Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

EFFECT OF LATE BLIGHT CAUSED BY *PHYTOPHTHORA INFESTANS* ON PHENOLIC CONTENTS OF POTATO ADVANCED LINES/CULTIVARS

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A B S T R A C T

Phenolic compounds are present in the healthy plants at concentrations sufficient to inhibit growth and sporulation of a pathogen; as a response to infection, their concentration is markedly increased imparting resistance against the invading microorganisms and phytoalexins which are not normally found in healthy plants, are synthesized or increase in amounts after infection. We have tested and found that there was a marked increase in the total phenols in all the 50 lines/cultivars due to late blight appearance as compared to the healthy ones. Results revealed that there was overall increase in the quantity of total phenols in all tested lines after disease appearance and this increase was ranged from 28.1 to 58.6 percent over the healthy plants of the same age group. Maximum increase in phenolic content after disease appearance was 58.6 percent in potato line FD 8-1 while minimum phenol increased in 28.1 percent in line 9619. This increase was also noted in the healthy potato plants which were not infected by the disease but this increase might be due to aging factor, was quite insignificant and unnoticeable. Although the total phenolic content was slightly increased more in those plants which were not diseased than that of healthy plants which were tested at the time when disease did not appear. It is apparent from the above figures that the increase in total phenols was more pronounced in diseased plants as compared to the healthy ones.

Keywords: Potato, late blight, resistance, phenols, disease index, disease severity.

INTRODUCTION

Late blight of potato can be successfully controlled by a combination of sanitary practices, resistant varieties and fungicide sprays. The ideal way to combat this disease is through cultivars resistant to this disease. Cultivation of resistant varieties is the most economic and valid option in the disease controlling strategies. Availability of the resistant cultivars is scanty and calls for extensive screening of potato germplasm against late blight of potato. None of the commercial varieties is found resistant to *P. infestans.* Many new varieties/advanced lines have been developed by breeders during the last few years which are required to be continuously tested for presence of resistance (Parvez *et al.,* 2003). Genetic resistance of potato cultivars can be utilized to lower the fungicide rates and the most resistant cultivars can be protected with half the rate of fungicide (Kapsa, 2002).

There are numerous reports on the role of phenolics (Kosuge, 1969) and Phytoalexins (Baily and Mansfield, 1982) in contributing resistance to the plants, by a number of host-parasite interactions. These substances act in the chemical defence of higher plants mainly in three ways. First, they are present in the healthy plants at concentrations sufficient to inhibit growth and sporulation of a pathogen (generally referred to as pre-formed resistance factors); Secondly, as a response to infection, their concentration is markedly increased imparting resistance against the invading microorganisms; thirdly, certain post-infection products (phytoalexins), which are not normally found in healthy plants, are synthesized or increase in amounts after infection (Manibushanrao *et al*., 1988). Phenolic compounds are essential for the growth as well as to confer resistance against plant pathogens as

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defense mechanism (Agrios, 2005). It has been established that phenolics and flavanoids are among the most frequent and widely distributed secondary metabolites in plant kingdom (Wahid and Ghazanfar, 2006). Deposition of phenolic compounds on epidermal cells was a general mechanism of resistance in potato leaves but it does not have any relation with resistance to penetration by *P. infestans*. Phenolic deposition was equally present in resistant as well as susceptible cultivars but was less in susceptible cultivars (Covarrubias, *et al.,* 2006).

Deposition of phenolic compounds on epidermal cells was a general mechanism of resistance in potato leaves but it does not have any relation with resistance to penetration by *P. infestans*. Phenolic deposition was equally present in resistant as well as susceptible cultivars (Covarrubias, *et al.,* 2006).

Level of salicylic acid in leaf tissues and chlorogenic acid and P-Cumaric acid at disease initiation stage was higher in leaf and root tissues of resistant varieties (Zeng, 2006). Hydroquinone and umbelliferone were present in higher amount in stem tissue of resistant varieties as compared to susceptible ones. Presence of different quantities of phenolic compounds in resistant and susceptible varieties suggested that phenols have active role in the resistance mechanism (Zeng, 2006).

Phenolic compounds are essential for the growth as well as to confer resistance against plant pathogens as defense mechanism (Agrios, 2005). It has been established that phenolics and flavonoids are among the most frequent and widely distributed secondary metabolites in plant kingdom (Wahid and Ghazanfar, 2006). Deposition of phenolic compounds on epidermal cells was a general mechanism of resistance in potato leaves but it does not have any relation with resistance to penetration by *P. infestans*. Phenolic deposition was equally present in resistant as well as susceptible cultivars (Covarrubias, *et al.,* 2006). Limited or no work has been done to see the role of phenolic compounds, which are produced as a result of pathogen attack.

MATERIALS AND METHODS

Experimental Details and treatments: Total phenols were tested from three different samples taken at different times, first after 50 days of planting of the crop when there was no disease appeared while second samples were taken from healthy and diseased plant individually, almost 30 days after the first appearance of the symptoms on late blight. Total soluble phenols were determined from leaf samples 0.5 g of each sample) of both healthy and diseased plants essentially according to the method of Julkunen-Tiitto (1985). Fresh potato leaf samples from healthy plants were taken after 50 days of sowing. Then waited for the appearance of the disease naturally and sampling was done almost 30 days of disease appearance both from diseased and healthy ones separately. These samples were weighed individually (0.5 g of each sample), frozen, and were ground in liquid nitrogen using mortar and pestle. The powder was scooped into 5 mL polystyrene tubes with cap. Extraction was carried out with 2mL of 80% acetone for 1 h at 50 °C in a water bath. Centrifuged for 10 min at 12,000 rpm and supernatant were taken in microfuge tube and stored at –20°C until used. Fifty microlitre (50 µL) of extract was diluted to 1 mL with distilled water in a 10 mL capacity test tube and mixed with 0.5 mL of 2 M Folin-Ciocalteau's phenol reagent (Sigma) and 2.5 mL of 20 % Na_2CO_3. The mixture was allowed to stand for 20 minutes at room temperature and then the absorbance of the samples was measured at 750 nm using the Hitachi U-2001 spectrophotometer, model 121-0032. The phenol concentration was determined from a standard curve prepared with Gallic acid. This experiment was run using four replications and individual sample was taken from at least 8 to 10 plants.

RESULTS AND DISCUSSION

Total phenols were tested from three different samples taken at different times, first after 50 days of planting of the crop when there was no disease appeared while second samples were taken from healthy and diseased plant individually, almost 30 days after the first appearance of the symptoms on late blight. The data regarding the total phenols of 50 potato lines/ cultivars is given in (Table 1). Results of present study revealed that there was overall increase in the quantity of total phenols in all tested lines after disease appearance and this increase was ranged from 28.1 to 58.6 percent over the healthy plants of that same age group. Maximum increase in phenolic content after disease appearance was 58.6 percent in potato line FD 8-1 while minimum phenol increased in 28.1 percent in line 9619.

This increase was also noted in the healthy potato plants which were not infected by the pathogen but this increase might be due to aging factor, was quit insignificant and unnoticeable.

Table 1. Total Phenols (µg g-1 fresh weight) lines/cultivars of Potato.

Lines/ Cultivars	Before disease appearance		After disease appearance				Per cent increase over the healthy plants
	Healthy Plants		Healthy Plants		Diseased Plants		
9619	65.86	a	68.41	a	96.93	a	28.1
CARDINAL	38.53	jklm	41.96	klmn	72.94	hijk	42.4
FD 1-10	46.83	cdefghijk	50.1	cdefghijk	81.24	bcdefghij	38.3
FD 1-3	40.53	ijklm	43.61	jklmn	74.94	fghij	41.7
FD 3-10	44.13	efghijkl	47.39	defghijklm	78.54	cdefghij	38.5
FD 32-2	57.73	b	60.72	b	92.14	ab	34.0
FD 35-25	47.43	cdefghijk	50.34	cdefghijk	81.84	bcdefghij	38.4
FD 35-36	48.26	cdefghij	51.01	cdefghijk	82.67	bcdefghi	38.3
FD 37-13	41.6	hijklm	44.52	hijklmn	76	efghij	41.4
FD 3-9	49.33	bcdefgh	52.15	bcdefghij	83.74	bcdefgh	37.1
FD 48-54	47.03	cdefghij	50.43	cdefghijk	81.44	bcdefghi	37.5
FD 49-28	49.83	bcdefgh	52.79	bcdefghi	84.24	bcdefg	37.0
FD 49-62	41.43	ghijklm	43.95	ghijklmn	75.84	defghij	41.3
FD 51-5	46.93	cdefghij	49.99	cdefghijk	81.34	bcdefghi	38.0
FD 51-6	52.53	bcdef	55.71	bcdef	86.94	bcdef	35.9
FD 52-2	55.46	bc	58.67	bc	89.87	abc	34.7
FD 53-6	42.66	ghijklm	45.18	ghijklmn	77.07	defghij	41.3
FD 53-7	44.4	efghijklm	47.18	fghijklm	78.8	cdefghij	40.1
FD 56-1	54.69	bcd	58.35	bcd	89.1	abcd	34.4
FD 61-3	39.1	ijklm	42.06	klmn	73.5	ghij	42.3
FD 63-2	47.13	cdefghijk	50.65	cdefghijk	81.54	bcdefghij	37.8
FD 63-4	44.06	efghijkl	46.36	fghijklm	78.47	cdefghij	40.1
FD 64-2	52.36	bcde	55.09	bcdef	86.77	abcde	36.4
FD 65-4	36.36	lm	39.62	mn	70.77	ijk	43.5
FD 65-6	48.26	cdefghij	50.69	cdefghijk	82.67	bcdefghi	38.6
FD 69-1	18.3	p	20.89	q	36.71	n	49.1
FD 70-1	47.8	cdefghij	50.66	cdefghijk	82.2	bcdefghi	38.3
FD 71-1	14.17	p	16.8	q	31.17	o	46.6
FD 76-59	54.36	bc	56.66	bcde	88.77	abc	36.0
FD 8-1	19.46	op	22.27	pq	53.87	m	58.6
FD 8-3	44.5	efghijklm	47.14	fghijklmn	78.9	cdefghij	40.2
FSD RED	48.5	cdefghi	51.7	bcdefghij	82.9	bcdefghi	37.6
FSD White	50.23	bcdefgh	53.13	bcdefgh	84.64	bcdefg	36.4
KARODA	45.8	defghijkl	49.38	cdefghijklm	80.2	cdefghij	38.3
MARATO	24.26	o	27.25	p	58.67	lm	53.4
N- 18	51.76	bcdefg	54.6	bcdefg	86.17	bcdefg	36.5
N- 22	42.33	fghijklm	45.65	fghijklmn	76.74	cdefghij	40.0
N- 30	46.1	cdefghijk	49.3	Cdefghijkl	80.5	bcdefghij	38.7
N- 37	42.96	fghijklm	45.95	Fghijklmn	77.37	defghij	40.5
N- 8	50.63	bcdefg	54.11	Bcdef	85.04	bcdefg	35.7
N- 13	43.3	efghijklm	45.96	Fghijklmn	77.7	cdefghij	40.9
N- 34	37.2	klm	39.7	Lmn	71.6	hijk	44.0
N- 39	43.53	fghijklm	46.83	Fghijklmn	77.94	cdefghij	39.9
RODIO	30.16	n	33.25	O	64.57	kl	48.5
SH- 692	54.53	bcd	57.57	Bcde	88.94	abcd	35.2
SH 788	39.36	ijklm	42.69	Ijklmn	73.77	fghij	41.4
SH-5	44.76	efghijklm	48.17	Efghijklm	79.17	cdefghij	39.1
SH-704	51.33	bcdefgh	53.45	Bcdefghij	85.74	bcdefg	37.6
SHANAN	35.76	M	38.18	No	70.17	jk	45.6
SIPLY RED	14.27	p	17.3	Q	26.61	o	33.8
CV	7.176%/		6.857%/		5.034%/		

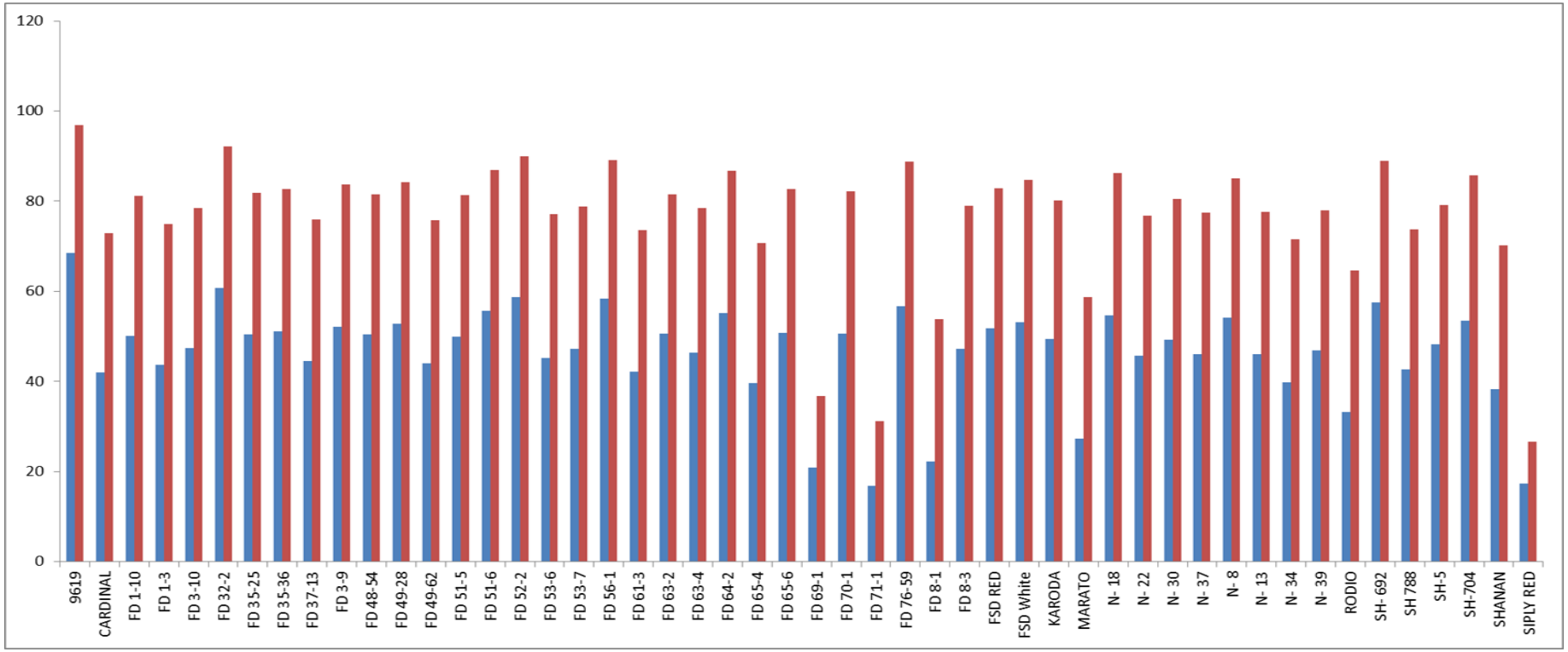

Figure 1. Total Phenols (µg g^{-1} fresh weight) healthy and diseased plants of potato lines/cultivars

This increase in phenols of diseased plant of all the lines was statistically significant. All though the total phenolic content were slightly higher in the plants which were not diseased than that of healthy plants which were tested at the time when disease was not appeared. It is apparent from the above figures that the increase in total phenols was more pronounced in case of diseased plants as compared with the healthy ones. These phenols were produced in reaction to the infection by the *Phytophthora infestans* not due to the aging. If some part of this quantity was produced due to aging factor was quite insignificant and unnoticeable. Our results are confirming the results of Reddy and Khare, 1984; Parashar and Sindhan, 1986; Jamil *et al.,* 1990; Randhawa, 1994 who found phenolic compounds were accumulated in the resistant cultivars due to host-parasite interactions.

Sahi et al. (2000) found that all the phenolic compounds may not impart role in resistance to plant pathogens but some of them may favour disease development on the other hand as well. As a result of inoculation with the pathogen, there was significant increase in the content of total phenols in both the reaction groups, increase being more pronounced in the lentil lines of resistant groups as compared with the susceptible group. There are numerous reports on the role of phenolics (Kosuge, 1969; Mehta and Mehta, 1989) and Phytoalexins (Baily and Mansfield, 1982) in contributing resistance to the plants, by a number of host-parasite interactions. Role of phenolics and Phytoalexins is too much prominent in contribution to resistance of host plants against parasite (Baena *et.al.* 2007).

These substances act in the chemical defense of higher plants mainly in three ways. First, they are present in the healthy plants at concentrations sufficient to inhibit growth and sporulation of a pathogen (generally referred to as pre-formed resistance factors); Secondly, as a response to infection, their concentration is markedly increased imparting resistance against the invading microorganisms; Finally, certain post-infection products (phytoalexins), which are not normally found in healthy plants, are synthesized or increase in amounts after infection (Manibushanrao et al., 1988).

Phenolic compounds are essential for the growth as well as to confer resistance against plant pathogens as defense mechanism (Agrios, 2005). It has been established that phenolics and flavanoids are among the most frequent and widely distributed secondary metabolites in plants kingdom (Wahid and Ghazanfar, 2005). According to a large number of reports the role of phenolics and Phytoalexins is too much prominent in contribution to resistance of host plants against parasite (Baena *et.al.* 2007). Phenolic compounds are essential for the growth as well as to confer resistance against plant pathogens as defense mechanism (Agrios, 2005).

Deposition of phenolic compounds on epidermal cells was a general mechanism of resistance in potato leaves but it does not have any relation with resistance to penetration by *P. infestans* (Covarrubias, *et al.,* 2006). It has been established that phenolics and flavanoids are among the most frequent and widely distributed secondary metabolites in plants kingdom (Wahid and Ghazanfar, 2006). Deposition of phenolic compounds on epidermal cells was a general mechanism of resistance in potato leaves but it does not have any relationship with resistance to penetration by *P. infestans*. Phenolic's deposition was equally present in resistant as well as susceptible cultivars (Covarrubias, *et al.,* 2006).

More production of phenolic compounds in diseased as compared to healthy potato plants was due to the interaction between the plant and pathogen as plant produce different type of compounds in reaction to *Phytophthora infestans* infection to safe guard itself so it is evident from our results that this increase was not due to aging of the plant. If some part of this quantity was produced due to aging factor was quite insignificant and unnoticeable. Further studies would be carried out to assess the quantity of phenols in resistant and susceptible lines/ cultivars at different stages of plant growth and disease development to establish the relationship between the quantity of phenols and resistance of the lines/cultivars individually.

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