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Biochemical Defense Mechanism in Potato Against Stem Canker and Black Scurf Disease

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Abstract: Potato cultivars (*Solanum tuberosum*) differed in their susceptibility to stem canker and black scurf disease caused by *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris* [Frank] Donk*)*. Disease reactions were determined for nine commercial potato cultivars under greenhouse conditions. The nine tested cultivars were divided to three groups. Lady Rosetta was the most susceptible cultivar followed by Monalisa and Mondial cvs. Spunta, Nicola and Hermes cultivars showed moderate susceptibility, While Draga followed by Cara and Diamont were the most resistant. The two potato cultivars Draga, the resistant cv. and lady Rosetta, the highly susceptible were selected to study the nature of disease resistance among potato cultivars. Fraction of phenolic compounds by HPLC, oxidative enzymes and thio-amino acid contents were determined in leaves and roots of these potato cultivars. The resistant Draga cultivar showed the highest amounts of free, conjugated and total phenols, fraction of phenolic compound (Coumarin, Caffic acid, p-Coumaric acid, Benzoic acid, Resorcinol and Apigenin), oxidative enzymes (peroxidase and polyphenoloxidase) and thio amino acids (Cysteine, Cystine and Methionine) compared with the highly susceptible lady Rosetta.

Key words: Potato, *Thanatephorus cucumeris*, thio-amino acids, phenolic compound, HPLC, varietal resistance

INTRODUCTION

Misaghi (1982) recorded that phenolic compounds, their oxidative products (quinones) and derivatives (certain phytoalexins) exhibited antibiotic properties and therefore were considered by some to play a role in disease resistance. Halloin (1994) found that a histochemical test employing nitrous acid, that produces red nitroso derivatives or some phenolic compounds was modified for use in the field and was used to demonstrate localization or phenolic compounds in healthy and *Rhizoctonia solani* infected sugar beet (*Beta vulgaris*) plants. Nitroso-reactive materials were present in petioles and around mature crown cavities or healthy plants. They were absent from tap roots, except that they occasionally were observed in lateral root tissue contained within tap roots.

Infection of tolerant/susceptible lentil (*Lens esculentum*) cultivars by *Fusarium oxysporum*, *F. solani*, *Sclerotium bataticola* and *Rizoctonia solani* led to a considerable increment in thio (Sulphur) amino acids, methionine, cystine and cysteine were obtained in all tested cultivars as a result of infection. The lowest decrease in thio-amino acids was recorded after infection in susceptible cultivars compared with the tolerant and moderately susceptible cultivars, El-Shaer (2002) found that.

Paranidharan *et al.* (2003) recorded that changes in the activities of peroxidase, ascorbate peroxidase, catalase and superoxide dismutase in rice in response to infection by *R. solani* were studied. A significant increase in peroxidase activity was observed in *R. solani* inoculated rice leaf sheaths 1 day after inoculation and the maximum enzyme activity was recorded 3 days after inoculation at which period a 3 fold increase in peroxidase activity was observed compared to the untreated control. Ascorbate peroxidase and catalase activities significantly increased 1-2 day after inoculation and the maximum enzyme activities were recorded 5 days after inoculation.

The aim of the present work was to study the cultivar reactions of nine common commercial cultivars of potato to infection with *Rhizoctonia solani* under controlled greenhouse conditions. Fractions of phenolic compounds by HPLC, oxidative enzymes and thio amino acid contents in the described resistant and susceptible potato cultivars were determined.

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MATERIALS AND METHODS

Varietal reactions under greenhouse conditions: Nine potato cultivars were screened for their reaction to black scurf and stem canker disease. The tested cultivars were Draga, Cara, Diamont, Hermes, Nicola, Spunta, Mondial, Monalisa and Lady Rosetta. Pure cultures of the selected *R. solani* isolates (10 days old) were used for cultivars reaction tests. Soil infestation was conducted with inocula of each isolate propagated on corn-meal sand medium. Loamy soil was sterilized with formalin solution 5% and left for two weeks then soil infestation was carried out at the level of 5% inoculum. Potato tubers of the nine cultivars were surface sterilized by immersing in 0.2% Sodium hypochlorite for 2 min and planting in pots was made in diameter 50 cm containing 1 tubers (Ranging in size between 5-7.5 cc) and soil previously infested with *R. solani* (Anastomosis group AG-3 and was obtained from Plant Pathology Research Institute, ARC, Egypt)with 4 replicates under greenhouse conditions were planted with each tested cultivar. Infection percentage was recorded 90 days after planting.

Biochemical studies: Biochemical studies associated with healthy and inoculated plants with *R. solani* on two cultivars Draga, the low susceptible cv. and Lady Rosetta, the highly susceptible cv. were carried out. The investigated parameter included changes in phenolic compounds, fraction of phenolic compounds, oxidative enzymes and fraction of thio amino acid at 20, 40 and 60 days after planting.

Determination of the phenolic compounds content: The phenolic compounds were determined in leaves and roots of the tested cultivars of potato which were inoculated separately with isolate of *R. solani* to determine total, free and conjugated phenolic compounds. The phenolic compounds content was colorimetrically determined using the folin reagent according to Snell and Snell (1953).

Fractionation of phenolic compounds: HPLC system (HP1050) was used to detect and to determine some phenolic compounds from the plant tissue (Coumarin, Caffic acid, p. coumaric acid, Benzoic acid, Resoricinal and Apegenin). Ten gm of fresh tissues of each sample were homogenized with methanol 40% and stirred on a shaker. The extract was filtered through a whatman filter paper No. 1 and the solvent was evaporated in vacuum. The dried residues containing phenol compounds were dissolved in a solution consists of methanol: water: Acetic acid, 40: 59.3: 0.7, v: v: v) and stored in vials. The separation and determination were performed on C18 column, according to Gertz (1990).

Determination of oxidative enzymes: The oxidative enzymes were determined in leaves and roots of the tested cultivars of potato which were inoculated separately with isolate of *R. solani* to determine peroxidase (PO) and polyphenoloxidase (PPO) activity. The enzyme extraction from all samples was prepared as recommended by Maxwell and Bateman (1967). Peroxidase activity was estimated according to the method of Allam and Hollis (1972) and the activity of polyphenoloxidase was measured using the colorimetric method of Maxwell and Bateman (1967).

Fractionation of thio amino acids: HPLC system (HP1050) was used to detect and determine thio amino acids from the plant tissue (Cysteine, cystine and Methonine). Free amino acids were extracted according to the method proposed by Shad *et al.* (2002). The separation and determination were performed on C18 column according to Gertz (1990).

Statistical analysis: The statistical analysis was computed using analysis of variance procedure described by Snedecor and Cochran (1980). The significant differences between treatment means were separated by Duncan,s Multiple Range Test (Duncan, 1955).

RESULTS

Reactions of different potato cultivars to *R. solani*: Nine Egyptian potato cultivars, i.e., Diamont, Draga, Hermes, Cara, Lady Rosetta, Monalisa, Mondial, Nicola and Spunta were evaluated, under greenhouse conditions, for their reactions against the most pathogenic *R. solani* Sharkia's isolates code No. 18. Data presented in Table 1 show significant differences between nine cultivars. The highest percentage of infection by stem canker was obtained on Lady Rosetta (18.6%) and followed by

Table 1: Reactions of potato cultivars against the highly pathogenic *Rhizoctonia solani* isolate under green house conditions

	Percentages of infection (Mean±SE)	
Cultivars	Stem canker	Black scurf
Draga	05.3±0.386^{f}	03.3±0.407^{d}
Kara	06.4±0.450^{f}	03.5±0.418^{d}
Diamond	06.6±0.438^{f}	05.0±0.581^{cd}
Hermez	08.4±0.669^{e}	05.3±0.684^{c}
Nicola	10.1±0.526^{d}	06.8±0.584b^{c}
Spounta	13.5±0.785^{c}	06.6±0.669^{bc}
Mondial	16.5±0.787^{b}	08.3±0.697^{b}
Monalisa	18.1±0.475^{a}	09.6±0.584^{a}
Lady Rosetta	18.6±0.515^{a}	10.3±0.493^{a}
$LSD_{0.05}$	1.706	1.709

Each value represents the Mean±SE (Standard Error) and mean of 4 replicates, Values in the same column with the same letter are not significant at ($p \leq 0$)

Monalisa (18.1%). The lowest percentage of infection was obtained with Draga (5.3%), Cara (6.4%) and Diamont (6.6%). On the other hand, the same trend was observed in reaction to black scurf symptom for the disease. The highest percentage of infection by black scurf was obtained on Lady Rosetta (10.3%) followed by Monalisa (9.6%). The lowest percentage of infection was obtained for Draga (3.3%) and Cara (3.5%).

Biochemical changes in susceptible and resistant potato cultivars:

Phenolic contents: Data presented in Table 2, reveal that leaves and roots attained higher levels of free, conjugate and total phenol contents in inoculated plants compared with uninoculated ones, in roots compared to leaves and in resistant cultivar (Draga) compared with susceptible cultivar (Lady Rosetta). Twenty days after inoculation the free, conjugated and total phenols content were higher in inoculated treatments compared to the uninoculated plants in each of Draga and Lady Rosetta cultivars. Forty days after inoculation, similar trend was observed, high content of free (9.0 mg), (7.7 mg), conjugate and total phenols (16.7) in Draga. The corresponding figures in healthy treatments were 8.3, 7.0 and 15.3 mg, respectively. In Lady Rosetta the same trend was observed in the inoculated leaves and root. Sixty days after inoculation, high content of free, conjugate and total phenols in Draga was observed being 9.6, 7.6 and 17.2 mg, respectively. The corresponding figures were 9.1, 6.8 and 15.9 mg, respectively. In Lady Rosetta the same trend was observed in the inoculated leaves and root.

Fractionation of phenolic compound: Data in Table 3, reveal that leaves attained higher levels of six phenolic compounds in inoculated plants compared to uninoculated ones. Twenty days after inoculation coumarin and caffic acid content were higher in inoculated leaves compared to the uninoculated ones in each of Draga and Lady Rosetta cultivars. Draga, the resistant cv, had higher percentage of phenolic compound than that in Lady Rosetta i.e., the susceptible cv.

Forty days after inoculation, a different trend was observed as the appearance of four phenolic compound (p-coumaric acid, benzoic acid, resorcinol and apigenin) in Draga and two phenolic compound (p-coumaric acid, benzoic acid) in Lady Rosetta were recognized. High percentage of p-coumaric acid and Benzoic acid in inoculated Draga leaves that showed 6.79 and 0.92 mg, compared to the uninoculated healthy ones 4.83 and 0.74 mg, respectively. In Lady Rosetta the same trend was observed in the inoculated leaves. Sixty days after inoculation, trend similar to that of 40 days was observed as the appearance of four phenolic compounds (p-coumaric acid, benzoic acid, resorcinol and apigenin) in Draga and two phenolic compounds (p-coumaric acid, benzoic acid) in Lady Rosetta. High percentage of p-coumaric acid and benzoic acid in Draga the inoculated leaves showed 2.87 and 0.59 mg, respectively than the uninoculated 1.92 and 0.48 mg, respectively. In Lady Rosetta the same trend was observed in the inoculated leaves but the amount and its increase were limited, compared to Draga cultivar.

Table 2: Free, conjugate and total phenol contents in inoculated and uninoculated resistant potato cv. Draga (R) and susceptible cv. Lady Rosetta (S) cultivars 20, 40 and 60 days after sowing under green house conditions

		Phenols content (mg g^{-1} fresh weight)					
		Draga (R)			Lady Rosetta (S)		
Days	Sample	Free	Con.	Total	Free	Con.	Total
20							
Inoculated	Leaves	7.9	6.9	14.8	6.1	5.8	11.9
	Root	109	37.0	146.0	65.0	27.0	92.0
Uninoculated	Leaves	7.2	6.3	13.5	5.6	5.7	11.3
	Root	62	33.0	95.0	36.0	42.0	78.0
40							
Inoculated	Leaves	9.0	7.7	16.7	6.6	6.6	13.2
	Root	168	66.0	234.0	125.0	44.0	169.0
Uninoculated	Leaves	8.3	7.0	15.3	5.9	6.5	12.4
	Root	124	46.0	170.0	94.0	41.0	135.0
60							
Inoculated	Leaves	9.6	7.6	17.2	6.9	7.4	14.3
	Root	246	49.0	295.0	173.0	73.0	246.0
Uninoculated	Leaves	9.1	6.8	15.9	6.2	7.3	13.5
	Root	189	78.0	267.0	123.0	81.0	204.0

Table 3: Phenolic compounds in inoculated and uninoculated resistant potato cv. Drage and susceptible cv. Lady Rosetta 20, 40 and 60 days after sowing under green house conditions

	Relative concentration (%) of phenolic compounds											
	Draga (R)						Lady Rosetta (S)					
	20 days		40 days		60 days		20 days		40 days		60 days	
Phenolic Compound	I*	H**	I	H	I	H	I	H	I	H	I	H
Coumarin	23.6	11.40	35.2	23.50	19.3	13.5	8.99	5.62	16.1	11.8	8.16	7.53
Caffic acid	8.55	6.91	17.9	12.4	11.2	8.63	3.46	2.87	6.83	4.77	2.90	1.48
p-coumaric acid	-	-	6.79	4.83	2.87	1.92	-	-	3.09	2.45	1.64	0.82
Benzoic acid	-	-	0.92	0.74	0.59	0.48	-	-	0.62	0.55	Tras.	Tras.
Resorcinol	-	-	0.87	0.69	0.71.	0.57.	-	-	-	-	-	-
Apigenin	-	-	0.81	0.52	0.62	0.43	-	-	-	-	-	-

I*: Inoculated, H**: Healthy (uninoculated)

Peroxidase and polyphenoloxidase activities: Data presented in Table 4, show that roots exhibited higher levels of PO and PPO activities than shoots in all sampling and in inoculated and uninoculated treatments. The activities of PO and PPO were higher in either inoculated leaves or roots compared to the healthy uninoculated plants, 20 days after inoculation in Draga and Lady Rosetta cultivars. The resistant Draga, the resistant cultivar, had higher levels of activities of PO and PPO than the susceptible Lady Rosetta one.

Forty days after inoculation, the PO and PPO activities in Draga were higher in root samples than in the leaves after inoculation. In Lady Rosetta, the inoculated leaves showed higher PO activity (2.52 mg) than the healthy uninoculated one (2.23 mg). On the other hand, PPO activities were almost alike in inoculated and uninoculated plants either in leaves or root samples. Sixty days after inoculation, in Draga the activity of PO in inoculated leaves was higher (2.68 mg) than that in uninoculated leaves (2.47 mg) and the same trend in inoculated and uninoculated roots. In Lady Rosetta the activity of PPO in inoculated leaves was higher (2.48 mg) than that in healthy uninoculated leaves (2.27 mg) and the same trend in inoculated and uninoculated roots. Generally, activity of PO was higher in the 3 intervals and in inoculated and uninoculated samples as well the activity of PPO showed a tendency of an increase but to a limited extent.

Thio-amino acids fractionation: Three thio-amino acids cysteine, cystine and methionine were chromatographically identified in the leaves and root of Draga and Lady Rosetta cultivars using the High Performance Liquid Chromatography (HPLC) technique. These thio-amino acids cysteine, cystine and methionine were determined at 20, 40 and 60 days after inoculated and uninoculated plants with *R. solani*. Data presented in Table 5 reveal that leaves and roots showed higher levels of thio-amino acids cysteine, cystine and methionine content in inoculated plants compared to uninoculated ones. Twenty days after inoculation, the thio-amino acids content were higher in inoculated leaves or roots compared to the uninoculated plants in each of Draga and Lady Rosetta cultivars. Forty days after inoculation, a similar trend was observed as higher content of cysteine, cystine and methionine in Draga, the inoculated leaves showed activity 0.78, 0.64 and 0.59 mg, respectively compared to the healthy uninoculated 0.67, 0.55 and 0.42 mg, respectively. The same trend was

Table 4: Activity of peroxidase and polyphenoloxidase in inoculated and uninoculated Draga, the resistant cv. and Lady Rosetta, the susceptible one with *R. solani* 20, 40 and 60 days after inoculation

		Activity (min^{-1})			
		Draga		Lady Rosetta	
Days	Sample	PO*	PPO**	PO	PPO
20					
Inoculated	Leaves	2.45	0.09	2.16	0.06
	Root	3.74	0.11	3.44	0.08
Uninoculated	Leaves	2.23	0.07	1.92	0.05
	Root	3.54	0.09	3.17	0.07
40					
Inoculated	Leaves	2.88	0.12	2.52	0.10
	Root	4.21	0.15	3.74	0.12
Uninoculated	Leaves	2.31	0.08	2.23	0.07
	Root	3.34	0.13	3.16	0.11
60					
Inoculated	Leaves	2.68	0.08	2.48	0.06
	Root	3.50	0.12	3.25	0.11
Uninoculated	Leaves	2.47	0.05	2.27	0.05
	Root	3.26	0.10	3.09	0.10

PO*: Peroxidase, PPO**: Polyphenoloxidase

Table 5: Relative concentration of cysteine, methionine and cystine in inoculated or uninoculated potato cultivars differed in their susceptibility to *R. solani* 20, 40 and 60 days after sowing under green house conditions

		Relative concentration (%) of thio-amino acids					
		Draga			Lady Rosetta		
Days	Sample	Cysteine	Cystine	Methionine	Cysteine	Cystine	Methionine
20							
Inoculated	Leaves	0.67	0.51	0.36	0.41	0.27	0.33
	Root	7.65	6.71	2.35	2.84	1.36	3.12
Healthy	Leaves	0.57	0.41	0.28	0.29	0.17	0.21
Uninoculated	Root	4.34	3.52	1.84	1.47	0.91	2.55
40							
Inoculated	Leaves	0.78	0.64	0.59	0.49	0.32	0.45
	Root	8.71	8.64	2.52	2.67	2.11	4.37
Healthy	Leaves	0.67	0.55	0.42	0.38	0.24	0.32
Uninoculated	Root	6.74	4.61	1.96	1.91	1.23	3.19
60							
Inoculated	Leaves	0.51	0.41	0.29	Tras	Tras	Tras
	Root	2.95	Tras	1.15	Tras	Tras	Tras
Healthy	Leaves	0.43	0.36	0.22	Tras	Tras	Tras
Uninoculated	Root	2.87	Tras	0.92	Tras	Tras	Tras

observed in the inoculated roots. In Lady Rosetta, similar conclusion may be recognized in the inoculated leaves and roots.

Sixty days after inoculation, high content of cysteine, cystine and methionine was observed in Draga, the inoculated leaves showed activity 0.51, 0.41 and 0.29, respectively than the healthy uninoculated 0.43, 0.36 and 0.22 mg, respectively and the same trend was observed in the inoculated roots. In Lady Rosetta the contents of cysteine, cystine and methionine were very low amount (tras) in the inoculated and uninoculated leaves and roots. In general, leaves and roots of Draga and Lady Rosetta cultivars recorded higher levels of thio-amino acids (cysteine, cystine and methionine) in inoculated plants 20, 40 and 60 days after planting, being more pronounced in the resistant Draga compared to the healthy uninoculated plants and the susceptible cultivar, Lady Rosetta, respectively.

DISCUSSION

Potato cultivars differed in their susceptibility to stem canker and black scurf. Cultivar reactions of the most commonly used commercial cultivars of potato were tested. Data obtained from the nine tested cultivars were grouped into three categories. Lady Rosetta was the most susceptible cultivars followed by the cultivars Monalisa and Mondial. Spunta, Nicola and Hermes cultivars showed moderate susceptibility while Draga, Cara and Diamant, were most resistance. Cultivar variation in disease resistant may be attributed to the differences in the plant morphology, anatomy and bio-chemical components of either tubers or plants. The results reported herein are in accordance with those mentioned by Hide *et al*. (1973), along with those of Carling and Leiner (1990) and Demirci and Doken (1993) who showed that most isolates of *Rhizoctonia solani* AG-3 were moderately to highly virulent on King Edward and Resy potato cultivars.

This study was undertaken in the principal to studies different changes in chemical components of the inoculated tubers and plants compared to the corresponding uninoculated ones.

Free, conjugated and total phenol contents were considered in leaves and root samples of the highly resistant cv. Draga and the highly susceptible cv. Lady Rosetta, 20, 40 and 60 days after planting and after inoculation with *R. solani.* Results showed that free, conjugated and total phenol contents were increased in the two potato cultivars but the increase in cv. Draga was higher than cv. Lady Rosetta.

It is well established that among the measures by which plants express resistance being through plant secondary metabolites. Among them, phenolic compounds are known to impart resistance against fungal diseases. This could be explained on base of the fact that phenolic compounds are toxic to several plant pathogens (Vidhyasekaran, 2004).

Phenols and their oxidation products are involved in various stages of host parasite relationship and are associated with disease syndrome of plant and plant resistant phenomena. There was a clear correlation between the oxidative enzymes, phenolic compound contents and resistance (Jindal, 2000).

Farkas and Kiraaly (1962) found that the participation of an endogenous supply of phenolic compounds in plant disease resistance is depended upon the activity of oxidative enzymes (polyphenol oxidase, peroxidase and catalase). Phenols are oxidized to quinone or semi-quinones which play a great role as antimicrobial agents. Some phenols have been found in all plants after infection as response to the ingress of pathogen and their appearance is considered as part of an active defense response. Since the first suggestion that phenolic intermediates have a role in the active expression of resistance the localization and timing of the host response (Stoessl, 1983).

Oxidative enzymes, i.e., peroxidase and polyphenoloxidase were considered in leaves and root samples of the highly resistant cv. Draga and susceptible cv. Lady Rosetta 20, 40 and 60 days after planting and inoculation with *R. solani.* The obtained results showed that the inoculated potato plants showed a higher peroxidase and polyphenoloxidase activity than the healthy uninoculated ones. Also, the moderately resistant cultivar had higher peroxidase and polyphenoloxidase activity than the susceptible one. These differences in enzymatic activities might be attributed to that oxidative enzymes play a partial role in activating inducible defence of plant (Vera-Estrella *et al*., 1994).

The high increment in enzymatic activity happened in early stages in resistant cultivar and successfully challenged the infection progress and by time, that enzymatic activity is being elevated to the normal levels (like in the healthy). While on the contrary, in the susceptible cultivar enzymatic activity increased gradually with time after inoculation but with low levels and failed to challenge the infection progress.

Fractionation of thio amino acids in leaves and root samples of resistant cv. Draga and susceptible cv. Lady Rosetta 20, 40 and 60 days after planting and inoculating with *R. solani* showed that thio amino acid contents increased in the two potato cultivars but the increasing in cv. Draga was higher than cv. Lady Rosetta.

The increasing in free amino acids contents in the inoculated tissues may be ascribed to the

decomposition of host protein or to the decrease in protein synthesis (Sempio and Marte, 1968).

On other hand, the increase in amount of tyrosine and phenylalanine were observed with inoculation, don't result of protein breakdown but the increase in concentration of the aromatic amino acids due to specific synthesis for phenolic compounds (Vir and Grewal, 1975).

Fractionation amino acids showed that the infection in general, caused several changes in these fractions such as absence or presence of certain components and increase or decrease in the amount of other ones. These data are in harmony with those obtained by Farag *et al.* (1986).

REFERENCES

Allam, A.L. and J.P. Hollis, 1972. Sulfide inhibition of oxidases in rice roots. Phytopathology, 62: 634-639.

Carling, D.E. and R.H. Leiner, 1990. Virulence of isolates of *Rhizoctonia solani* AG3 collected from potato plant organs and soil. Plant Dis., 74: 901-903.

Demirci, E. and M.T. Doken, 1993. Anastomosis groups and pathogenicity of *Rhizoctonia solani* Kuehn isolates from potatoes in Erzurum-Turkey. J. Turk. Phytopathol., 22: 95-102.

Duncan, D.B., 1955. Multiple ranges and multiple F Tests. Biometrics, 11: 1-42.

El-Shaer, A.H.I., 2002. Advanced studies on root infecting fungi of lentil and their control. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Cairo, Egypt.

Farag, R.S., S.M. Mohsen, F.A. Khalil and A.E. Basyony, 1986. Effect of certain fungi on the lipids of wheat kernel, sesame and soybean. Egypt. J. Food Sci., 14: 131-145.

Farkas, G.L. and Z. Kiraaly, 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. J. Phytopathol., 44: 105-150.

Gertz, C., 1990. HPLC Tips and Tricks. Great Britain at the Iden Press, Oxford, pp: 608.

Halloin, J.M., 1994. Localization of phenolic compounds in crowns and roots of healthy and *Rhizoctonia solani*-infected sugar beets. Plant Sci., 99: 223-228.

Hide, G.A., J.M. Hirst and O.J. Stedman, 1973. Effects of black scurf (*Rhizoctonia solani*) on potatoes. Ann. Applied Biol., 74: 139-148.

Jindal, P.C., 2000. Biochemical disease resistance studies against anthracnose of grape (*Sphaceloma ampelinum* De Barry). Haryana J. Hortic. Sci., 29: 11-14.

Maxwell, D.P. and D.F. Bateman, 1967. Changes in the activity of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. Phytopathology, 57: 132-136.

Misaghi, I.J., 1982. Physiology and Biochemistry of Plant Pathogen Interactions. Plenum Press, New York, USA., pp: 287.

Paranidharan, V., A. Palaniswami, P. Vidhyasekaran and R. Velazhahan, 2003. Induction of enzymatic scavengers of active oxygen species in rice in response to infection by *Rhizoctonia solani*. Acta Physiol. Plant., 25: 91-96.

Sempio, C. and M. Marte, 1968. Effect of infection on the development of bean organs susceptible and resistance to rust with reference to nitrogen metabolism in the roots. Phytopathology, 62: 288-299.

Shad, M.A., T.M. Ansari, H. Pervez, M. Rubab and T. Mahmood, 2002. Changes in sugar, amino acid and mineral contents of leaves of two mango varieties affected by quick decline disease. J. Biol. Sci., 2: 694-696.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Edn., Iowa State University Press, Ames, Iowa, USA., Pages: 507.

Snell, F.D. and C.T. Snell, 1953. Colorimetric Methods of Analysis. D. van Nostrand Co. Inc., Toronto, Canda, pp: 606.

Stoessl, A., 1983. Secondary Plant Metabolites in Preinfectional and Postifectional Resistance. Academic Publisher, New York, Pages: 221.

Vera-Estrella, R., B.J. Barkla, V.J. Higgins and E. Blumwald, 1994. Plant defense response to fungal pathogens. Plant Physiol., 104: 209-215.

Vidhyasekaran, P., 2004. Concise Encyclopedia of Plant Pathology. Taylor and Francis, London, UK., ISBN-13: 9781560229438, Pages: 619.

Vir, S. and S. Grewal, 1975. Role of free amino acids in disease resistance to gram blight. Indian Phytopathol. J., 8: 206-208.