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RESEARCH ARTICLE

Defense responses to *Fusarium oxysporum* f. sp. *ricini* infection in castor (*Ricinus communis* L.) cultivars

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Abstract

The biophysical and biochemical basis of resistance in castor to wilt caused by *Fusarium oxysporum* f. sp. *ricini* was studied. Entry and colonization of fungal mycelium in susceptible (JI-35) and resistant (48-1) cultivars was studied histopathologically by employing bright field, scanning and transmission electron microscopy. Browning of xylem vessels of vascular bundles in susceptible cultivar can be taken as an anatomical parameter for early detection of the wilt disease. Electron microscopy revealed more mycelium and spores in the susceptible cultivar. Cell wall was thick in resistant cultivar than the susceptible cultivar. Biochemical activity of antioxidant enzymes and pathogenesis related (PR) proteins in the roots of resistant and susceptible genotypes of castor were studied at 24, 48, 72 and 96 h after inoculation till 7 days. Studies pertaining to the activity of defense enzymes viz, superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and β-1,3-glucanase revealed that the level of defense related enzyme activity of SOD, GR and β-1,3-glucanase was higher in resistant cultivar. However, the activity of CAT reached a maximum at 4 days post inoculation and decreased by 7 days. In contrast, the activity of APX enzyme was higher in susceptible cultivar. Release of these enzymes related to expression of resistant mechanism restricted the browning of xylem vessels in resistant cultivar. The restricted growth of mycelium, absence of browning in xylem vessels and increase in activity of defense related enzymes in resistant cultivar indicates the resistance mechanism in the plant.

Keywords Castor · Wilt · Histopathology · Antioxidant enzymes · Resistance mechanism

Introduction

Castor (*Ricinus communis* L.), belonging to the family Euphorbiaceae, is the most important non-edible oilseed crop of arid and semi arid regions of India. The major castor growing states in India during 2016–2017 were Gujarat, Rajasthan, Telangana, Andhra Pradesh, Karnataka, Tamil Nadu and Odisha covering an area of 8.3 lakh ha with a production of 14.2 lakh tonnes and productivity of 1713 kg/ha (Anonymous 2017). The major diseases affecting castor cultivation are gray mold, wilt and root rot. Wilt caused by *Fusarium oxysporum* f. sp. *ricini* is one of the most important diseases of castor and occurs in all castor growing areas in India. The extent of yield loss depends on the stage at which plant wilts with the losses ranging from 77% at flowering to 63% at 90 days and 39% in later stages on secondary branches (Pushpavathi et al. 1998). Lakshminarayana and Raoof (2006) reported that 10 to 40% reduction in yield and also 8–14% in seed weight and 1–2% in seed oil content.

Breeding of resistant castor cultivars is the most promising option for wilt management which requires knowledge on host–pathogen interaction and its resistance mechanism (Lavanya et al. 2011). A wide range of biotic and abiotic factors induce the generation of Reactive Oxygen Species (ROS) and disturb the redox environment of plant cells leading to oxidative stress (Hameed and Sheikh 2007). Production of these harmful substances is controlled by the anti oxidative enzymes and pathogen related proteins which are used by the host in developing defense mechanism against pathogen. ROS have several direct and indirect roles in plant defense and they are directly toxic to invading microorganisms. Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX),

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catalase (CAT), peroxidase (POX) that can protect them against the detrimental effects of activated oxygen (Kapadia et al. 2013). SOD is a major scavenger of O_{27} (reductive radical) and its enzymatic action results in the formation of hydrogen peroxide. With the increase of peroxidase enzyme activity in the infection process the hydrogen peroxide is generated which either inhibits the pathogen or generate other free radicals which are harmful to the pathogen. CAT, APX and a variety of general POX catalyse the breakdown of hydrogen peroxide. Therefore, these enzymatic systems eliminate the damaging effects of toxic oxygen species. Both SOD and POX exist in multiple isoforms in plant tissues. In these protection processes, POX enzyme is directly involved in lignin biosynthesis.

β-1,3-Glucanase is recognized as one of the pathogen related proteins and its accumulation has been observed in many plant species up on infection by viruses, bacteria and fungi as per Stintz et al. (1993). Kapadia et al. (2013) described the defense responses of resistant and susceptible genotypes of castor to wilt disease.

In this paper, we present the results of studies pertaining to histopathology and activity of defense related enzymes in resistant and susceptible castor cultivars to *F. oxysporum* f. sp. *ricini* to understand the mechanism of resistance.

Materials and methods

The *F.oxysporum f.sp.ricini* culture was prepared by isolating it from infected root samples of castor wilt susceptible JI-35 cultivar. Pure culture of it was prepared by sub culturing it on potato dextrose agar medium. It was then maintained and preserved in paraffin oil at − 20 °C (Nakasone et al. 2004) for further studies. Seven days old *F.o.r* culture in Petri dish was flooded with sterile distilled water and filtered through a muslin cloth. The spore suspension was prepared with the concentration of 1×10^6 spores/ml.

Seed material and inoculation of seedlings

The experiments were conducted at ICAR-Indian Institute of Oilseeds Research, Hyderabad, India by the method of Santha Lakshmi Prasad et al. (1999) with little modification. Seeds of resistant (48-1) and susceptible (JI-35) castor cultivars were surface sterilized with 0.1% sodium hypochlorite and raised in moistened germination towels. The seed varieties 48-1, JI-35 were reported as resistant and susceptible to castor wilt (Santha Lakshmi Prasad et al. 2008). Ten days old seedlings were kept in Hoagland solution for 3 days in test tubes to enable establishment and acclimatization. Root tips of seedlings were trimmed for about 2–3 cm, dipped in the spore suspension for 5–10 min and transferred to fresh Hoagland solution. Samples were drawn at different time intervals after inoculation and subjected to histopathological studies and biochemical analysis.

Histopathological studies

Root samples were taken for histopathological studies at 24, 48, 72 and 96 h after inoculation. Bright field, scanning and transmission electron microscopy images were observed for histological differences in resistant and susceptible castor cultivars.

Sample preparation for bright field microscopy

Root samples from *F.o.r* inoculated plants were observed in Olympus CX41 model bright field microscope with 40× magnification. For the staining of the roots the method described by Vierheilig et al. (1998) with modification was followed. The healthy and inoculated roots were rinsed well in tap water; thin sections were cut with a sterile blade, boiled in 10% KOH for 5 min and stained with 0.1% lactophenol cotton blue and viewed under microscope.

Sample preparation for scanning electron microscopy (SEM)

The sample preparation procedure for SEM was followed according to the technique developed by Bozzola and Russell (1999). Castor root samples collected from *F.o.r* inoculated plants were cut into 2 mm pieces. The solution of 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) was used as fixative and the sample was fixed for 24 h at 4 °C and then post fixed for 4 h in 2% aqueous osmium tetroxide solution. Dehydration procedure was carried out in series of different concentrations of alcohol and then it was dried to critical point drying under silica desiccation. The samples were then processed and mounted on to the stubs with double sided carbon conductivity tape. Samples were then coated with gold layer of very thin size using automated sputter coater for 3 min and viewed under SEM.

Sample preparation for Transmission electron microscopy (TEM)

The *Fusarium* inoculated root samples were collected and fixed in the solution of 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2). Further, they were fixed for 24 h at 4 °C then washed for 4 times with 45 min each in phosphate buffered saline and they were fixed in 1% aqueous osmium tetroxide for 2 h. The samples were then given six washes of deionized distilled water 45 min each and dehydrated in different concentrations of alcohol and it was infiltrated and embedded in spur resin and incubated at 80° for 72 h for total polymerization. The 60 nm thin sections were prepared

on ultra microtome with a glass knife, and mounted with copper grids. The sections were stained with aqueous urenyl acetate (UA) and counter stain used was reynolds lead citrate. The samples were observed under TEM at required magnifications as per the standard procedures at RUSKA Laboratory, College, of Veterinary Sciences, Hyderabad, India. This technique was developed by Bozzola and Russell (1999).

Biochemical studies

The *F.o.r* inoculated and healthy control castor root samples of JI-35 and 48-1 were collected at an interval of 24 h i.e., 1, 2, 3, 4, 5, 6, 7 days after inoculation (DAI). 1 g of root tissue ground in prechilled mortar and pestle in specific extraction buffer (5 ml) for each enzyme as per Sunil et al. (2011). The enzymes studied were viz., peroxidase (POX), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), β-1,3-glucanase and superoxide dismutase (SOD).

Enzyme extraction

The extraction buffer for POX and SOD contained 0.1 M Tris–HCl buffer (pH 7.5) with 3% (w/v) polyvinyl pyrrolidone, 1 mM EDTA and 1 mM $CaCl_2$. The buffer Potassium phosphate (0.1 M, pH 7.5) was taken in place of Tris–HCl buffer for CAT, APX and GR enzymes. One gram of root tissue was ground in 5 ml of extraction buffer in prechilled mortar and pestle. The suspension was filtered through muslin cloth and the filtrate was then centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was used as crude enzyme source as per the method of sunil et al. (2011). 1 g of root tissue was ground in 5 ml of 0.05 M sodium acetate buffer (pH 5.0) and the suspension was filtered through muslin cloth. The filtrate was centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was used as the crude enzyme source for extraction of the enzyme β-1,3-glucanase as per the method of Pan et al. (1991).

Enzyme assays

SOD enzyme activity was measured by following the method of Beauchamp and Fridovich (1971). Photochemical reduction of nitroblue tetrazolium (NBT) was inhibited by this enzyme and its inhibition ability can be calculated as the SOD enzyme activity. POX enzyme activity was measured estimating the rate of oxidation of guaiacol in presence of H_2O_2 at 470 nm (Rao et al. 1996). The guaiacol and H_2O_2 acts as substrates in the reaction. This enzyme activity can be expressed as guaiacol oxidized/min at certain assay conditions. The method given by Sinha (1972) was followed to estimate the enzyme activity of catalase. The standard graph of H_2O_2 was prepared. The units of H_2O_2 oxidized/min were determined by knowing its concentration in the samples of inoculated and control treatments. The enzyme activity of APX was calculated by the method given by Nakano and Asada (1981). The units of ascorbate oxidized/min is expressed as its activity. The enzyme activity of GR was calculated by the method given by Halliwell and Foyer (1978). NADPH is used as substrate here and the activity of enzyme is expressed in units following the NADPH oxidised/min. The enzyme activity of β-1,3-glucanase was estimated by the method given by Pan et al. (1991) i.e., laminarin dinitrosalicylic acid. The laminarin (sigma) is used as substrate in the reaction. The optical density values are taken at 500 nm.

Data was subjected to three factor factorial analysis of variance to work out the significant effect of each enzyme activity levels and their interaction in resistant and susceptible castor genotypes till 7 days after inoculation through online source i.e., Web Access Statistical Package (http://icargoa.res.in/waspnew.html).

Results and discussion

Bright field microscopy

Stained sections of root tissues under bright field microscope revealed that at the time of infection, the fungus enters the castor root through specialized penetration hyphae developed from conidia. These specialized hyphae attached to root hair of JI-35 at 24 h after inoculation (Fig. 1a–c). The hyphae was penetrated into the epidermal cells (Fig. 1d). The epidermal layer was slightly distorted because of toxins released by the pathogen (Fig. 1e). The hyphae entered into hypodermis within 48 h after inoculation (Fig. 1f) and colonized into the adjacent cells of cortex layer in root tissue (Fig. 1g). The mycelium was observed in inner layers of root tissue at 72 h after inoculation (Fig. 1h). Heavy colonization was observed in infected roots of the susceptible cultivar JI-35 (Fig. 1i). Four days after inoculation, the mycelium entered into inner pith layer followed by browning of vascular bundle with the entry of mycelium into xylem vessels (Fig. 1j). Browning of xylem vessels was not observed in healthy control, JI-35 (Fig. 1k).

The occlusion of xylem vessels of root tissue with gum like substances was observed in JI-35 (Fig. 2a) at 4 DAI. Growth of mycelium was observed in the tissues (Fig. 2b). The cells of vascular bundle in susceptible cultivar was noticed with browning and distorted structure (Fig. 2c, d). In untreated control of JI-35, mycelium was not observed in the root tissue and the tissue was healthy (Fig. 2e).

Entry and penetration of the pathogen in the resistant cultivar 48-1 was also similar to the susceptible genotype. Although there was no browning of the tissue, sparse hyphae and mycelium were observed in the root

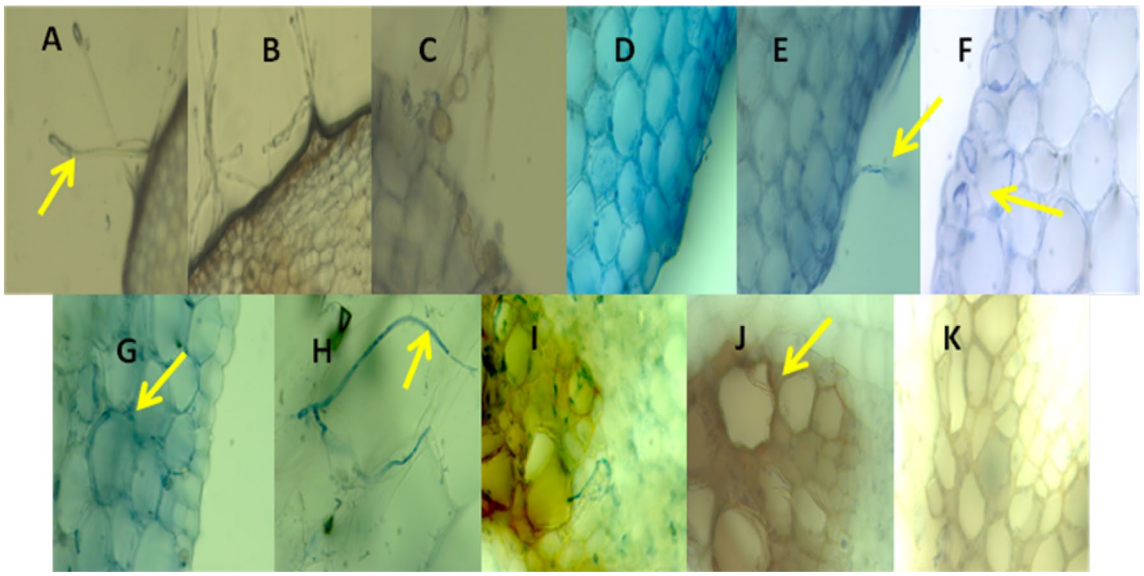

Fig. 1 Entry of fungal mycelium and spores into epidermal and hypodermal layers and its colonization in JI-35 (susceptible cultivar) through bright field microscopy. **a** Penetration hyphae on root hair (24 hai). **b** Mycelium enters into root tissue (24 hai). **c** Distorted structure of root tissue (48 hai). **d**, **e** Mycelium enters epidermis (48 hai). **f** Mycelium in hypodermis (48 hai). **g**, **h** Mycelium in cortical cells (72 hai). **i** Colonization of mycelium in root tissue (72 hai). **j** Mycelium in xylem vessels (72 hai). **k** Healthy control with no browning. Arrow indicate Mycelium

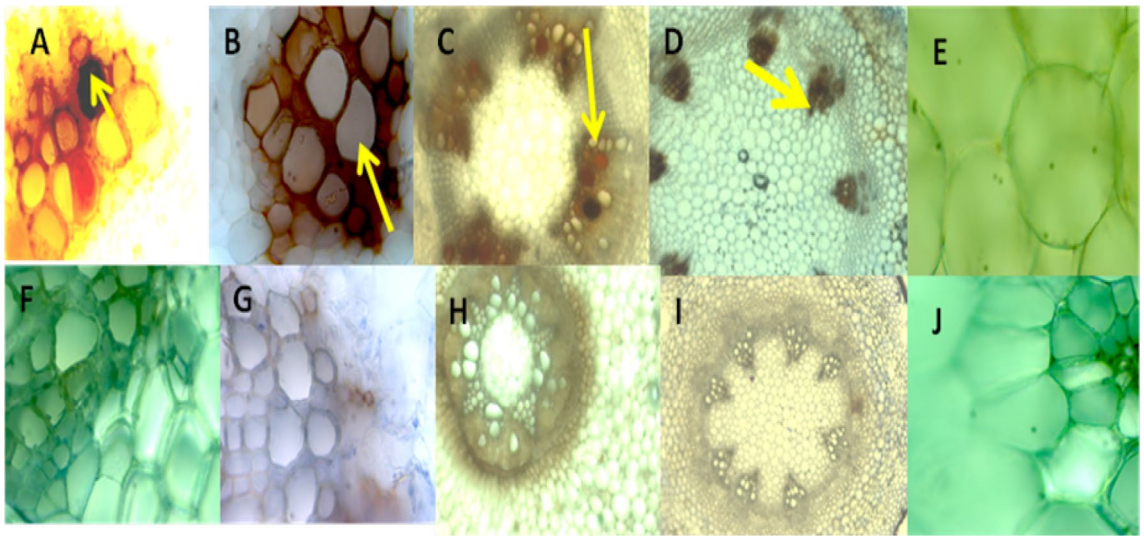

Fig. 2 Browning of xylem vessels in castor cultivars through bright field microscopy at 4 DAI. **a**–**d** Browning of xylem vessels in root vascular bundle in JI-35 (susceptible). **e** JI-35, healthy control. **f**–**i** Clear xylem vessels in root vascular bundle 481 (resistant). **j** 48-1, healthy control. Arrow indicates browning of xylem vessels

tissue (Fig. 2f, g). Thick and dense cell wall was observed in resistant genotype (Fig. 2h) than susceptible one. The root tissue was clear without browning in inoculated 48-1 (Fig. 2i). Browning of the vascular bundle was not observed in untreated control of 48-1 (Fig. 2j). Similar observations were reported by Brammall and Higgins (1988) in tomato cultivars infected with the pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* which limited fungal colonization in resistant tomato cultivars.

Scanning electron microscopy (SEM)

Increased colonization of fungal mycelium was observed on the surface of root tissue of inoculated JI-35 susceptible

genotype (Fig. 3). In control seedlings of both resistant and susceptible genotypes, one day after inoculation (DAI), the tissue was sterile and healthy without mycelium as shown in Fig. 3d, h. Entry of mycelium at one DAI in inoculated JI-35 root tissue was observed (Fig. 3a). The presence of germ tube and the increased mycelium was observed at 2 DAI (Fig. 3b, c), the tissue was distorted with more mycelium and spores (Fig. 3i, j). In inoculated seedlings of resistant cultivar (48-1), the root tissue was less colonized with mycelium and less number of spores at both one DAI and two DAI (Figs. 3e–g, k, l) than inoculated susceptible genotype.

Wafaa-Hagga et al. (2010) also reported more fungal mycelia, micro and macro conidia were observed in the stem and root vessels of susceptible genotype of *Mangifera indica* infected with *f. sub glutinans* through the SEM images, while percent colonization of Fusarium was significantly higher in susceptible genotype.

Transmission electron microscopy (TEM)

The susceptible and resistant cultivars of castor inoculated with *F.o.r* revealed that the more number of spores observed in inoculated root cells of susceptible cultivar (JI-35) (Fig. 4a, b). Less number of spores were observed in root tissue of 48-1, resistant cultivar (Fig. 4e, f) while spores were not observed in control root tissue of susceptible (Fig. 4c, d) and resistant cultivar (Fig. 4g, h).

The cell wall was thick in root tissue of 48-1 while thin cell wall was observed in root tissue of susceptible cultivar (JI-35) which also can be taken as a reason for the restriction of resistant cultivar to pathogen attack. In roots of JI-35, susceptible cultivar there was heavy colonization than roots of resistant cultivar and there were only fewer conidia produced in resistant cultivars compared to susceptible roots. Similar observations were also noticed in the wilt resistant and susceptible roots of lettuce (Vallad and Subbarao 2008).

In susceptible cultivar (JI-35) the infection led to the necrosis of the internal tissue and clogging of vascular bundles which may restrict the movement of water and nutrients to the plant. Our results can be compared by the works of Xu et al. (1997) who presented the entry of infecting hyphae and its penetration in the host epidermal, cortical and xylem vessels in the susceptible and resistant cucumber cultivars inoculated with *F. oxysporum* f. sp. *cucumerinum*.

Browning of vascular xylem vessels was observed in our experiment and this can be taken as a anatomical parameter for early detection of the wilt disease. The results were also compared with reports of Scott et al. (2013), that browning was observed in susceptible lettuce cultivars. Less colonized cultivars may prove to be good sources of

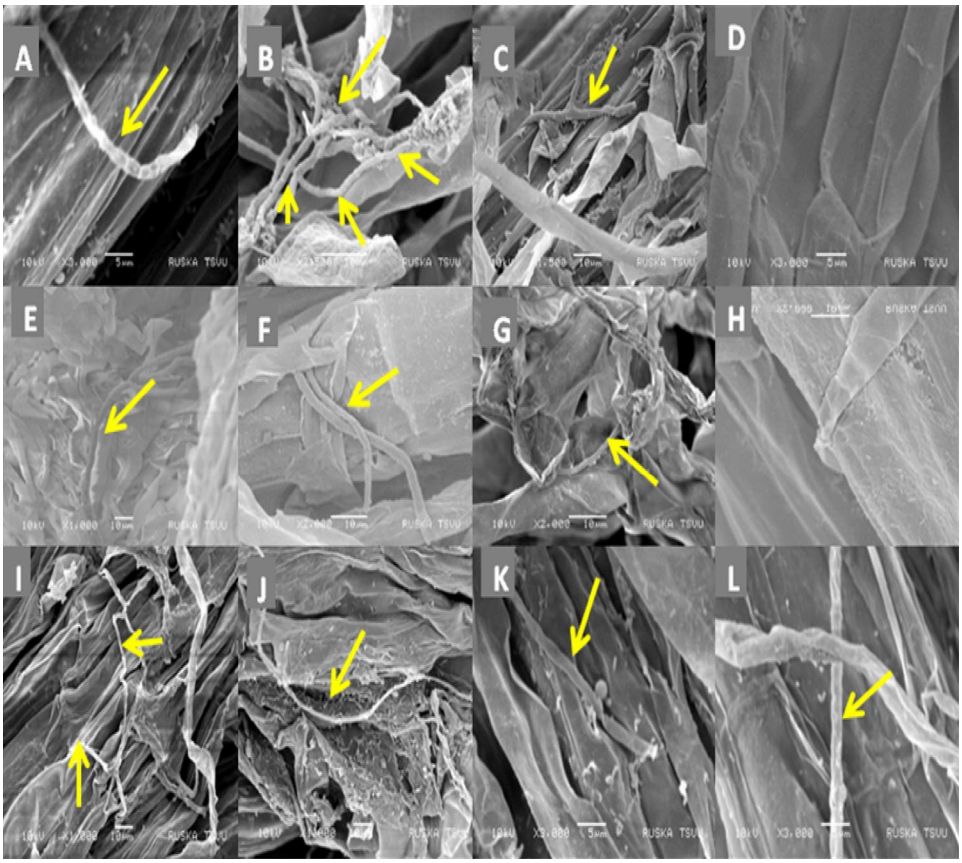

Fig. 3 Scanning electron microscopy images of Mycelium on root surface of resistant and susceptible castor cultivars. **a** JI-35, 24 h after inoculation. **b**, **c** JI-35, 48 h after inoculation **d** JI-35 control, **e** 48-1, 24 h after inoculation. **f**, **g** 48-1,48 h after inoculation, **h** 48-1 control. **i**, **j** JI-35, distubed root structure and more mycelium on it. **k**, **l** 48-1, clear root structure and less mycelium on it. Arrow indicates Mycelium

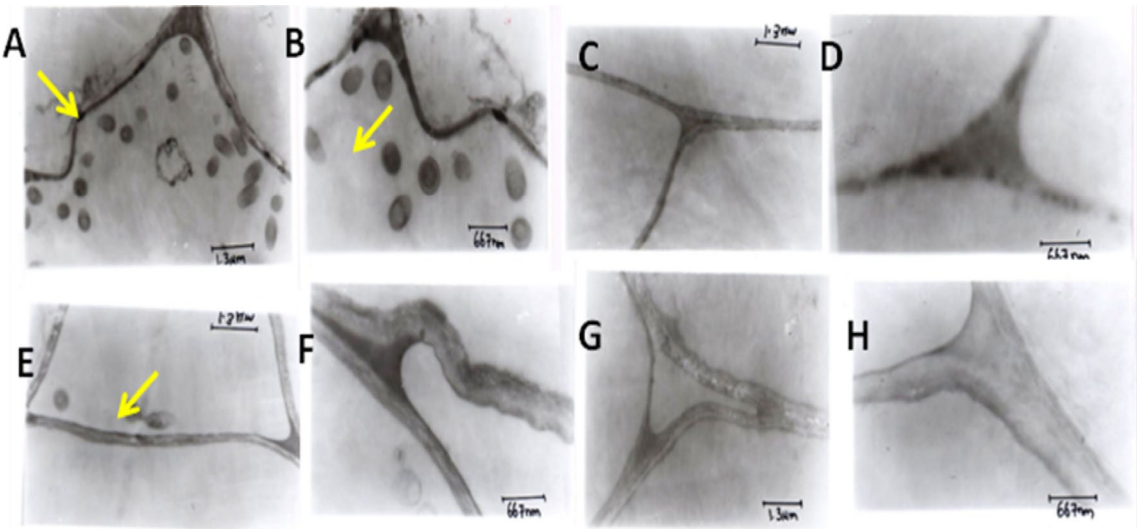

Fig. 4 Transmission electron microscopy images of Fusarium spores observed in root cells of resistant and susceptible castor cultivars at 48 h after inoculation. **a**, **b** Spores in JI-35 root tissue. **c**, **d** Control in JI-35. **e**, **f** spores in 48-1 root tissue. **g**, **h** control in 48-1. Arrows indicates Spores

resistance to Fusarium wilt to use as parental lines in breeding programmes.

Garcia-Sanchez et al. (2010) observed similar patterns of root colonization between resistant and susceptible cultivars of bean infected with *F. oxysporum* f. sp. *phaseoli* with increased amount of mycelium in susceptible cultivar. Jimenez-Fernandez et al. (2013) also observed that resistant genotype was colonized with less mycelium than susceptible genotype in chickpea crop infected with *F. oxysporum* f. sp. *ciceris.*

Biochemical studies

The values of enzyme activity levels for replicated values are subjected to factorial anova (analysis of variance). The different enzymes activity in castor cultivars inoculated wilt *Fusarium oxysporum* f. sp. *ricini.* were statistically significant for two way and three-way interaction between three factors along with main factors viz., genotype (G), days after inoculation (D), and enzyme concentration (C).

Superoxide dismutase (SOD)

The activity of the defense enzymes in root tissue of resistant and susceptible castor cultivars after inoculation with *F.o.r* was studied. The activities of SOD in roots of susceptible and resistant castor cultivars subjected to pathogen infection are presented in Table 1. The levels of SOD in Fusarium inoculated 48-1 ranged from 49.0 µmol/g FW (1 DAI) to 65.3 µmol/g FW (7 DAI) and in JI-35, it ranged from 49.0 µmol/g FW (1 DAI) to 59.70 µmol/g FW (7 DAI). The SOD activity in roots was observed to be significantly higher in castor wilt resistant genotypes compared to the susceptible genotypes. In inoculated, SOD activity increased continuously throughout the infection period in both genotypes. The enzyme activity was more in resistant genotype as compared to the susceptible one throughout infection period. In contrast, the activity increased up to 4 DAI in control roots and thereafter decreased gradually in both genotypes. The presence of defense enzymes released by the host restricts the pathogens activity in resistant genotype. These results are in agreement with the observation of Kapadia et al. (2013) who reported higher SOD activity recorded in the resistant castor plants when inoculated with *Fusarium oxysporum* f. sp. *ricini*. Garcia-Limones et al. (2002) suggested that the increased SOD activity in roots can be associated with resistance to Fusarium wilt in chickpea.

Peroxidase (POX)

Peroxidase (POX) activity in castor wilt susceptible and resistant genotypes was represented in Table 1. The levels of POX in pathogen inoculated JI-35, susceptible genotype ranged from 5.6 (1 DAI) to 2.6 nmol/min/g FW (7 DAI). In pathogen inoculated resistant genotype, the levels of POX ranged from 7.7 (1 DAI) to 4.9 nmol/min/g FW (7 DAI). In comparison, POX activity was more in resistant genotype than compared to inoculated susceptible genotype. POX activity decreased continuously in control plants in both the genotypes.

Our results can be compared with findings of Kumar et al. (2015) who reported the resistant cultivar (ICP-8863) showed significantly high enzyme activity in super oxide dismutase catalase and peroxidase than compared with

Table 1 Activity of enzymes in resistant and susceptible castor cultivars

Enzyme	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI
SOD	Superoxidedismutase (µmol/gFW[a])						
JI-35 con	47	52.8	54	71	58.5	61.6	53.6
JI-35 ino	49	52.6	56.4	71.9	64.4	56	59.7
48-1 con	47	48.3	53.9	54.6	61.1	57.5	61.2
48-1 ino	49	50.6	52	56	54.6	57.5	65.3
POX	Peroxidase (nmol/min/gFW[a])						
JI-35 con	6.2	5.8	5.3	4.3	3.9	3.2	2.4
JI-35 ino	5.6	5.5	4.9	3.6	3.4	2.8	2.6
48-1 con	8.6	7.9	7.5	6.0	5.6	5.1	4.5
48-1 ino	7.7	7.0	6.4	6.0	5.5	5.1	4.9
CAT	Catalase (µmol/min/gFW[a])						
JI-35 con	77	80	87	94	96	98	117
JI-35 ino	82.6	95	100	120	106	95	97
48-1 con	70	86	95	125.3	111.3	102.6	108
48-1 ino	85	90	100	125	120	118	117
APX	Ascorbate peroxidase (nmol/min/gFW[a])						
JI-35 con	267.9	276.4	285.7	294.6	296.4	324.3	286.2
JI-35 ino	345.7	312.5	305.4	267.9	255.4	253.6	232.1
48-1 con	250.0	257.7	260.7	309.1	267.9	263.9	262.5
48-1 ino	267.1	252.5	240.5	236.6	234.3	232.1	214.3
GR	Glutothione reductase (nmol/min/gFW[a])						
JI-35 con	20.4	22.4	22.9	24.5	26.1	28.6	31.9
JI-35 ino	35.0	33.8	33.3	30.2	24.5	21.2	19.6
48-1 con	21.2	27.1	34.0	36.8	32.7	30.8	21.5
48-1 ino	22.1	25.3	28.6	29.4	30.2	31.9	28.6
B-1,3 Glu	B-1,3-glucanase (mmol/min/gFW[a])						
JI-35 con	2.1	2.5	2.6	3.2	2.4	2.1	1.9
JI-35 ino	1.6	1.7	1.8	2.2	2.9	2.7	2.6
48-1 con	2.2	2.2	2.3	2.5	2.7	2.3	2.1
48-1 ino	2.1	2.4	2.5	3.0	3.2	3.3	3.4

Source of variation	CD 5%	F prob
Genotype (G)	0.63	0.000
Days after inoculation (D)	0.833	0.000
Enzyme concentration (E)	0.772	0.000
G×D	1.667	0.000
G×E	1.543	0.000
D×E	2.041	0.000
G×D×E	4.082	0.000

Values are mean values of two replications

DAI days after inoculation, *JI-35* susceptible genotype, *48-1* resistant genotype, *Con* control, *Ino* inoculated

[a]Units

susceptible cultivar (GS-1) infected with *Fusarium udum* in pigeonpea cultivars.

Catalase (CAT)

The levels of catalase enzyme in *Fusarium* inoculated JI-35, ranged from 82 (1 DAI) to 120 µmol/min/g FW (4 DAI) and decreased to 97 µmol/min/g FW (7 DAI). In 48-1, it ranged from 85 (1 DAI) to 125 µmol/min/g FW (4 DAI) and

then decreased to 117 µmol/min/g FW (7 DAI) as shown in Table 1. In inoculated plants, catalase activity increased at 4 DAI and then consistently decreased towards 7 DAI, throughout the infection period in both genotypes. In roots of control plant (JI-35) activity increased continuously from 77 (1 DAI) to 117 µmol/min/g FW (7 DAI), in 48-1, it ranged from 70 (1 DAI) to 125.3 µmol/min/g FW (4 DAI) and decreased to 108 µmol/min/g FW (7 DAI). More enzyme activity of catalase recorded in the inoculated 48-1 resistant plants than JI-35, susceptible plants.

These results are in agreement with the observation of Garcia-Limones et al. (2002) that the increase in CAT activity in roots of resistant plant can be associated with resistance to Fusarium wilt in chickpea. Kapadia et al. (2013) also reported that the enzyme activity of peroxidase and superoxide dismutase infected with *F. oxysporum* increased in the roots of susceptible castor genotypes, while the activity of catalase was more in the roots of the castor resistant genotypes. Similar results were reported earlier in banana against *F. oxysporum cubense* reported by Li et al. (2011), chickpea against *F. oxysporum* f. sp. *ciceris* reported by Garcia-Limones et al. (2002). More enzyme activity observed in resistant pearl millet plants infected with downy mildew fungus reported by Mahatma et al. (2011) and also, the tomato plants infected with *F. oxysporum* f. sp. *lycopersici* was reported by Mandal et al. (2008).

Ascorbate peroxidase (APX)

The levels of ascorbate peroxidase (APX) activity in inoculated JI-35 genotype ranged from 345.7 (1 DAI) to 232.1 nmol/min/g FW (7 DAI). In 48-1, resistant genotype, it ranged from 267.1 nmol/min/g FW (1 DAI) to 214.3 nmol/min/g FW (7 DAI) towards seventh day as shown in Table 1, while in inoculated roots, the APX activity decreased towards 7 DAI in both the genotypes through out infection period. The increase was more prominent in susceptible cultivar.

In control roots of JI-35, susceptible genotype, the enzyme activity increased from 276.4 (1 DAI) to 324.3 nmol/min/g FW (6 DAI) constantly and decreased towards 286.2 nmol/min/g FW (7 DAI). In 48-1, resistant genotype, the enzyme activity ranged from 250.0 (1 DAI) to 309.1 nmol/min/g FW (4 DAI) and decreased constantly to 262.5 nmol/min/g FW (7 DAI) towards seventh day. However the APX enzyme activity was more prominent in susceptible genotype than resistant genotype in both inoculated and control plants through out the infection period. However, the increase was more enhanced in susceptible genotype. Similar results were also reported by Garcia-Limones et al. (2002) in chickpea crop against *Fusarium oxysporum* f. sp. *ciceris* pathogen infection. Similar results were obtained by Arias et al. (2005) in susceptible sunflower cultivar causing chlorotic mottling caused by poty virus.

The increase in APX activities are rather more associated with establishment of compatible interaction of host and pathogen (Garcia-Limones et al. 2002). These reports are in accordance with our results that the susceptible genotype released more enzyme through out infection period.

Glututhione reductase (GR)

The levels of Glututhione reductase (GR) activity in inoculated JI-35 decreased from 33.8 (2 DAI) to 19.6 nmol/min/g FW (7 DAI) towards seventh day. In 48-1, it increased from 22.1 nmol/min/g FW (1 DAI) to 31.9 nmol/min/g FW (6 DAI) and slightly decreased to 28.6 nmol/min/g FW at (7 DAI) as shown in Table 1. In inoculated plants the enzyme activity was more in resistant genotype. In control roots, the JI-35 activity ranged from 20.4 (1 DAI) to 31.9 nmol/min/g FW (7 DAI). In 48-1, it increased from 21.2 (1 DAI) to 36.8 nmol/min/g FW (4 DAI) and decreased to 21.5 nmol/min/g FW (7 DAI). However the GR enzyme activity was more prominent in resistant genotype than susceptible genotype in both control and inoculated plants throughout the infection period. Molina et al. (2002) and Mandhania et al. (2006) also reported that the GR activity in resistant cultivars indicated that plants exhibit a more active ascorbate–glutathione cycle than the susceptible cultivars.

β-1,3-Glucanase

The levels of the enzyme β-1,3-glucanase in inoculated JI-35 reduced from 2.9 (5 DAI) to 2.6 mmol/min/g FW at (7DAI). In 48-1, it increased from 2.1 (1 DAI) to 3.4 mmol/min/g FW towards 7DAI shown in Table 1. In inoculated plants, the enzyme activity was more in resistant genotype. In control roots, the JI-35 activity increased from 2.1 (1 DAI) to 3.2 mmol/min/g FW (4 DAI) and drastically decreased to 1.9 mmol/min/g FW towards 7DAI. In 48-1, it increased from 2.2 on (1 DAI) to 2.7 mmol/min/g FW up to fifth day and decreased to 2.1 mmol/min/g FW by seventh day. The inoculated 48-1 plants showed more enzyme activity of β-1,3-glucanase. These results are in agreement with the observation of Kasprzewska (2003) where β-1,3-glucanase activity was higher in the resistant genotypes compared to susceptible genotypes. This result was also supported by the findings of Koretsky (2001) in soybean against Fusarium. In comparison the activity of enzyme in inoculated sample, it was more in resistant cultivar than in susceptible one.

The role of these enzymes in regulating the stress induced by pathogens in different crops revealed the increased levels of these enzymes in resistant genotypes and its important role in imparting resistance. These enzymes acted as resistance enhancer in castor genotypes against wilt disease.

In conclusion, the pathogen enters into the root by producing number of penetration hyphae and germ tubes in JI-35, susceptible genotype while less number of penetration hyphae and germtubes were observed in resistant genotype 48-1. Browning of xylem vessel was observed in JI-35, susceptible cultivar. The presence of resistance mechanism in resistant cultivar restricted the browning of xylem vessels in 48-1, resistant cultivar. Increased amount of mycelium and more spores were observed on the surface of root tissue in susceptible genotype than resistant genotype.

In the susceptible cultivar, JI-35 the dark browning started from 4th day and the plant started to detoriate and completely wilted within 7–8 days after inoculation. However, the resistant cultivar is highly stronger in appearance than the susceptible, throughout the infection period in both healthy and inoculated plants. These antioxidant enzymes are important in conferring resistance against pathogen. This study indicated that the activity levels of antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR) and β-1,3-glucanase were significantly higher in the resistant genotype than in susceptible genotype. The enzyme activity of ascorbate peroxidase was less in resistant genotype because of increased oxidative stress caused by the pathogen. The growth of pathogen was faster in susceptible genotype, than the resistant genotype. The restriction of growth of pathogen in resistant genotype is evident that specific resistance mechanism is functioning in this genotype.

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