Research Article
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The activity of pathogenesis related proteins in smut resistant and susceptible sugarcane (GT54–9) mutants induced by gamma radiation

Abstract

The activity of six pathogenesis related (PR) proteins (polyphenole oxides, phenylalanine ammonia lyase, peroxidase, esterase, chitinase and β 1,3 glucanase) in sugarcane were used to detect the variation between smut susceptible and resistant sugarcane clones generated from the moderately resistant sugarcane cultivar GT 54–9 using gamma radiation. Activity of PR proteins was monitored in healthy and artificially infected plants. A dramatic increase in the tested enzymes (phenylalanine ammonia lyase, peroxidase, esterase and chitinase) was noticed in the resistant infected (RI) plants compared to the susceptible infected (SI) plants and to the control. Generally, the levels of the tested enzymes in the (SNI) plants were lower than those recorded in the (RNI) or the moderately resistant (GT 54–9 cultivar) control plants.

Keywords: sugarcane smut, ustilago scitaminea, pathogenesis related proteins, polyphenole oxidase, phenylalanine ammonia lyase, peroxidase, esterase, chitinase, β1,3 glucanase

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Abbreviations: PR, pathogenesis related proteins; PPO, polyphenol oxidase; PAL, phenylalanine ammonia lyase; EST, esterases; POX, peroxidase; IDOC, % of increase/decrease over control; RI, resistant infected; SI, susceptible infected; Syd, sporisorium scitamineum; RNI, resistant non–infected

Introduction

Smut is an important economic disease of sugarcane. The causal organism, *Ustilago scitaminea* Syd.; (*Sporisorium scitamineum* (Syd.) M. Piepenbr., M. toll & Oberw.) was first reported in Natal in 1877 in South Africa and now smut occurs in most of the sugarcane producing areas of the world.[1] The disease aggressively affects plant growth and cane yield.[2]

Plants producing a number of compounds and proteins in response to pathogen infection. These compounds and proteins are believed to have a high importance in protecting them from the deleterious effects of the pathogen. These include the accumulation of antimicrobial compounds[3–5] and the physical strengthening of plant cell walls through increased production of hydroxyproline–rich glycoproteins, lignin and suberin.[6–8] The produced proteins (pathogenesis–related (PR) proteins) are known to be highly resistant to proteolytic degradation.[9] The PR proteins have been classified into 14 families based on the amino acid sequences, serological relationship and/or enzymatic or biological activity.[10] Many PR proteins exhibit direct antifungal activity against a wide range of fungal pathogens.[11]

Resistance to smut has been associated with the accumulation of free or conjugated polyamines in sugarcane tissues.[12,13] Several glycoproteins produced in sugarcane tissues during the infection process[2] found affecting the fungal spore germination negatively.[14–16] Some of glycoproteins have the ability to affect the cytoplasmic polarity during spore germination[14] and impede cell polarization by inhibiting the protrusion of the germ tube and spore germination. The inhibition of teliospore germination constitutes a defense mechanism involved in the resistance of sugarcane to smut.[15,16]

The aim of this study was to investigate the PR proteins changes in young sugarcane plants and to relate susceptibility or resistance to smut with changes in the levels of PR proteins produced by inoculated smut sporidia.

Materials and methods

Plant materials

Ten smut resistant sugarcane clones were obtained from (Sugar Crops Research Institute, Giza, Egypt) (GT 54–9, C9/0.5Kr–11, C9/0.5Kr–32, C9/0.5Kr–42, C9/0.5Kr–46, C9/1Kr–3, C9/2Kr–1, C9/3Kr–63, C9/3Kr–69 and C9/3Kr–8) and 10 susceptible clones (C9/2Kr–19, C9/2Kr–4, C9/3Kr–38, C9/3Kr–45, C9/3Kr–47, C9/3Kr–52, C9/3Kr–56, C9/3Kr–70, C9/1Kr–3 and C9/3Kr–79). All clones were selected from a former mutation induced clones by gamma radiation to the moderately resistant sugarcane cultivar GT 54–9.

Sugarcane stalks of each mutant were stripped of all leaves, cut into one bud setts, then given a hot water treatment for 10min at 52°C to stimulate growth. The setts were dipped into 30mg a.i./L fungicide solution (Benomyl). The setts were cultivated (3cm depth) in 25cm diameter pots filled with sand, pitmos and soil (1:1:1) each pot contained 3setts. The greenhouse temperature was 28°C±2.[17]

After the germinating shoots reach 20cm long (45days old), pots of each mutant divided to two groups over three replicates (3pots each) the plants of the first group inoculated with *U. scitaminea* and the plants of the second group inoculated with sterile distilled water to serve as a control.

Fungal material

Single haploid plus (+) and minus (–) spores from germinated teliospores of *U. scitaminea*–isolated from commercial field located in Quena governorate, Egypt were prepared using the method described by Benhamou et al.[18] Two isolates of single haploid (+) and (–) yeast–

like culture of *U. scitaminea* grown on GYC medium for 72h. at 28°C. After incubation the cultures centrifuged at 8000 rpm for 5minutes. The supernatant discarded and the collected cells washed 2times using sterile distilled water and adjusted to reach 2x105 spores per ml using a haemocytometer. Equal volumes of haploid (+) and (–) yeast–like spore suspension were mixed and incubated for 3h. at 30°C before inoculation.[18] To reduce surface tension, Tween 20 was added at a rate of 100 ul per 100ml of spore suspension prior inoculation.[17]

Inoculation

The hypodermic injection technique according to[17] was used to inoculate the emerged plants. Plant Shoots were inoculated when they reached 20cm long using 50µl of a suspension containing 2×105sporidia/ml of a 1:1 mixture (plus and minus) of the isolated mating cell types (inoculated plants) or with 50µl of sterile water (non–inoculated). Inoculation was carried out in the apical portion of the stem through the leaf sheath to ensure its contact with the meristematic region of the stem, which is the specific site of mycelium penetration and development in nature, when airborne dispersed teliospores are deposited on vegetative buds. Inoculum was infected into the stem 3cm above the first leaf with a visible dewlap. Smut inoculated and non–inoculated plants were sampled at 120h post–inoculation. Stem segments of 6cm long (3cm above and below the point of inoculation) of the inoculated and non–inoculated plants sampled at different time intervals were ground in liquid nitrogen. Total protein in each clone was determined according to.[19] Samples of 1.0g of the fine powder were extracted for each enzyme system. The percentage of increase/decrease over control (%IDOC) of each enzyme in infected and non–infected plants was calculated using the formula: ((T–C)/C)×100 Where:

C=enzyme level in infected or non infected control GT 54–9 cultivar.

T=enzyme level in infected or non infected clones.

Analysis of Polyphenol Oxidase (PPO) activity

Sugarcane samples were extracted according to the method described by Malik et al.[20] The enzyme extract was prepared by suspending 1.0g sample in 0.1M sodium phosphate buffer pH 7 (2ml/g fresh weight), then centrifuged at 6000rpm for 30min. under 4°C, the clear extract was collected, completed to 15ml volume using phosphate buffer and used as a crude enzyme source. The reaction mixture contained 0.2ml of crude enzyme source, 1ml of phosphate buffer pH 7;1ml of 10–3M catechol and completed with distilled water up to 6ml. The reaction was incubated for 30min. at 30°C. One unit of PPO was expressed as the change in absorbance at 420nm, and expressed as unit's min^{-1} g^{-1} fresh wt.[21]

Analysis of Phenylalanine Ammonia Lyase (PAL) activity

A procedure described by Zucker[22] was used; one gram of sample was suspended in 5ml of 0.1M borate buffer (pH 8.8) and 54mM β–mercaptoethanol. The mix centrifuged at 10,000rpm at 4°C for 10minutes then 1ml of the supernatant was mixed with 1ml 30mM of phenylalanine and 1ml borate buffer and incubated for one hour at 40°C for reaction termination, 0.2ml of 5NHCl was added then the volume increased up to 4ml using distilled water. The amount of transcinnamic acid formed in the reaction was measured at 290nm and calculated according to Saunders et al.,[23] as the change in absorbance of 0.01 is equivalent to the production of 3.09 n moles of cinnamic acid. The specific activity of the enzyme was expressed as moles of cinnamic acid produced per hour/gm of the tissue.

Extraction procedure for peroxidases and esterases

For estimation of peroxidases and esterases, one gram of sugarcane sample was suspended in 2ml of cold freshly prepared 10% polyvinylpyrrolidone in 0.5M tris HCl buffer (pH 7.2) and the ratio was kept 2:1 (v/w) for buffer and material. The slurry was centrifuged at 14000rpm for 20minutes at 4°C, and the resulted supernatant was used for enzyme assay.

Estimation of Esterases (EST)

Oneml of crude enzyme was added to 5ml of the reaction mixture and kept at 37°C for 1h. The reaction mixture was prepared by dissolving 200mg α–napthyl acetate in 10ml of 50% acetone and 200mg Fast Blue RR salt in 90ml of 0.2M of phosphate buffer (pH 7.0). The components of the reaction mixture were mixed together and filtered through Whatman No.1 filter paper in the dark at 4°C. The activity was measured at 600nm and expressed as unit's min^{-1} g^{-1} fresh wt.[24]

Estimation of peroxidases

The reaction mixture was prepared as previously described by Malik et al.[20] The mixture contained, 0.5ml phosphate buffer pH 7; 0.2ml enzyme source; 0.3ml of 0.05 M pyrogallol; 0.1ml of 1%(v/v) H_2O_2. The total mixture volume was raised to 3ml using distilled water. The reaction mixture was incubated at 30°C for 5min. Then the reaction stopped by adding 0.5ml of 5% (v/v) H_2O_2.[21] One unit of peroxidase activity was expressed as the changes in absorbance at 425nm and expressed as unit's min^{-1} g^{-1} fresh wt.

Determination of Chitinase

One gram of sample was suspended in an extraction buffer consisting of 0.1M acetate buffer (pH 5.0) containing 0.1% (W/V) each of ascorbic acid and sodium sulphite and 5% PVP. The homogenates were centrifuged at 12,000rpm at 4°C for 30min., and then the supernatant was used for enzyme assay. A mixture of crude enzyme source (1 ml) and suspension of colloidal chitin (1ml; 0.1% in 50mM sodium acetate buffer; pH 5) was incubated at 38°C in a water bath with constant shaking. After 2hr, the release of *N*–acetylglucosamine in the reaction mixture was estimated by the method of Reissig et al.[25] The enzyme activity was determined using *N*–acetylglucosamine (Sigma) as the standard. Absorbance was measured at 660nm. One unit of chitinase is defined as the amount of enzyme producing 1µmol *N*–acetylglucosamine/min in 1ml of reaction mixture under standard assay conditions. Specific activity was expressed as unit's min^{-1} g^{-1} fresh wt.

Determination of ß–1, 3 glucanase

One gram of sample was suspended in an extraction buffer consisting of 0.1M acetate buffer (pH 5.0) containing 0.1% (W/V) each of ascorbic acid and sodium sulphite and 5% PVP. The homogenates were centrifuged at 12,000rpm at 4°C for 30min., the supernatant was used for enzyme assay.[26]

Total activity of ß–1, 3 glucanase was determined by measuring the released reducing sugar from laminarin (Sigma–Aldrich) as a substrate.[26] The assay mixture was consisted of 0.8ml of 0.1M

acetate buffer pH 5.0 containing 1% laminarin and 0. 4ml of enzyme extract. After 30minutes incubation 30°C, the reducing substances were colorimetrically estimated according to[27,28] at 660nm. The standard curve of glucose was used as reference. Specific activity was expressed as unit's min^{-1} g^{-1} fresh wt.

All the chemicals and reagents used in this work were produced by Sigma Aldrich and Amresco. The colorimetric assays were carried out using Spectronic 601; Milton Roy, Rochester, NY spectrophotometer. Data of the present work were statistically analyzed by analysis of variance using a complete randomize design with 3 replicates according to[29] using SPSS system version 8[30]

Results and discussion

The germination of *Ustilago scitaminea* spores occurs on the internode surface and it is followed by appressoria formation, mainly on the inner scale of young buds and on the bases of emerging leaves.[31] The entry of the germ tube into the bud meristem occurs between 6 and 36h., after the teliospores are deposited on the surface.[32] After the infection, the fungal hyphae grow throughout the infected plant, but mostly in the parenchyma cells of the lower internodes. In the upper internodes, hyphal growth concludes with the formation of the whip (sori with teliospores). It has been proposed that varied resistance of sugarcane is determined by several morphological features of buds.[31] In this work we used injection as an inoculation method to determine the physiological resistance.

Polyphenoloxidase (PPO) activity

The levels of polyphenoloxidase in the resistant infected (RI) and non–infected (RNI) sugarcane clones (Table 1) were significantly higher than in the susceptible infected (SI) and non–infected (SNI) sugarcane clones. In susceptible clones, it was noticed that the levels of PPO were lower than in the control (GT 54–9 cultivar) in both infected and non–infected treatments while in resistant varieties the levels of PPO were higher in non–infected treatment except the clones C9/3Kr–63 and C9/3Kr–8 and the clones C9/0.5Kr–46, C9/1Kr–3, C9/3Kr–63, C9/3Kr–69 and C9/3Kr–8 in infected treatment. In susceptible clones, the percentage of PPO level reduction compared to that in control (% IDOC) ranged from 9.61 to 41.98% in non infected plants and from 37.99% to 52.31%, while in resistant clones the increase of PPO level in non–infected clones ranged from 0.27% and 26.74% and the reduction was less than 3% in two clones and in infected clones the increase ranged from 2.63 to 5.51% and the reduction ranged from 0.56 to 8.1% (Figure 1).

Table 1 Specific activity of poly phenol oxidase (PPO) in healthy and *U. scitaminea* infected sugarcane resistant and susceptible mutants

Resistance	Clone	Polyphenoloxidase (Units min^{-1} g^{-1} fresh wt.)				Mean
		Non-Infected	% IDOC*	Infected	% IDOC*	
Susceptible	C9/2Kr-19	22.48lmn	↓33.38	25.48lm	↓48.86	24.4231 B
	C9/2Kr-4	19.58n	↓41.98	25.85l	↓48.12	
	C9/3Kr-38	20.90mn	↓38.09	25.28lm	↓49.26	
	C9/3Kr-45	20.93mn	↓37.98	24.68lm	↓50.46	
	C9/3Kr-47	22.87lmn	↓32.25	26.78kl	↓46.23	
	C9/3Kr-52	22.31lmn	↓33.91	24.26lmn	↓51.31	
	C9/3Kr-56	23.78lmn	↓29.55	26.98kl	↓45.85	
	C9/3Kr-70	23.50lmn	↓30.38	23.76lmn	↓52.31	
	C9/1Kr-13	22.34lmn	↓33.82	25.24ijk	↓49.34	
	C9/3Kr-79	30.51jk	↓9.61	30.89ijk	↓37.99	
	Mean	**22.9234**		**25.9228**		
	C9 Cont.	33.76ij		49.83abcd		
Resistant	C9/0.5Kr-11	42.55fgh	↑26.06	51.78abc	↑3.91	43.9238 A
	C9/0.5Kr-32	40.97h	↑21.36	53.85a	↑8.06	
	C9/0.5Kr-42	42.79fgh	↑26.74	51.14abc	↑2.63	
	C9/0.5Kr-46	41.17gh	↑21.96	48.95bcd	↓1.76	
	C9/1Kr-3	33.85 ij	↑0.27	47.50cde	↓4.67	
	C9/2Kr-1	44.14efgh	↑30.74	52.58ab	↑5.51	
	C9/3Kr-63	32.88ij	↓2.59	47.09cdef	↓5.49	
	C9/3Kr-69	35.43i	↑4.94	45.79defg	↓8.10	
	C9/3Kr-8	32.83ij	↓2.74	49.55abcd	↓0.56	
	Mean	**38.0400**		**49.8075**		
Mean		**34.2631 B**		**43.1359 A**		
LSD at (0.05) for:						
Resistance (R):		**6.361**				
Infection (I):		**5.724**				
Clones (C):		**5.724**				
RxIxC:		**4.217**				

*IDOC, % of Increase/Decrease over control; up arrows (↑), increase; down arrow (↓), decrease
*Different letters in means indicate a significant difference PPO, polyphenoloxidase

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Many workers have reported the increase in the activity of polyphenole oxidase in resistant varieties. Polyphenol enzymes (catecholase and cresolase) have been reported to be responsible for in vivo synthesis and accumulation of phenolic compounds.[33,34]

The importance of polyphenoloxidase activity in disease resistance is due to its property to oxidize phenolic compounds to quinines which are often more toxic to microorganisms than the original phenols. It is reasonable to assume that an increased activity of polyphenoloxidase results in higher concentration of toxic products of oxidation and therefore causes greater degree of resistance to infection.[35] Sundar et al.,[36] reported that polyphenoloxidase activity is related to resistance against red rot disease in sugarcane.

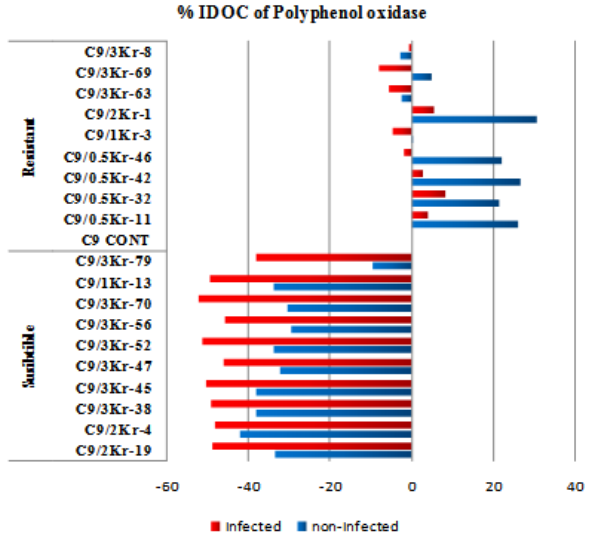

Figure 1 Percentage of Increase/Decrease over control (%IDOC) of polyphenoloxidase (PAL) in healthy and *U. scitaminea* infected sugarcane resistant and susceptible GT54-9 mutants.

Phenylalanine Ammonia lyase (PAL) activity

The results demonstrated that the specific activity of Phenylalanine ammonia lyase showed a similar pattern of increase as for the activity of PPO in selected resistant and susceptible clones of sugarcane. In the present study, it was noticed that the PAL activity was significantly higher in infected and non infected resistant clones compared to the susceptible ones (Table 2).

In susceptible clones the percentage of PAL level reduction than in control GT 54–9 cultivar. The % IDOC decreased in susceptible clones and ranged from 37.66 to 55.84% in non–infected plants and from 2.48 to 18.73% in infected plants except for four clones (C9/3Kr–45, C9/3Kr–47, C9/3Kr–52 and C9/3Kr–56) the PAL activity increased by (11.11, 27.69, 16.08 and 0.16% respectively) compared to the control. On the other hand, in resistant non–infected clones the % IDOC decreased compared to the control. The reduction of PAL activity ranged from 8.22 and 22.07%. In resistant non–infected clones PAL activity significantly increased to the maximum 32.0% and a minimum of 22.55% (Figure 2).

Phenylalanine ammonia lyase activity is fundamental to maintain or increase the synthesis of all these phenolics and according to De Armas et al.,[37] resistance to smut in sugarcane is associated with the possibility of maintaining high levels of PAL activity. Singh et al.,[38] reported higher activity of PAL in red rot resistant cultivars of sugarcane than the susceptible ones. On the other hand,[39–41] reported that the increase of PAL is associated with the increase in lignin synthisis in disease resistant plants.

Crude elicitor prepared from *S. scitamineum* mycelium induces high phenylalanine ammonia–lyase activity without accumulation of free hydroxyl cinnamic acids and moderately high peroxidases activity, mainly in resistant cultivars.[37]

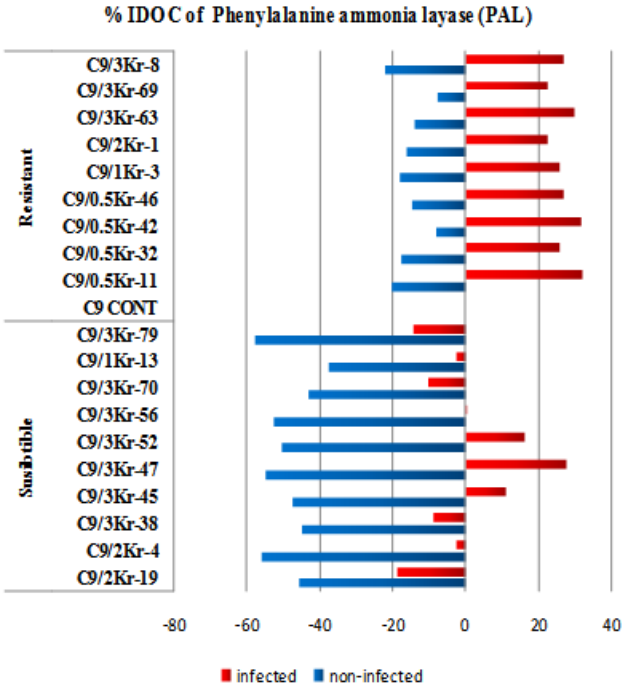

Figure 2 Percentage of Increase/Decrease over control (%IDOC) of Phenylalanine ammonia layase (PAL) in healthy and *Ustilago scitaminea* infected sugarcane.

Analysis of Esterase (EST)

The levels of esterases were significantly increased in the (RI) and (RNI) clones compared to the infected and non–infected control, respectively, as well as the (SI) and (SNI) control. The percentage of IDOC in the resistant non–infected clones ranged from 1.69% to 41.13% compared to the non–infected control while in the (RI) clones, the increase ranged from 5.99% to 23.62% compared to (RNI) control. In infected susceptible clones the enzyme level decreased significantly compared to the infected control, the reduction ranged from 2.58% to 20.47% (Table 3).

Kim et al.,[42] reported that esterase of Capsicum annum inhibit the appressorium formation of *Glomerella cingulata*. Zhang et al.,[43] reported a detoxification gene for Albicidin from Pantoea dispersa. The gene encodes an esterase and it abolishes the capacity of *Xanthomonas albilineans* to release albicidin toxin and incite the symptoms of leaf scald disease in sugarcane. Koretsky[44] suggested the role of esterases in the development of resistance to fusarium infection in soy bean (Figure 3).

Analysis of Peroxidase (POX)

Data presented in Table 4 show that the enzyme levels significantly

increased in the (RI) and (RNI) control compared to the (SI) and (SNI) clones. In the resistant non–infected control the enzyme level increased 29.96% to 66.07% over the non–infected control. On the other hand the increase of enzyme levels in the (RI) clones ranged from 5.20% to 27.02% over the (RI) control. The enzyme level in the (SI) clones almost decreased 2 folds compared to the infected control. The range of enzyme reduction ranged from 29.73% to 46.43%). The obtained data suggest the presence of an association between resistance and the increase of peroxidase level in the plant (Figure 4).

Table 2 Specific activity of phenyl alanine ammonia layase (PAL) in healthy and *Ustilago scitaminea* infected sugarcane resistant and susceptible mutants

Resistance	Clone	PAL (moles of transcinnamic acid/h^{-1} g^{-1} fresh wt)				Mean
		Non-Infected	% IDOC	Infected	% IDOC	
Susceptible	**C9/2Kr-19**	0.0084lmn	↓45.67	0.0163gh	↓18.73	**0.0140 B**
	C9/2Kr-4	0.0068mn	↓55.84	0.0196de	↓2.48	
	C9/3Kr-38	0.0085 lm	↓44.80	0.0183def	↓8.78	
	C9/3Kr-45	0.0081lmn	↓47.61	0.0223c	↑11.11	
	C9/3Kr-47	0.0069lmn	↓54.97	0.0257a	↑27.69	
	C9/3Kr-52	0.0076lmn	↓50.43	0.0233bc	↑16.08	
	C9/3Kr-56	0.0073lmn	↓52.59	0.0201d	↑0.16	
	C9/3Kr-70	0.0088 l	↓43.07	0.0180efg	↓10.28	
	C9/1Kr-13	0.0096 n	↓37.66	0.0196fgh	↓2.48	
	C9/3Kr-79	0.0065 n	↓57.79	0.0172fgh	↓14.42	
	Mean	**0.0078**		**0.0201**		
Resistant	**C9 Cont**	0.0154hi	-	0.0201d	-	**0.0191 A**
	C9/0.5Kr-11	0.0123jk	↓20.12	0.0265a	↑32.00	
	C9/0.5Kr-32	0.0127jk	↓17.74	0.0253a	↑25.87	
	C9/0.5Kr-42	0.0141ij	↓8.22	0.0265a	↑31.84	
	C9/0.5Kr-46	0.0131jk	↓14.71	0.0255a	↑27.03	
	C9/1Kr-3	0.0126jk	↓18.18	0.0253a	↑25.70	
	C9/2Kr-1	0.0129jk	↓16.01	0.0246ab	↑22.55	
	C9/3Kr-63	0.0133jk	↓13.85	0.0261a	↑30.01	
	C9/3Kr-69	0.0142ij	↓7.57	0.0246ab	↑22.55	
	C9/3Kr-8	0.0120k	↓22.07	0.0255 a	↑27.03	
	Mean	**0.0133**		**0.0250**		
Mean		**0.0118 B**		**0.0232 A**		
LSD at (0.05) for:						
Resistance (R):		**0.00232**				
Infection (I):		**0.00562**				
Clones (C):		**0.00232**				
RxIxC:		**0.001709**				

*IDOC, % of Increase/Decrease over control; up arrows (↑), increase; down arrow (↓), decrease

*Different letters in means indicate a significant difference

In plants, the increased production of both the superoxide radical and H_2O_2 is a common feature of defence response to challenge by the microbial pathogen and elicitors.[45] It has been proposed that, the rapid increase in either intra or extra cellular H_2O_2 is involved in the induction of execution of the hypersensitive response.[46] Bestwick et al.,[47] reported that, Cytochrome c peroxidase is a key enzyme during the synthesis of phytoalexin which has some inhibitory effect on disease.

Plant peroxidases can be directly involved in defence mechanisms acting as a catalyst for the polymerization of phenolic compounds to form lignin and suberin in the cell wall, which can act as mechanical barriers to block the spread of the pathogen in the plant.[48] The importance of peroxidases during plant resistance against pathogens has been demonstrated for the interaction between rice and Xanthomonas oryzae pv. oryzae[49] and between cotton and X. campestris pv. Malvacerum.[50]

Another important difference was the enhancement in the resistant cultivar of peroxidase, an enzyme that uses free phenolics as substrates for the activation of the important mechanisms of resistance of sugar cane leaves to the fungal pathogen.[51]

Turk[52] and Que et al.,[53] reported that cytochrome c peroxidase is newly induced after infection, and the author believed that hydrogen peroxide redox type cytochrome c reaction (2 cytochrome c (Fe2+)+H_2O_2+2H+⟶2 cytochrome c (Fe^{3+})+2H_2O) was catalyzed by the up–regulated expression of cytochrome c peroxidase, which

improved the increasing synthesis of phytoalexin and inhibited the growth of *S. scitamineum* and thus reduced the harm of *S. scitamineum*.

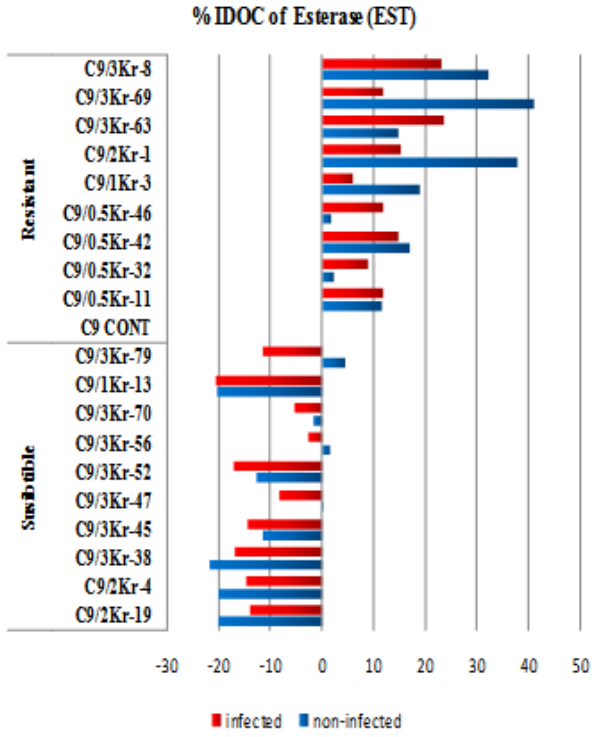

Figure 3 Percentage of Increase/Decrease over control (%IDOC) of Esterase (EST) in healthy and *Ustilago scitaminea* infected sugarcane resistant and susceptible GT54-9 mutants.

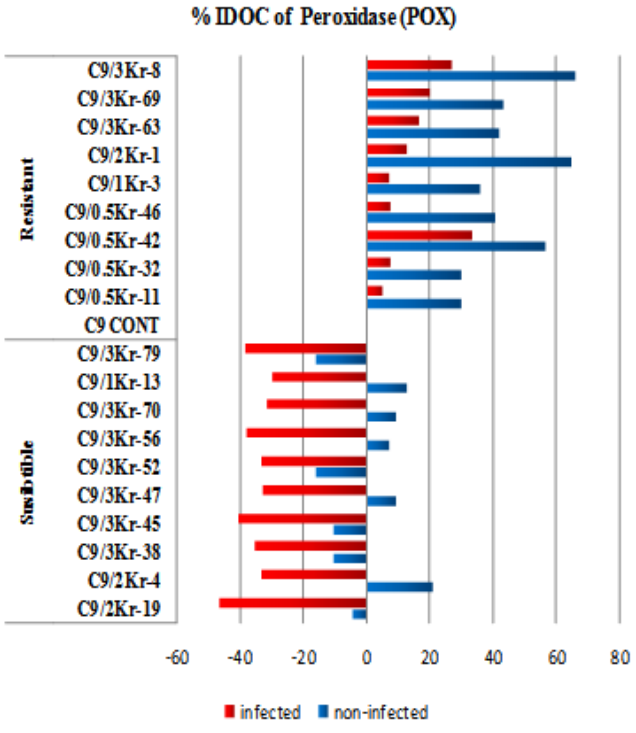

Figure 4 Percentage of Increase/Decrease over control (%IDOC) of Peroxidase (POX) in healthy and *U. scitaminea* infected sugarcane resistant and susceptible GT54-9 mutants.

Analysis of chitinase

Data presented in Table 5 show that all the (SI) and (SNI) clones have a low level of chitinase compared to the infected and non infected control. The decrease percentage of chitinase over control IDOC in the susceptible clones ranged from 40.17% to 51.84%, while in the infected clones the decrease over control ranged from 52.89% to 67.67% (Figure 5).

In resistant non infected clones the level of chitinase significantly increased in some of the tested clones while it was nonsignificant with the others compared to the non infected control (1.181% to 20.997%). In (RI) clones the level of chitinase increased over control in some clones and decreased in the others.

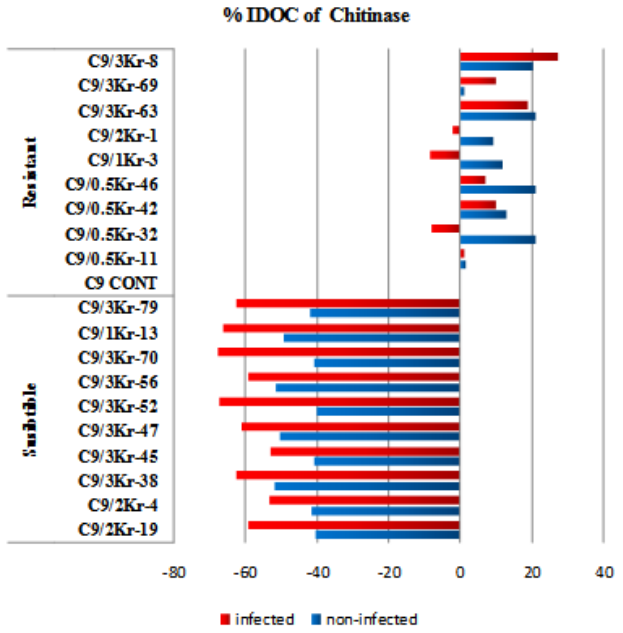

Figure 5 Percentage of Increase/Decrease over control (%IDOC) of Chitinase in healthy and *U. scitaminea* infected sugarcane resistant and susceptible GT54-9 mutants.

Analysis of β–1,3 glucanase

The results of the present study demonstrated that, the specific activity of β–1,3 glucanase showed similar pattern of decrease as for the activity of chitinase in the (SI) and (SNI) clones compared to the infected and non infected control (Table 6). In contrast to chitinase pattern of activity of the (RI) and (RNI) clones the specific activity of β–1,3 glucanase in all the tested colones decreased compared to the non infected control while in the infected clones some of the tested clones showed a reduction in the specific activity and the others showed increases (Figure 6).

Kuc[54] reveled that, the plant response phase against the infection includes the accumulation of different compounds such as phytoalexins (i.e. low molecular mass antimicrobial compounds that accumulate at sites of infection), systemic enzymes that degrade pathogens (e.g. chitinases, β–1,3–glucanases and proteases). Wu et al.,[55] reported, two defense related genes encoding the anionic peroxidase and acidic chitinase were induced in transgenic *Solanum tuberosum* by the action of a broad range of fungal pathogens.

Mauch et al.,[56] reported that, Chitinase and glucanase are the most

widely used approach of developing fungus resistant plants they found that, both enzymes showed over–expression in transgenic plants. They stated that, chitin and glucan comprise major components of the cell wall of most of the fungi. Over–expression of these hydrolytic enzymes in the plant cells is postulated to cause hyphal lysis, thereby inhibiting fungal growth. Broque et al.,[57] constitutively expressed bean chitinase in tobacco and *Brassica napus* to enhance resistance towards *Rhizoctonia solani*. Among the PR proteins hydrolytic enzymes (chitinase and glucanase), Osmotins, Thionins and Defensins are specially important.

Table 3 Specific activity of Esterase (EST) in healthy and *U. scitaminea* infected sugarcane resistant and susceptible mutants

Resistance	Clone	Esterase healthy (units min^{-1} g^{-1} fresh wt)				Mean
		Non- Infected	% IDOC	Infected	% IDOC	
Susceptible	**C9/2Kr-19**	0.1868 [	↓20.05	0.2988m	↓13.96	
	C9/2Kr-4	0.1865 [	↓20.19	0.2963n	↓14.67	
	C9/3Kr-38	0.1827 /	↓21.83	0.2885o	↓16.92	
	C9/3Kr-45	0.2070y	↓11.42	0.2970n	↓14.48	
	C9/3Kr-47	0.2342w	↑0.19	0.3184k	↓8.32	
	C9/3Kr-52	0.2037z	↓12.85	0.2873o	↓17.26	**0.2568 B**
	C9/3Kr-56	0.2370v	↑1.41	0.3383h	↓2.58	
	C9/3Kr-70	0.2298x	↓1.65	0.3284i	↓5.44	
	C9/1Kr-13	0.1864[	↓20.23	0.2772p	↓20.47	
	C9/3Kr-79	0.2442t	↑4.47	0.3077l	↓11.41	
	Mean	**0.2098**		**0.3037**		
	C9 Cont	0.2337w		0.3473g		
Resistant	**C9/0.5Kr-11**	0.2607s	↑11.53	0.3883d	↑11.79	
	C9/0.5Kr-32	0.2392u	↑2.33	0.3783e	↑8.93	
	C9/0.5Kr-42	0.2735q	↑17.03	0.3981c	↑14.63	
	C9/0.5Kr-46	0.2377uv	↑1.69	0.3880d	↑11.71	
	C9/1Kr-3	0.2777p	↑18.81	0.3681f	↑5.99	**0.3333 A**
	C9/2Kr-1	0.3222j	↑37.85	0.4007b	↑15.36	
	C9/3Kr-63	0.2680r	↑14.67	0.4293a	↑23.62	
	C9/3Kr-69	0.3298i	↑41.13	0.3879d	↑11.69	
	C9/3Kr-8	0.3090 l	↑32.22	0.4279a	↑23.19	
	Mean	**0.2751 *B***		**0.3914 *A***		
Mean		**0.2587**		**0.3664**		
LSD at (0.05) for:						
Resistance (R):		**0.0023**				
Infection (I):		**0.0036**				
Clones (C):		**0.0023**				
RxIxC:		**0.0017**				

*IDOC, % of Increase/Decrease over control; up arrows (↑), increase; down arrow (↓), decrease

*Different letters in means indicate a significant difference

EST: Esterase

Table 4 Specific activity of Peroxidase (POX) in healthy and *U. scitaminea* infected sugarcane resistant and susceptible mutants

Resistance	Clone	Peroxidase (units min^{-1} g^{-1} fresh wt)				Mean
		Non- Infected	% IDOC	Infected	% IDOC	
Susceptible	**C9/2Kr-19**	0.0163 t	↓4.483	0.0283 q	↓46.433	
	C9/2Kr-4	0.0207 r	↑20.857	0.0353mn	↓33.207	
	C9/3Kr-38	**I.** tu	↓10.331	0.0341 no	↓35.448	
	C9/3Kr-45	0.0153 tu	↓10.331	0.0313 p	↓40.719	
	C9/3Kr-47	0.0187 s	↑9.1618	0.0356mn	↓32.638	
	C9/3Kr-52	0.0143 u	↓16.179	0.0352mn	↓33.428	**0.0255 B**
	C9/3Kr-56	0.0183 s	↑7.2125	0.0328 op	↓37.941	
	C9/13Kr-70	0.0187 s	↑9.1618	0.0362 lm	↓31.471	
	C9/1Kr-3	0.0193 u	↑12.865	0.0371 op	↓29.734	
	C9/3Kr-79	0.0143 u	↓16.179	0.0326 op	↓38.257	
	Mean	**0.0171**		**0.0338**		

Citation: Esh AMH, Guirgis AA, El-kholi MMA, et al. The activity of pathogenesis related proteins in smut resistant and susceptible sugarcane (GT54–9) mutants induced by gamma radiation. *Adv Plants Agric Res.* 2014;1(4):146–156. DOI: 10.15406/apar.2014.01.00024

Table Continued..

Resistance	Clone	Peroxidase (units min^{-1} g^{-1} fresh wt)				Mean
		Non- Infected	% IDOC	Infected	% IDOC	
Resistant	**C9 Cont**	0.0277 q		0.0528 g		**0.0495 A**
	C9/0.5Kr-11	0.0360lmn	↑29.963	0.0556 f	↑5.2083	
	C9/0.5Kr-32	0.0360lmn	↑29.963	0.0567 f	↑7.3864	
	C9/0.5Kr-42	0.0433 i	↑56.438	0.0703 a	↑33.207	
	C9/0.5Kr-46	0.0390 jk	↑40.794	0.0568 f	↑7.5442	
	C9/1Kr-3	0. 0377 kl	↑35.980	0.0566 f	↑7.2601	
	C9/2Kr-1	0.0457 h	↑64.861	0.0596 e	↑12.878	
	C9/3Kr-63	0.0393 jk	↑41.997	0.0616 d	↑16.635	
	C9/3Kr-69	0.0397 j	↑43.201	0.0634 c	↑20.138	
	C9/3Kr-8	0.0460 h	↑66.065	0.0671 b	↑27.020	
	Mean	**0.0390**		**0.0601**		
Mean		**0.0328 B**		**0.0529 *A***		
LSD at (0.05) for:						
Resistance (R):		**0.00232**				
Infection (I):		**0.00613**				
Clones (C):		**0.00232**				
RxIxC:		**0.0017**				

*IDOC, % of Increase/Decrease over control; up arrows (↑), increase; down arrow (↓), decrease

*Different letters in means indicate a significant difference

POX: Peroxidase

Table 5 Specific activity of Chitinase in healthy and *U. scitaminea* infected sugarcane resistant and susceptible mutants

Resistance	Clone	Chitinase (units min^{-1} g^{-1} fresh wt)				Mean
		Non-Infected	% IDOC	Infected	% IDOC	
Susceptible	**C9/2Kr-19**	5.1288 o	↓40.3043	13.7268i	↓59.3330	**8.9131 B**
	C9/2Kr-4	5.0156 o	↓41.6216	15.7488h	↓53.3428	
	C9/3Kr-38	4.1374 p	↓51.8439	12.6970j	↓62.3841	
	C9/3Kr-45	5.0831 o	↓40.8367	15.9011h	↓52.8917	
	C9/3Kr-47	4.2670 p	↓50.3352	13.1216j	↓61.1261	
	C9/3Kr-52	5.1407 o	↓40.1660	11.0142k	↓67.3694	
	C9/3Kr-56	4.1642 p	↓51.5317	13.7392i	↓59.2966	
	C9/3Kr-70	5.0872 o	↓40.7887	10.9125k	↓67.6708	
	C9/1Kr-13	4.3564 o	↓49.2947	11.3433j	↓66.3945	
	C9/3Kr-79	4.9858 o	↓41.9693	12.6920j	↓62.3989	
	Mean	**4.7366**		**13.0897**		
Resistant	**C9 Cont**	8.5916 n		33.7543e		**22.6195 A**
	C9/0.5Kr-11	8.7170 n	↑1.4599	34.1254e	↑1.0994	
	C9/0.5Kr-32	10.4055 l	↑21.1131	31.0864g	↓7.9037	
	C9/0.5Kr-42	9.7082 m	↑12.9969	37.0859c	↑9.8703	
	C9/0.5Kr-46	10.3956 l	↑20.9971	36.1056d	↓6.9660	
	C9/1Kr-3	9.6148 m	↑11.9091	30.8785g	↓8.5197	
	C9/2Kr-1	9.3880 m	↑9.2698	32.9592f	↓2.3556	
	C9/3Kr-63	10.3986 l	↑21.0317	40.0965b	↑18.789	
	C9/3Kr-69	8.6931 n	↑1.1810	37.1152c	↑9.9569	
	C9/3Kr-8	10.3336 l	↑20.2759	42.9358a	↑27.200	
	Mean	**9.6246**		**35.6143**		
Mean		**8.2437 B**		**28.8700 A**		
LSD at (0.05) for:						
Resistance (R):		**0.684**				
Infection (I):		**0.669**				
Clones (C):		**0.684**				
RxIxC:		**0.504**				

*IDOC, % of Increase/Decrease over control; up arrows (↑), increase; down arrow (↓), decrease

*Different letters in means indicate a significant difference

Citation: Esh AMH, Guirgis AA, El-kholi MMA, et al. The activity of pathogenesis related proteins in smut resistant and susceptible sugarcane (GT54–9) mutants induced by gamma radiation. *Adv Plants Agric Res.* 2014;1(4):146–156. DOI: 10.15406/apar.2014.01.00024

Table 6 Specific activity of β-1,3 glucanase in healthy and *U. scitaminea* infected sugarcane resistant and susceptible mutants

Resistance	Clone	β 1,3 Glucanase (units min^{-1} g^{-1} fresh wt)				Mean
		Non-Infected	% IDOC	Infected	% IDOC	
Susceptible	C9/2Kr-19	151.66 u	↓21.00	189.57n	↓65.52	
	C9/2Kr-4	145.68 v	↓24.11	197.08l	↓64.16	
	C9/3Kr-38	151.72 u	↓20.97	182.46o	↓66.82	
	C9/3Kr-45	154.66 t	↓19.44	184.06o	↓66.53	
	C9/3Kr-47	165.71 r	↓13.68	234.78i	↓57.31	
	C9/3Kr-52	154.66 tu	↓19.44	194.75lm	↓64.58	**178.11 B**
	C9/3Kr-56	148.73 v	↓22.53	193.07m	↓64.89	
	C9/3Kr-70	146.70 v	↓23.58	225.21j	↓59.04	
	C9/1Kr-13	153.64 t	↓19.97	230.74k	↓58.04	
	C9/3Kr-79	155.67 t	↓18.91	201.74k	↓63.31	
	Mean	**152.88**		**203.35**		
Resistant	C9 Cont	191.99 m		549.97f		
	C9/0.5Kr-11	173.10 q	↓9.83	661.21a	↑20.22	
	C9/0.5Kr-32	166.03 r	↓13.51	594.98e	↑8.18	
	C9/0.5Kr-42	158.08 s	↓17.66	642.16b	↑16.76	
	C9/0.5Kr-46	174.99 q	↓8.85	550.26f	↑0.05	
	C9/1Kr-3	173.07 pq	↓9.85	631.14c	↑14.75	**379.32 A**
	C9/2Kr-1	178.32 p	↓7.11	483.09h	↓12.15	
	C9/3Kr-63	176.11 p	↓8.26	526.53g	↓4.26	
	C9/3Kr-69	179.03 p	↓6.74	591.98e	↑7.63	
	C9/3Kr-8	177.05 p	↓7.78	607.33d	↑10.43	
	Mean	**174.78**		**583.87**		
Mean		**168.33 B**		**478.55 A**		
LSD at (0.05) for:						
Resistance (R):		**4.238**				
Infection (I):		**6.636**				
Clones (C):		**4.238**				
RxIxC:		**3.122**				

*IDOC, % of Increase/Decrease over control; up arrows (↑), increase; down arrow (↓), decrease

*Different letters in means indicate a significant difference

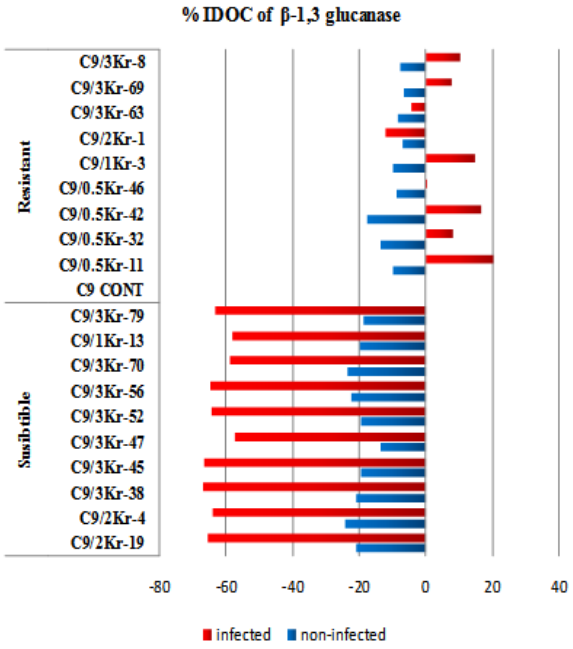

Figure 6 Percentage of Increase/Decrease over control (%IDOC) of β 1,3 glucanase in healthy and *U. scitaminea* infected sugarcane resistant and susceptible GT54-9 mutants.

Citation: Esh AMH, Guirgis AA, El-kholi MMA, et al. The activity of pathogenesis related proteins in smut resistant and susceptible sugarcane (GT54–9) mutants induced by gamma radiation. *Adv Plants Agric Res.* 2014;1(4):146–156. DOI: 10.15406/apar.2014.01.00024

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Conflict of interest

The author declares no conflict of interest.

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