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Biochemical characterization of defense responses in rose genotypes in response to artificial inoculation with black spot pathogen *Diplocarpon rosae*

Saidulu Y.[1]*, Tejaswini P., Upreti K.K.[2], Sriram S.[2], Seetharamu G.K.[1], Devappa V.[1] and Mythili J.B.[2]

[1]College of Horticulture, Bengaluru-65, University of Horticultural Sciences, Bagalkote, Karnataka, 587 104, India.
[2] ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru, Karnataka, 560 089, India.
*Corresponding author Email : saidulugowd08@gmail.com

ABSTRACT

Resistance responses in the leaves of eight rose genotypes, Knock Out (highly resistant), Arka Nishkant (moderately resistant), *R. multiflora* (highly susceptible), Arka Swadesh (highly susceptible), IIHRR 13-4 (susceptible), Arka Parimala (susceptible), *R. indica* (susceptible) and IIHRR 4-15-12 (moderately susceptible), exhibiting varied levels of resistance against black spot were investigated post artificial inoculation with black spot pathogen, *Diplocarpon rosae*. There was consistent increase in the activities of defense related enzymes such as catalase, peroxidase, polyphenol oxidase, superoxide dismutase and phenylalanine ammonia lyase and other defense related secondary metabolites like phenols and flavonoids at different phases of black spot progression and increase was high in resistant genotypes Knock Out and Arka Nishkant. The peak activity of defense enzymes and high concentration of other metabolites was witnessed during early stages of infection in the resistant genotypes while it was during later phase in the susceptible genotypes. These results suggested that the faster and stronger activation of defense system is associated with the resistance against black spot in the rose genotypes.

Keywords: Artificial inoculation, diplocarpon rosae, enzymes, flavonoids, phenols, resistance and rose

INTRODUCTION

Black spot, caused by *Diplocarpon rosae* is the major destructive and dominant disease among different fungal diseases in rose. Owing to the demand and popularity of roses in current flower trade and landscape gardening, breeding for the resistant varieties or developing the varieties that require less care in terms of management against the destructive diseases is the need of the hour. Plants protect themselves through various means of defenses through accumulation of several defense related biochemical compounds following infection by pathogens. Reactive Oxygen Species (ROS) that are produced as initial cellular responses following successful pathogen recognition (Ashry and Mohamed, 2011) have major roles in cell signaling and these are the secondary messengers for activation of genes that encode for protective proteins (Lamb and Dixon, 1997 and Mendoza, 2011). However, the increased ROS production causes cellular damage through peroxidation of membrane fatty acids (Lamb and Dixon, 1997) and plants defend against this with up regulation of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Mittler *et al.,* 2004). Phenolics are toxic to microbes in nature and increase the physical and mechanical strength of the host cell wall. The oxidation of these toxic phenolic compounds by polyphenol oxidase (PPO) produces quinones (antimicrobial compounds) that are highly toxic to invading fungi thereby offering resistance against a wide range of pathogens (Cahill and Mccomb, 1992). Phenylalanine ammonia lyase (PAL), one of the key enzymes in the phenyl propanoid pathway, has a role in synthesis of phytoalexin and salicylic acid. Increase in the PAL activity subsequently increases the phenolic contents offering disease resistance to plants (Klessig and Malamy, 1994).

Understanding of plant resistance mechanism against pathogens at various levels provides new opportunities to breed improved cultivars with better resistance to the diseases. There were not many studies in this line on black spot disease infection in rose. Thus, the study was conducted to investigate the role of various defense related enzymes and compounds at different progression periods post infection by black spot pathogen in different rose genotypes possessing differential resistance.

MATERIAL AND METHODS

Location and climate of experimental site

The present investigation was carried out during 2016 at ICAR-IIHR, Bengaluru, which is geographically located at 130 58' N Latitude, 780 E Longitude and at an elevation of 890 m above mean sea level with an average annual rainfall of about 890 mm.

Plant material

The rose genotypes evaluated in the present study were part of rose breeding program at ICAR-Indian Institute of Horticultural Research, Bengaluru. A total of eight rose genotypes were used for the study in completely randamized design (CRD). The plants were provided with all inputs as per the package of practices for rose cultivation except for fungicidal sprays during the period of investigation. Young and healthy leaves from 4th to 6th node from apex of the shoot were collected (Dong, 2014) ran damly from three plants of the selectedfrom the selected genotypes *viz., R. multiflora* (highly susceptible), Arka Swadesh (highly susceptible), IIHRR 13-4 (susceptible), Arka Parimala (susceptible), *R. indica* (susceptible), IIHRR 4-15-12 (moderately susceptible), Arka Nishkant (moderately resistant) and Knock Out (highly resistant) in three replications. The collected leaves were cleaned with deionized sterile water and wiped with sterile tissue paper.

Preparation of conidia suspension

Rose leaves, severely infected with black spot, preferably with yellow halo around the spots were collected and surface cleaned with sterile tissue paper. Later, the infected leaf portions were cut and submerged in deionized sterile distilled water in the sterile tubes. The tubes were kept in orbital shaker for one minute after adding two drops of Tween-20. The suspension was filtered and the concentration of conidia in the filtrate was adjusted to 2×10^4 conidia/ml (Leus, 2005) using haemocytometer. This filtrate was used for inoculation of leaves. The spores from pure culture of the pathogen maintained on detached leaves was used for further inoculation of healthy leaves in the present study.

Artificial inoculation of leaves

The inoculation of excised leaves with *D. rosae* was performed as described by Debener *et al*. (1998). The cleaned healthy leaves were placed on moist blotting paper, with their petioles wrapped in moist cotton plugs in glass petri plates to maintain 100 per cent humidity. On each leaflet surface, 4-6 droplets of 10µL conidial suspension (2×10^4 conidia/ml) was pipetted out in laminar air flow to avoid contamination. The inoculated leaves were then incubated at ~25ºC under 10 h photoperiod for two weeks (Dong, 2014).

Enzyme assays:

The infected and control leaves were analyzed for the activities of following defense related biochemical compounds on every third day *i.e.,* on 0, 3, 6, 9, 12 and 15 days after inoculation (DAI).

POX activity (EC 1.11.1.7) (Enzyme units/ g fresh weight -EU/g FW): The POX activity was determined by following same method described by Chander (1990) and enzyme activity was expressed as enzyme units g/ FW.

PPO activity (EC 1.10.3.1) (EU/g FW): The PPO activity was determined following the method of Selvaraj and Kumar (1989) without any modifications and the enzyme activity was expressed as EU/g FW.

CAT activity (EC 1.11.1.6) (EU/mg FW): The CAT activity was determined by following the same procedure as per Masia (1998) and activity was expressed as EU/mg FW.

PAL activity (EC 4.3.1.24) (EU/g FW): The PAL activity was estimated as per the same procedure followed by Hodgins (1971) and activity was expressed as EU/g FW.

SOD activity (EC 1.15.1.1) (EU/mg FW): The SOD activity was estimated as per the same procedure followed by Du and Bramlage (1994) and activity was expressed as EU/mg FW.

Total phenols (mg/ g fresh weight): Total phenol content was estimated by same procedure followed by Singleton and Rossi (1965) by spectrophotometric method using Folin-Ciocalteau's Phenol Reagent (FCR) and the phenol content was expressed as mg/g FW.

Total flavonoids (mg/ g fresh weight): Total flavonoid content was estimated by the spectrophotometric method using same procedure as followed by Chun *et al.* (2003) and total flavonoid content was expressed as mg/g FW.

RESULTS AND DISCUSSION

a) POX activity

Analysis of data revealed that POX activity increased significantly in inoculated leaves of all genotypes after infection with the pathogen (Fig S1, S2). Among the inoculated leaves (I_2) of all genotypes, highest peroxidase activity (1.84 EU/ g FW) was observed in highly resistant variety Knock Out, on 6th day after inoculation (G_8D_3) which was almost 1.4 times to the maximum activity found in highly susceptible genotype, *R. multiflora* (1.29 EU/ g FW) that was observed on 9th day after inoculation (G_1D_4) (Table 1). In the variety Arka Nishkant, which was moderately resistant, peak enzyme activity was observed (1.76 EU/ g FW) on 12th day (G_7D_5). No significant changes in enzyme activity were found in un-inoculated leaves (I_1) of all genotypes during entire observation period (Fig. S1, S2) (data not presented). Peroxidase is involved in biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989). Peroxidase mediates the oxidation of phenols to oxidized phenols that are highly toxic to the pathogen (Sequeira, 1983). Thus, the increased activity of peroxidase in infected tissues contributes to resistance by inhibiting the pathogen growth.

In the present study, resistant genotypes have recorded quick and high peroxidase activity compared to susceptible ones. Due to the increased activity of peroxidase at early stages of infection, the pathogen growth was hindered and thus offered resistance against black spot. Similar increased activity of peroxidase in resistant genotypes in response to pathogen infection has been reported in *Fusarium* infection in melon (Hanifei *et al.*, 2013), *Botrytis* infection in faba bean (El-Komy, 2014) and brown rust infection in wheat (Riaz *et al.*, 2014).

b) PPO activity

PPO activity increased in inoculated leaves of all genotypes in response to the pathogen inoculation (Fig. S3, S4). At a given time period on 3rd (D_2) and 6th (D_3), highest PPO activity (2.62 EU/ g FW and 3.13 EU/ g FW respectively) was found in highly resistant variety Knock Out (G_8D_2 and G_8D_3) respectively) whereas the lowest activity (0.85 EU/ g FW and 1.84

Table 1. POX activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	POX activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	0.47	0.68	1.22	1.29	1.18	0.71
2	Arka Swadesh (G_2)	0.38	0.69	1.25	1.38	1.23	0.8
3	IIHRR 13-4 (G_3)	0.46	0.71	1.3	1.38	1.22	1.02
4	Arka Parimala (G_4)	0.55	0.93	1.32	1.56	1.39	1.04
5	*R. indica* (G_5)	0.47	1.01	1.44	1.52	1.62	1.65
6	IIHRR 4-15-12 (G_6)	0.49	0.78	1.31	1.7	1.59	1.65
7	Arka Nishkant (G_7)	0.44	1.48	1.69	1.68	1.76	1.71
8	Knockout (G_8)	0.46	1.81	1.84	1.74	1.71	1.64
	S.Em ±	0.06	-	-	-	-	-
	C.D. @ 5%	0.18	-	-	-	-	-

EU/ g FW respectively) was recorded in highly susceptible genotype *R. multiflora* (G_1D_2 and G_1D_3 respectively) (Table 2). This revealed that the enzyme activity in resistant genotype increased immediately in response to the pathogen infection whereas the enzyme activity increased slowly and gradually in the susceptible genotype. Among all genotypes, highest activity of PPO in inoculated leaves (I_2) (3.64 EU/ g FW) was recorded in moderately resistant genotype Arka Nishkant on 9th day after inoculation (G_7D_4). No significant changes in enzyme activities were found in un-inoculated leaves (I_1) of all genotypes during entire observation period (Fig. S3, S4) (data not presented). PPO catalyzes the oxidation of phenols released due to membrane damage (Siddique *et al.*, 2014) during microbial invasion into oxidized phenols *i.e.*, quinones that are more reactive and highly toxic (Batsa, 2004) which creates toxic environment for pathogen development (Jockusch, 1966 and Mohamed *et al.*, 2012). Thus increase in PPO activity is associated with resistance. In present study, PPO activity increased quickly with pathogen inoculation in resistant genotypes whereas the increase in susceptible genotypes was slow and less. This early increase in activity of PPO in resistant genotypes inhibited the fungal growth and thereby contributed for resistance in resistant genotype. These results are in conformity with the findings of Khatun *et al.* (2009) who reported increased activity of PPO in black spot (*Alternaria tenuis*) infected resistant rose leaf tissues during progression of disease. Highest activity of PPO was also reported in *Fusarium* infected resistant melon genotypes compared to susceptible ones (Hanifei *et al.*, 2013).

c) CAT activity

The inoculated leaves of all genotypes showed significantly higher levels of CAT activity during the period of observation than those of un-inoculated controls (Fig. S5, S6). In highly resistant genotype Knock Out, the CAT activity increased sharply and reached its peak (19.83 EU/ mg FW) on 12th day after inoculation (G_8D_5), whereas in *R. multiflora* which was highly susceptible, the CAT activity increased comparatively at a slower pace and reached its peak (8.71 EU/ mg FW) on 9th day (G_1D_4), followed by a decrease by 15th day (7.15 EU/ mg FW) (G_1D_6). When the CAT activity of all genotypes was compared on third day (D_2) immediately after pathogen inoculation, the highest activity (16.06 EU/ mg FW) was observed in highly resistant genotype Knock Out (G_8D_2) whereas lowest activity (7.64 EU/ mg FW) was found in Arka Swadesh (G_2D_2) which was highly susceptible to the disease. No significant changes were detected in control leaves (I_1) throughout the observation period (Fig. S5, S6) (data not presented).

CAT is one of the important H_2O_2 scavenging enzymes that eliminate the toxic effects of H_2O_2 through a mechanism known as Halliwell–Asada–Foyer pathway

Table 2. PPO activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	PPO activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	0.59	0.85	1.84	2.65	2.45	2.19
2	Arka Swadesh (G_2)	0.53	0.99	2.05	2.82	2.68	2.35
3	IIHRR 13-4 (G_3)	0.57	1.02	2.16	2.30	2.52	2.44
4	Arka Parimala (G_4)	0.57	1.53	2.41	3.09	3.24	3.33
5	*R. indica* (G_5)	0.69	1.13	2.32	2.88	2.60	2.46
6	IIHRR 4-15-12 (G_6)	0.74	2.11	2.82	3.35	3.47	3.54
7	Arka Nishkant (G_7)	0.74	2.27	2.94	3.64	3.44	3.40
8	Knockout (G_8)	0.70	2.62	3.13	3.29	3.39	3.42
	S.Em ±	0.07	-	-	-	-	-
	C.D. @ 5%	0.18	-	-	-	-	-

(Hanifei *et al.*, 2013). It protects the plant cells from oxidative damage caused by ROS (Gill and Tuteja, 2010). The results of present study revealed that inoculated leaves of all genotypes showed sig-nificantly higher levels of CAT activity than those of un-inoculated controls. In inoculated leaves of both resistant and susceptible genotypes, the CAT activity increased during the progress of infection. However, induced levels of CAT were significantly higher during progression of infection in the inoculated leaves of resistant genotypes compared to those of susceptible genotypes. These differences in CAT activity in present study suggested that the low enzyme activity in susceptible genotypes made them less efficient in reducing the high levels of H_2O_2 produced during *D. rosae* infection. These results are in agreement with the findings of El-Komy (2014) who reported increased activity of CAT in resistant genotypes over susceptible ones after inoculation with chocolate spot pathogen of faba bean. Mandal *et al.* (2008) have also reported that a less efficient enzymatic ROS scavenging system, mainly a decrease in CAT activity caused high level of damage caused by *F. oxysporum* f. sp. *Lycopersici*, in tomato. (El-Komy, 2014).

d) PAL activity

The inoculated leaves of all genotypes showed significantly higher levels of PAL activity than those of un-inoculated controls (Fig S7, S8). PAL activity changed significantly in inoculated leaves (I_2) of all genotypes with progression of time after inoculation. In inoculated leaves of all genotypes, PAL activity increased in response to pathogen infection and reached peak by 9th day in all genotypes except in highly resistant variety Knock Out where the peak activity was observed on 6th day itself. Further, the enzyme activity got decreased slightly by 15th day in all genotypes after reaching peak (Fig. S7). In highly resistant variety Knock Out, maximum activity that was recorded on 6th day was 2.95 EU/ g FW (G_8D_3) whereas in *R. multiflora* which was highly susceptible, peak PAL activity was recorded as 1.48 EU/ g FW (G_1D_4) which was observed on 9th day. No significant changes were detected in control leaves (data not presented) throughout the observation period (Fig. S7, S8).

PAL is primary enzyme in the phenylpropanoid metabolism and plays a significant role in the synthesis of several defense-related secondary compounds such as phenols and lignin (Hemm *et al.* 2004; Tahsili *et al.* 2014). The activation of PAL and subsequent increase in phenolic content in plants is a general response associated with disease resistance (Siddique *et al.* 2014). Results of present study revealed that PAL activity was high in resistant genotype compared to susceptible genotypes. This increased activity of PAL in resistant genotypes have lead to more production of defense related secondary compounds which conferred protection against disease. The increased activity of PAL in defense against fungal pathogens in resistant genotypes was also reported in case of brown rust interactions in wheat (Riaz *et al.*, 2014)

e) SOD activity

The SOD activity changed significantly in inoculated leaves of all genotypes with progression in days after inoculation (Fig S9, S10). At a given time period on third day (D_2) immediately after inoculation, highest SOD activity (2.99 EU/ mg FW) was found in moderately resistant genotype Arka Nishkant (G_7D_2) where the lowest activity (1.50 EU/ mg FW respectively) was recorded in highly susceptible genotype *R. multiflora* (G_1D_2). The highly resistant genotype Knock Out (G_8) recorded SOD activity equivalent to 2.91 EU/ mg FW on third day (G_8D_2). On sixth day (D_3) after inoculation, highest SOD activity among all genotypes (3.76 EU/ mg FW) was found in highly resistant genotype Knock Out (G_8D_3) where the lowest activity (2.08 EU/ mg FW) was recorded in highly susceptible genotype *R. multiflora* (G_1D_3). This revealed that the enzyme activity in resistant genotype increased immediately in response to the pathogen infection whereas the enzyme activity increased gradually at a slower pace in susceptible genotype. The SOD activity in highly resistant genotype Knock Out reached its peak on 6th day (3.76 EU/ mg FW) (G_8D_3) after inoculation and thereafter decreased by 15th day (2.21 EU/ mg FW) (G_8D_6) whereas in highly susceptible genotype *R. multiflora*, the activity remained increasing throughout the observation period and reached peak on 15th day (2.60 EU/ mg FW) (G_1D_6). No significant changes in enzyme activity were detected in control leaves throughout the observation period (Fig S9, S10) (data not presented).

SOD is one of the important reactive oxygen species scavenging enzymes which catalyzes the dismutation of superoxide anion radicals (O^{2-}) into H_2O_2 and O_2

(Smirnoff, 1993; Khan and Panda, 2008). H_2O_2 generation in infected plants is considered one of the important defense strategies of plants against the invading necrotrophic pathogen (Hanifei *et al.*, 2013). Results of present study revealed that increased SOD activity was observed in both resistant and susceptible genotypes but the increase was more and quick in resistant ones, in response to pathogen inoculation. In case of susceptible genotypes, though there was increase in enzyme activity, it may not be adequate and quick enough to counter pathogen development, making them susceptible to the disease. Similar results of higher SOD activity in resistant cultivar over susceptible cultivar, after pathogen inoculation were reported in case of chocolate spot disease of faba bean (El-Komy, 2014) and *Mycosphaerella fragariae* infection in strawberry (Ehsani-Moghaddam *et al.* 2006).

f) Total phenols

The inoculated leaves of all genotypes showed significantly higher levels of total phenols during the period of observation than those of un-inoculated controls (Fig S11, S12). In inoculated leaves (I_2) of all genotypes, total phenols changed significantly with progression in time period after inoculation and reached their peak on 9th day inoculation (D_4) and thereby decreased by 15th day (D_6) (Table 6). In highly resistant genotype Knock Out, the total phenols increased sharply and reached peak (81.94 mg/g FW) on 9th day (G_8D_4) whereas in *R. multiflora* which was highly susceptible, total phenols increased comparatively at a slower rate and reached its peak (49.84 mg/g FW) on 9th day (G_1D_4). When the total phenols content of all genotypes was compared on third day (D_2) immediately after pathogen inoculation, highest accumulation (71.94mg/g FW) was observed in highly resistant genotype Knock Out (G_8D_2) whereas lowest accumulation (31.59 mg/g FW) was found in IIHRR 13-4 (G_3D_2) which was susceptible to the disease. No significant changes in enzyme activity were detected in control leaves throughout the observation period (Fig S11& S12) (data not presented).

Phenols enhance the mechanical strength of host cell walls by synthesis of lignin and suberin which are involved in the formation of physical barriers that can block the spread of pathogens (Ngadze *et al.* 2012; Singh *et al.* 2014). Further, Khatun *et al.*, 2009 reported that the phenols are fungitoxic in nature. In the present study, the amount of total phenols was significantly higher in inoculated leaves of resistant genotypes, while it was significantly lower in susceptible genotypes. Thus, high accumulation of phenols in resistant genotypes may be playing role in eliciting resistance response against black spot pathogen. The increased phenolic content in resistant genotypes after pathogen inoculation was also reported in case of chocolate spot disease of faba bean (El-Komy, 2014) and in cotton interaction with cotton leaf curl Burewala virus (Siddique *et al.*, 2014).

g) Total flavonoids

The results revealed that inoculated leaves of all genotypes showed significantly higher levels of total flavonoids during the period of observation than those of un-inoculated controls (Fig. S13, S14). Total flavonoids changed significantly in inoculated leaves (I_2) of all genotypes with increase in number of days after inoculation and showing their peak on 9th day after inoculation (D_4) and further decreased by 15th day (D_6) (Table 7). In highly resistant Knock Out genotype, total flavonoids increased sharply and reached peak (35.11 mg/g FW) on 9th day after inoculation (G_8D_4) whereas in *R. multiflora* which was highly susceptible, total flavonoids increased comparatively at a slower rate and reached peak (18.33 mg/g FW) on 9th day (G_1D_4). When the total flavonoids of all genotypes were compared on third day (D_2) immediately after pathogen inoculation, highest accumulation (28.73 mg/g FW) was observed in highly resistant genotype Knock Out (G_8D_2) whereas lowest accumulation (10.57 mg/g FW) was found in IIHRR 13-4 (G_3D_2) which is susceptible to the disease. No significant changes were detected in control leaves throughout the observation period (Fig. S13, S14) (data not presented).

Flavonoids are very important in plant resistance against pathogenic bacteria and fungi. Antipathogenic properties of flavonoids can be non-specific in nature and partly could be the result of their antioxidative properties. Flavonoid compounds are transported to the site of infection and induce the hypersensitivity reaction, which is the earliest defense mechanism employed by the infected plants and programmed cell death (Mierziak *et al.*, 2014) thus restrict the spread

Table 3. CAT activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	CAT activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	6.78	8.10	8.10	8.71	7.73	7.15
2	Arka Swadesh (G_2)	5.08	7.64	9.65	10.71	9.44	8.91
3	IIHRR 13-4 (G_3)	5.38	8.65	7.87	6.54	5.68	5.88
4	Arka Parimala (G_4)	6.60	9.40	10.18	10.63	9.61	8.70
5	*R. indica* (G_5)	5.58	9.83	8.39	7.65	6.10	5.83
6	IIHRR 4-15-12 (G_6)	6.69	12.08	15.02	16.77	17.44	18.28
7	Arka Nishkant (G_7)	6.55	13.12	16.27	17.86	18.88	17.39
8	Knockout (G_8)	4.50	16.06	18.01	18.48	19.83	17.21
	S.Em ±	0.18	-	-	-	-	-
	C.D. @ 5%	0.51	-	-	-	-	-

Table 4. PAL activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	PAL activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	0.60	0.86	1.36	1.48	1.41	1.33
2	Arka Swadesh (G_2)	0.71	1.18	1.51	1.80	1.68	1.48
3	IIHRR 13-4 (G_3)	0.88	1.41	1.60	1.91	1.73	1.46
4	Arka Parimala (G_4)	0.85	1.51	1.79	2.07	1.98	1.68
5	*R. indica* (G_5)	0.85	1.40	1.76	1.86	1.70	1.59
6	IIHRR 4-15-12 (G_6)	0.57	1.87	2.03	2.22	2.14	2.10
7	Arka Nishkant (G_7)	0.63	1.94	2.25	2.36	2.24	2.21
8	Knockout (G_8)	0.69	2.48	2.95	2.91	2.71	2.51
	S.Em ±	0.02	-	-	-	-	-
	C.D. @ 5%	0.06	-	-	-	-	-

Table 5. SOD activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	SOD activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	1.18	1.50	2.08	2.31	2.41	2.60
2	Arka Swadesh (G_2)	1.20	1.75	2.35	2.27	2.08	1.62
3	IIHRR 13-4 (G_3)	1.23	1.86	2.44	2.53	2.60	2.57
4	Arka Parimala (G_4)	1.18	1.97	2.53	2.76	2.59	2.63
5	*R. indica* (G_5)	1.09	2.02	2.67	2.82	2.81	2.68
6	IIHRR 4-15-12 (G_6)	1.32	2.80	3.30	3.48	3.55	3.58
7	Arka Nishkant (G_7)	1.17	2.99	3.42	3.60	3.30	3.43
8	Knockout (G_8)	1.19	2.91	3.76	3.10	2.61	2.21
	S.Em ±	0.03	-	-	-	-	-
	C.D. @ 5%	0.07	-	-	-	-	-

Table 6. Total phenols (mg/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	Total phenols (mg/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	35.78	42.27	47.07	49.84	45.72	39.14
2	Arka Swadesh (G_2)	28.45	36.40	40.48	43.36	41.33	36.17
3	IIHRR 13-4 (G_3)	26.84	31.59	37.27	46.66	41.25	37.03
4	Arka Parimala (G_4)	34.31	44.79	52.81	56.63	51.49	45.72
5	*R. indica* (G_5)	28.38	41.27	46.46	52.84	49.78	41.42
6	IIHRR 4-15-12 (G_6)	35.20	54.72	67.07	74.04	68.35	57.41
7	Arka Nishkant (G_7)	26.99	54.27	58.09	62.84	59.61	49.93
8	Knockout (G_8)	33.15	71.94	76.77	81.94	71.11	65.91
	S.Em ±	0.66	-	-	-	-	-
	C.D. @ 5%	1.84	-	-	-	-	-

Table 7. Total flavonoids (mg/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	Total flavonoids (mg/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	11.86	14.48	17.10	18.33	17.62	15.41
2	Arka Swadesh (G_2)	7.45	12.93	14.11	16.34	14.19	12.96
3	IIHRR 13-4 (G_3)	4.46	10.57	14.46	15.36	12.82	11.11
4	Arka Parimala (G_4)	13.92	20.73	24.89	26.38	24.91	19.48
5	*R. indica* (G_5)	10.85	18.81	24.63	27.45	24.12	19.41
6	IIHRR 4-15-12 (G_6)	13.13	22.87	27.10	29.55	28.89	21.14
7	Arka Nishkant (G_7)	4.60	18.45	21.57	23.85	20.94	18.85
8	Knockout (G_8)	12.38	28.73	32.63	35.11	29.54	19.95
	S.Em ±	0.25	-	-	-	-	-
	C.D. @ 5%	0.68	-	-	-	-	-

of pathogen. In the present study, the amount of total flavonoids was significantly higher in inoculated leaves of resistant genotypes, while it was significantly lower in susceptible genotypes. This high accumulation of flavonoids in resistant genotypes might have contributed for the resistance. Resistance against the fungal infection due to increased accumulation of flavonoids in leaves was also reported in interaction of cedar-apple rust pathogen and apple trees (Lu *et al.*, 2017).

CONCLUSION

The changes in activity of defense related enzymes like CAT, POX, PPO, SOD and PAL and accumulation of plant defense related secondary compounds like phenols and flavonoids were distinguished clearly in inoculated leaves compared to un-inoculated leaves. Further, the trend of either increase or decrease in activity of defense related biochemical compounds was more prominent and varied significantly among the studied genotypes with progression in time period of black spot disease. All studied defense related biochemical compounds increased drastically faster in higher quantities in resistant genotypes compared to susceptible genotypes during disease progression contributing for resistance.

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