest amount of total phenol (1.83, 1.72 and 1.58mg\g leaf), in healthy and infected leaves got increased (5.10, 4.80 and 4.38mg\g leaf), respectively, those genotypes have higher amount of phenol they reduced diseases and showed resistant against powdery mildew like Pant P-498, IPF 20-17 and HFP 9907 B had high level of increased phenol content and showed minimum disease intensity and other genotypes like Pant P-42, IPF 20-11 and Local check had low level of increased phenol content and showed maximum disease intensity (61.87, 60.12 and 59.31), respectively.

Reducing sugar

Reducing sugar (Table 1) in leaves of healthy and diseased *(Erysiphe polygoni)* plants of pea genotypes were estimated at two times, first at 45 DAS (before disease appearance) and second at 75DAS (after disease appearance), respectively. The reducing sugar was decreased in all the infected plants as compared to healthy plants or reducing sugar was decreased minimum in Pant P-498 (15.7%) followed by IPF 20-17 (16.89%), HFP 9907 B (18.54%) respectively. The reduced sugar was decreased (15 – 31%) in all the infected plants as compared to healthy plants.

The reducing sugar was decreased maximum (31.01%) in infected plants of Pant P-42 followed by IPF 20-11 (29.14%).

It is reported (Table 1) that the reducing sugar was decreased maximum in susceptible genotypes like Pant P-42, IPF 20-11 and Local check.

CONCLUSION

Peroxidase, total phenol and reducing sugar were considered as biochemical relations to develop the disease. The biochemical investigation was carried out to study the host parasite relationship in pea genotypes infected with *Erysiphe polygoni* and found that peroxidase activity was higher in infected genotypes than in healthy genotypes. Low disease incidence was recorded in genotypes showing increased levels of peroxidase activity, and phenol content. Reducing sugar levels decreased minimum in resistant genotypes, this may impart resistant ability of plants to resist against the pathogen.

Table – 1 Biochemical Changes in Pea Due to powdery mildew

S. No.	Name of genotypes	Peroxidase (µmol/min/g)			Phenol (mg/g)			Reducing sugar (mg/g)			#PDI %	Host reaction
		Healthy*	Infected**	Per cent increase	Healthy*	Infected**	Per cent increase	Healthy*	Infected**	Per cent decrease		
1	AMAN	0.83	2.17	61.44	1.35	3.63	68.49	10.57	8.34	26.73	46.79(43.16)	MS
2	HFP 1607	1.85	3.20	72.97	1.48	4.06	74.88	8.67	7.10	22.11	25.01 (30.01)	MR
3	HFP 1702	0.98	2.61	66.32	1.38	3.74	71.8	9.67	7.71	25.42	36.70 (36.70)	MS
4	HFP 9907 B	1.89	3.30	74.6	1.58	4.38	77.74	7.35	6.20	18.54	20.89 (27.20)	MR
5	HUPT 1709	1.87	3.25	73.79	1.52	4.20	76.86	7.89	6.60	19.54	21.25 (27.45)	MR
6	IPF 20- 11	0.65	1.53	35.38	1.10	1.59	45.07	12.94	10.02	29.14	60.12 (50.84)	S
7	IPF 20-17	2.15	3.84	78.6	1.72	4.80	78.9	6.78	5.80	16.89	11.86 (20.14)	MR
8	IPF 20-21	1.73	2.96	71.09	1.43	3.91	73.23	8.98	7.25	23.86	26.29 (30.85)	MS

© 2024 AATCC Review. All Rights Reserved.

9	LOCAL CHECK	0.68	1.63	39.7	1.10	2.75	49.77	12.25	9.55	28.27	59.31 (50.37)	S
10	PANT P 42	0.32	1.32	12.5	0.86	1.20	40.35	13.56	10.35	31.01	61.87 (51.87)	S
11	PANT P 497	0.93	2.45	63.44	1.36	3.65	69.37	10.12	7.99	26.65	42.50 (40.69)	MS
12	PANT P 498	2.38	4.30	80.67	1.83	5.10	79.29	6.19	5.35	15.7	9.75 (18.19)	R
13	PANT P 501	0.72	1.80	50	1.18	3.10	59.017	11.89	9.29	27.98	58.85 (50.10)	S
14	RFP 2010-1	1.86	3.23	73.65	1.49	4.10	75.16	8.12	6.75	20.29	22.96 (28.63)	MR
15	RFPG 180	0.94	2.50	65.95	1.37	3.71	70.4	9.97	7.92	25.88	38.40 (38.29)	MS
16	RFPG 181	0.99	2.66	68.68	1.39	3.78	72.72	9.23	7.42	24.39	35.89 (36.89)	MS
17	VL 72	0.81	2.11	60.49	1.22	3.20	62.94	11.05	8.70	27.01	55.30 (48.04)	S
	SEm+	0.03	0.06		0.03	0.08		0.33	0.20		1.14	
	CD (P=0.05)	0.09	0.18		0.09	0.24		0.94	0.58		3.27	

*Leaves collected at 45 DAS (before disease appearance)
**leaves collected at 75 DAS (after disease appearance)
#Average of three replication, figure in parenthesis are angular transformed values

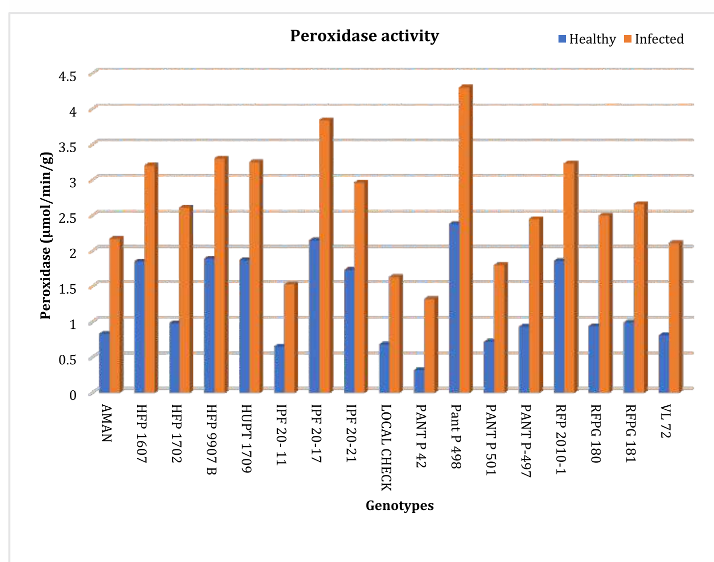

Fig-1 Biochemical changes (peroxidase activity) in leaves of healthy and infected (Erysiphepolygoni) plants of pea genotypes

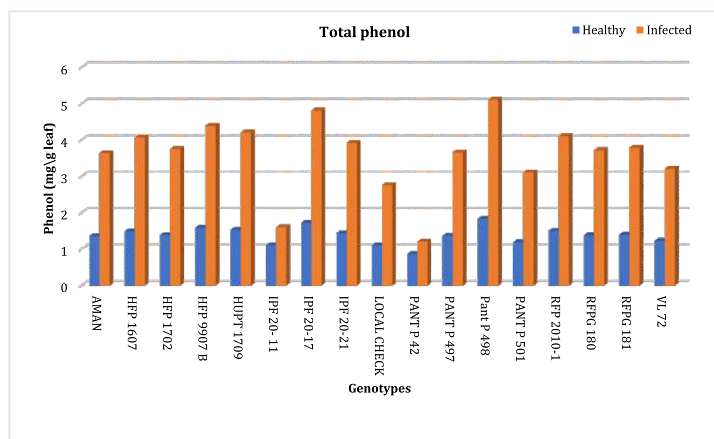

Fig-2 Biochemical changes (Total phenol) in leaves of healthy and infected (Erysiphepolygoni) plants of pea genotypes

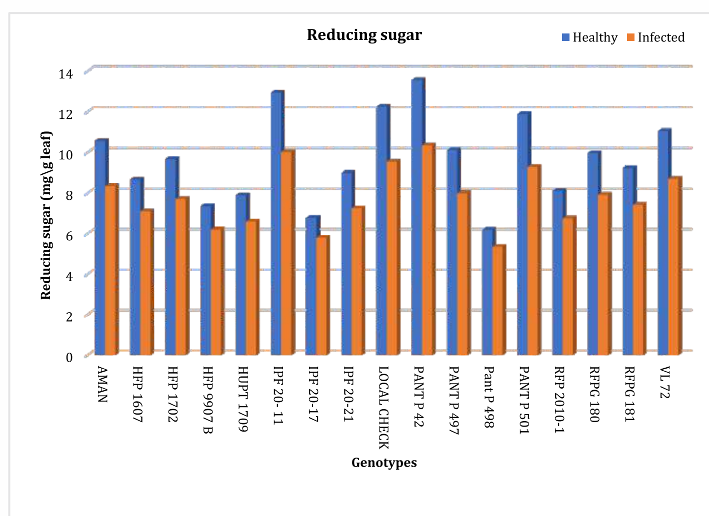

Fig-3 Biochemical changes (Reducing sugar) in leaves ofhealthy and infected (Erysiphepolygoni) plants of peagenotypes

REFERENCES

1. Duke JA (1981) Hand book of legumes of world economic importance. Plenum Press, New York Phytopathlogy: 199-265

2. Hules JH (1984) Nature, composition and utilization of food legumes. pp. 77-97. In: Expanding the production and use of cool season food legumes. (Eds.): F.J. Muehlbauer and W.J. Kasir.Kluwer Acad. Pub. Dordrech, Netherland.

3. Khosla HK, Naik SL, Mandloi SC, Goray SC (1988) Control of powdery mildew of mung and urid in relation to losses and disease development. Indian Phytopath. 41 (1): 59-63

4. Kumar KB, Khan PA (1982) Peroxidase and polyphenol oxidase in excised ragi leaves during senescence. Indian Journal of Experimental Botany., 20: 412-416

 © 2024 AATCC Review. All Rights Reserved.

5. Ma Z, Boye JI, Hu X (2018) Nutritional quality and techno functional changes in raw, germinated and fermented yellow field pea (*Pisumsativum* L.) upon pasteurization. LWT-Food Science and Technology, 92, 147–154

6. Malik CP, Singh MB (1980) Plant Enzymology and Histo-Enzymology: A text manual. Kalyani Publication, New Delhi/ Ludhiana., pp. 434

7. Melchior S, Calligaris S, Bisson G, Manzocco L. (2020) Understanding the impact of moderate-intensity pulsed electric fields (MIPEF) on structural and functional characteristics of pea, rice and gluten concentrates. Food and Bioprocess Technology, 13(12), 2145–2155

8. Pramod P, Dwivedi SN (2007) Fungicidal management of field pea (*Pisumsativum* L.) powdery mildew caused by *Erysiphepolygoni* DC. Progressive Research, 2(1/2): 116-118

9. Somogyi M (1952) Notes of sugar determination. Journal Biological Chemistry., 195: 1-23

10. Surwase AG, Badgire DR, Suryawanshi AP (2009) Management of pea powdery mildew by fungicides, botanicals and bioagents. Ann. PI. Protec. Sci., 17 (2): 384-388

11. Tiwari KR, Penner GA, Warkentin TD, Rashid KY (1997) Pathogenic variation in Erysiphe pisi, the causal organism of powdery mildew of pea. Can J Plant Pathol 19:267–271

© *2024 AATCC Review.* All Rights Reserved.