SUMMARY

Chickpea genotypes (ten desi and eight kabuli), differing in their reaction to Fusarium wilt, were grown in Fusarium infested soil and screened for wilt resistance and susceptibility. The root and leaf tissues of highly resistant (desi WR 315 and kabul KAK 2) and highly susceptible (desi JG 62 and kabul L 550) chickpea genotypes were collected during disease development stages under pathogen infestation and non-infestation conditions. They were analyzed for antioxidant enzyme activities and isozymes profiling. The specific activities of ROS scavenging enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and catalase (CAT) were found higher in the root and leaf tissues under compatible interactions (infection) compared with incompatible (non-infested or non-infected?) during disease development stages. The susceptible compatible interactions of desi and kabuli chickpea genotypes induced systemic antioxidant bursts (SOD, GPX, APX, PPO, CAT) efficiently after Fusarium infection. The isozymes profiling of SOD, APX, PPO, CAT agreed with the enzyme activities and also showed differences in GPX zymogram pattern distinctive in the root of kabuli resistant KAK 2 during infection stage (S2). However, native-PAGE electrophoregram revealed distinctive bands for infected root tissues of desi resistant WR 315 (novel) and kabuli resistant KAK 2 (correlated with GPX isoforms) at S2 stage. The SOD, GPX, and PPO isoforms are more diverse and associated with Fusarium interactions during disease development stages. The results explained production of an oxidative burst and related responses during Fusarium wilt infection of desi and kabuli chickpea genotypes.

Keywords: Cicer arietinum L., Fusarium wilt, systemic resistance, antioxidant enzymes, isozymes pattern.

INTRODUCTION

Chickpea (Cicer arietinum L.) is an important pulse crop for a good source of carbohydrates and proteins. Protein quality is considered to be better than other pulses. Chickpea proteome profiling demonstrated the significance of essential amino acids with limited sulphur-containing amino acids. The same can be supplemented by adding cereals to the daily diet. As with other pulses, chickpea seeds also contain anti-nutritional factors which can be reduced or eliminated by different cooking techniques (Van der Poel, 1989). The minerals like Ca, Mg, P and K are also present in chickpea seeds. The chickpea is a good source of important vitamins such as β-carotene of vitamin A and vitamin B complex like riboflavin, niacin, thiamin, folate (Mishra and Kumar, 2005). Starch is the major storage carbohydrate in chickpea seeds followed by dietary fibre, oligosaccharides and monosaccharides. Although lipids are present in low amounts, chickpea is rich in nutritionally important unsaturated fatty acids such as linoleic, oleic acids. β-sitosterol, campesterol and stigmasterol are important sterols present in chickpea oil. Therefore, chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits (Jukanti et al., 2012).

Fusarium wilt is the most yield reducing disease of chickpea (Cicer arietinum L.) and wide-spread in the growing areas of Asia, Africa, southern Europe and the Americas between latitudes 30°N and 30°S, where the crop growing season is dry and warm (Nene et al., 1980). The main reason for low productivity in chickpea is the adverse ecologies in which it is cropped and its vulnerability to abiotic and biotic stresses (Dwivedi et al., 2005). Annual yield losses due to wilt have been estimated at 10 to 90%. The causative agent of this disease has been classified as Fusarium oxysporum f. sp. ciceri (FOC) (Jimenez-Gasco et al., 2001). The FOC is seed-born and is found as chlamydospore-like structures in the hilum region of the seed (Haware et al., 1978).

Fusarium wilt of chickpea was first noticed in 1918 and later Prasad and Padwick (1939) isolated Fusarium from wilted chickpeas and conducted pathogenicity tests by
Antioxidant enzymes induced by *Fusarium* in chickpea

MATERIALS AND METHODS

**Plant materials and *Fusarium* isolates.** The seeds of ten ‘desi’ (WR 315, JCP 27, GG 1, GG 2, GG 4, GIG 3, JG 16, D, YELLOW, CHAFFA and JG 62) and eight ‘kabuli’ (KAK 2, JGK 1, BGD 128, PHULE G 0517, PKV 4, IPCK 2002-29, IPCK 2004-29 and L 550) genotypes of chickpea (total eighteen) were obtained from the Pulse Research Station, Junagadh Agricultural University, Junagadh. The fungal pathogen was isolated from infected roots of JG 62 chickpea genotypes using tissue isolation techniques and pure culture of phytopathogen was sent for identification at Indian type culture collection (ITCC), Division of plant pathology, IARI, New Delhi. The pathogen was identified as *Fusarium oxysporum* f. sp. *ciceri* based on morphology and microscopy characters (Burgess *et al.*, 1994). The chickpea genotypes were screened for susceptibility to *Fusarium* wilt under pathogen infestation; most resistance and susceptible genotypes were used for antioxidant profiling at different disease development stages.

**Soil inoculation, *FOC* infestation and *Fusarium* wilt incidence.** The sand maize meal medium was used for preparation of mass inocula in the laboratory. It was prepared in 250 ml flask using 80 g fine sand and 20 g maize meal per flask, and 20 ml of distilled water was added to each flask to moisten the mixture properly. The medium in flasks was then sterilized at 1.036 kg/cm² (15 lb psi) for one h for three consecutive days. The flasks were inoculated with mycelial plug of fungus *FOC* (4 mm) and incubated at 28°C for 7 to 9 days. The culture was then used for soil inoculation. Earthen pots (18 cm diameter) were used for pot culture studies. Pots were sterilized with 5% formaldehyde solution. Field soil with farm yard manure (FYM) and sand were mixed in the proportion of 1:2 and sterilized in autoclave at 1.036 kg/cm² for one h for three consecutive days. *FOC* culture (cfu $3 \times 10^{-6}$) was then added to the soil in the proportion of 1:10 (inoculum: sterilized soil). Pots were filled with non-infected and pathogen infected soil and watered for three days. Before sowing of chickpea genotypes (25 seeds per pot), the infection level of pathogen in infested pots were measured and it was found about $4 \times 10^{-5}$ cfu g$^{-1}$. Disease incidence of eighteen chickpea genotype was measured under pathogen infected soil culture in three replications (*Supplementary Fig. S1a and S1b*). Percentage of disease incidence was recorded during pre-infection (S1-10 DAS), infection (S2-18 DAS), and post-infection (S3-21 DAS) stages using following formula (Dubey and Singh, 2004).

\[
\% \text{ Disease incidence} = \left( \frac{\text{Diseased plants}}{\text{Total plants}} \right) \times 100
\]

**Antioxidant enzymes activities and isozymes profile.** Total 18 chickpea genotypes (ten desi and eight kabuli) were screened for *Fusarium* wilt incidence. The root and leaf tissues of highly resistant and susceptible genotypes of each group were examined for antioxidant enzymes and isozymes profile under pathogen infestation and non-infestation condition during disease development stages. The root and leaf samples were collected from pot culture study and kept under ice cold condition until buffer extraction.

**Sample preparations.** The root and leaf tissues of highly resistant (desi WR 315 and kabuli KAK 2) and highly susceptible (desi JG 62 and kabuli L 550) chickpea genotypes were collected during disease development stages (10 DAS as pre-infection stage-S1, 18 DAS as infection stage-S2 and 21 DAS as post-infection stage-S3) under infestation and non-infestation conditions. The root and leaf tissues (0.5 g)
were homogenized with a pre-chilled mortar and pestle under ice cold condition in 5 ml of extraction buffer, containing 50 mM sodium phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 rpm for 20 min and the supernatant was used for the assay (Joshi et al., 2012). The protein content was determined from extracts using Folin Lowry method for expression specific activity of enzymes.

**Enzyme activity assay.** SOD (EC 1.15.1.1) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA and 0.05 ml enzyme extract followed by riboflavin (Van Rossun et al., 1997). The tubes were shaken and placed 30 cm below from a light consisting of four 15-W fluorescent lamp. The reaction was allowed to lamp for 10 min. The photoreduction in NBT was measured as increase in absorbance at 560 nm. Blank and control were run the same way but without illumination and enzyme, respectively. One unit of SOD was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50% in a reaction mixture. Enzyme unit of SOD was calculated according to formula given by Constantine and Stanley (1977).

Glutathione peroxidase (GPX) (EC 1.11.1.7) activity was determined in the homogenates by measuring the increase in absorption at 470 nm due to the formation of tetraguaiacol (ε = 26.6 mM−1 cm−1) in a reaction mixture containing 50 mM sodium phosphate buffer pH 7.0, 0.1 mM EDTA, 25 μl enzyme extract, 10 mM guaiacol and 10 mM H2O2 (Costa et al., 2002). APX (EC 1.11.1.11) activity was measured immediately in fresh extract and was assayed as described by Nakano and Asada (1981). The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer, pH 7.0, 0.1 mM H2O2, 0.5 mM ascorbic acid, 0.1 mM EDTA and 0.1 ml enzyme extract for APX. The hydrogen peroxide dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (ε = 2.8 mM−1 cm−1). Polyphenol oxidase (PPO, EC 1.14.18.1) activity was measured using reaction mixture containing 2.9 ml of catechol (10 mM catechol in 10 mM phosphate buffer, pH 6.5, and reaction was initiated by the addition of 100 μl of enzyme extract. The changes in the colour due to the oxidized catechol were read at 490 nm for one minute at an interval of 15 second. Blank was carried out without substrate. The enzyme activity was expressed as U mg−1 protein and unit activity was defined as Δ OD min−1 g−1 Fr.Wt. tissues (Malik and Singh, 1980). The CAT (EC 1.11.1.6) activity was determined in the homogenates by measuring the decrease in absorption at 240 nm as H2O2 (ε = 39.4 mM−1 cm−1). The enzyme activity was measured in reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 10 mM H2O2 and 10 μl enzyme extract and expressed as μmol H2O2 oxidized min−1 g−1 protein as described by Aebi (1984).

**Native PAGE and isozymes staining.** Native-PAGE and isoenzymes analysis are based on the charge and size of the protein molecules (Laemmli, 1970). Since the native proteins are small and exist in quaternary structure, a 12% polyacrylamide gel was used for native protein profiling (Sambrook et al., 2001). Isozymes of SOD were separated on a 10% non-denaturing polyacrylamide gels. After electrophoresis, SOD isoforms were visualized by following the method of Beauchamp and Fridovich (1971). Gels were stained in 50 mM sodium phosphate buffer, pH 7.8, containing 0.24 mM NBT and 28 μM riboflavin for 20 min in the dark followed by immersion in 50 mM sodium phosphate buffer, pH 7.8, containing 28 mM TEMED, then exposed to a light source at room temperature until white bands appeared in blue background.

The GPX isozyme profile was carried out in 4% stacking and 8% separating gel. GPX was stained according to method described by Srivastava and Huystee (1977). Gel were equilibrated with 100 mM potassium phosphate buffer, pH 6.5, for 15 min, then incubated in 12.5 mM guaiacol solution containing 1.7 mM benzidine and 12 mM H2O2 up to appearance of brown-orange bands against a clear background. The APX was carried out in 4% stacking and 10% separating gel. APX stained according to method described by Mittler and Zilinskas (1993), based on the inhibition of NBT reduction by ascorbate. Following electrophoretic separation, gels were equilibrated with 50 mM potassium phosphate buffer, pH 7.0, containing 2 mM sodium ascorbate for 30 min (the buffer was changed by each 10 min incubation). Then, gels were incubated in the buffer given above amended with 4 mM sodium ascorbate and 2 mM H2O2 for 20 min, stained in 50 mM potassium phosphate buffer alone pH 7.8, amended with 28 mM TEMED and 2 mM NBT, and agitated gently for 2-3 min up to appearance of clear bands on an intense blue background due to NBT reduction by ascorbate.

The PPO isozymes were separated on 10% non-denaturing polycrylamide gels. Isoforms of POX were visualized by incubating the gel in 100 mM sodium phosphate buffer (0.025 M, pH 6.0) containing 100 μl of 30% H2O2, for 5 min with gentle shaking. After this, o-dianisidine (50 mg dissolved in 1 ml methanol) was added and kept in dark with occasional shaking until bands appeared (Sadasivam and Manickam, 1996). CAT isozymes were separated on 6% non-denaturing polyacrylamide gels at 4°C and were visualized following the method of Woodbury et al. (1971). Gels were rinsed three times with Millipore water for 15 min each followed by incubation in 0.003% (v/v) H2O2 for 10 min and rinsed briefly with Millipore water. To detect the CAT isoforms gels were stained in 1% ferric chloride and 1% potassium ferricyanide solution (prepared separately and mixed fresh before using) with shaking, then washed with Millipore water.

Isozyme banding patterns were recorded on the basis of number and the relative front (Rf) values of the bands. The Rf value is the mobility of each isozyme band that
travelled from the origin divided by the distance travelled by the front tracking dye. The Rf value of each respective bands on isozyme patterns was determined to allow precise comparisons among the various treatments of resistant and susceptible genotypes.

**Statistical analysis.** Growing, inoculation and sampling of plants were made in three independent experiments or replications. Analyses were performed in duplicate for each parameter within replications. The disease incidence was analyzed by complete randomized design (CRD) as statistical tool for interpretation of data using statistical package for the social sciences (SPSS) software. The variation in enzyme activities was presented in graphical forms with bars indicating the standard error between three replications.

**RESULTS**

**Fusarium wilt incidence.** The ten desi and eight kabuli chickpea genotypes (total 18) were found symptomless during pre-infection stage (S1-10 DAS). The chickpea genotypes showed 80 to 100% germination as recorded on 10 DAS and absence of wilt disease symptoms. Plants developed first symptoms characteristics of Fusarium wilt during 15 to 18 days after sowing (DAS) under pathogen infestation (**Supplementary Fig. S1a and S1b**). Disease incidence was recorded in the range of 11 to 70% in different desi and kabuli genotypes at the infection stage (S2-18 DAS). Resistant genotypes WR 315 (desi) and KAK 2 (kabuli) genotypes showed 18.94% and 11.11% disease incidence under pathogen infestation. The percent disease incidence was up to 90% during the post-infection stage of disease development (S3-21 DAS). Per cent disease incidence was recorded 89.86% and 90.48% in susceptible JG 62 (desi) and L 550 (kabuli) genotypes, respectively (Table 1).

**Antioxidant enzyme activities.** Out of 18 chickpea genotypes, highly resistant WR 315 (desi) and KAK 2 (kabuli) and highly susceptible JG 62 (desi) and L 550 (kabuli) genotypes were examined for antioxidant enzymes and isozymes profile under pathogen infestation and non-infestation environment during disease development stages. The specific activity of SOD was influenced by FOC infection under compatible interactions and increased during S1 to S3 stage of disease development in chickpea genotypes. The root tissues of susceptible desi JG 62 showed the highest SOD activity during disease infection stage (S2). In general, the SOD activity of infected root tissues was higher compared with infected leaf tissues (Fig. 1A). In case of GPX activity, susceptible desi JG 62 and kabuli L 550 genotypes showed 1.32 fold and 1.22 fold higher activities, respectively, in their infected root tissues (compatible interactions) compared with non-infected root tissue during infection stage (S2). The root and leaf tissues of susceptible genotypes JG 62 and L 550 elevated

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**Table 1. Percent disease incidence of Fusarium wilt at different disease development stages in chickpea genotypes.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Chickpea genotypes</th>
<th>Germination (%)</th>
<th>Pre-infection 10 DAS</th>
<th>Pre-infection 10 DAS</th>
<th>Infection 18 DAS</th>
<th>Post infection 21 DAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Desi</td>
<td></td>
<td>96 (24)</td>
<td>0.00</td>
<td>18.94</td>
<td>27.15</td>
</tr>
<tr>
<td>2</td>
<td>JCP 27</td>
<td></td>
<td>92 (23)</td>
<td>0.00</td>
<td>28.99</td>
<td>53.62</td>
</tr>
<tr>
<td>3</td>
<td>GG 1</td>
<td></td>
<td>92 (23)</td>
<td>0.00</td>
<td>26.09</td>
<td>55.07</td>
</tr>
<tr>
<td>4</td>
<td>GG 2</td>
<td></td>
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<td>0.00</td>
<td>27.78</td>
<td>40.28</td>
</tr>
<tr>
<td>5</td>
<td>GG 4</td>
<td></td>
<td>100 (25)</td>
<td>0.00</td>
<td>37.33</td>
<td>56.00</td>
</tr>
<tr>
<td>6</td>
<td>GJG 3</td>
<td></td>
<td>96 (24)</td>
<td>0.00</td>
<td>36.11</td>
<td>59.72</td>
</tr>
<tr>
<td>7</td>
<td>JG 16</td>
<td></td>
<td>96 (24)</td>
<td>0.00</td>
<td>58.33</td>
<td>77.78</td>
</tr>
<tr>
<td>8</td>
<td>D.YELLOW</td>
<td></td>
<td>96 (24)</td>
<td>0.00</td>
<td>61.11</td>
<td>79.17</td>
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<tr>
<td>9</td>
<td>CHAFFA</td>
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<td>92 (23)</td>
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<td>68.12</td>
<td>82.61</td>
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<tr>
<td>10</td>
<td>JG 62</td>
<td></td>
<td>92 (23)</td>
<td>0.00</td>
<td>66.67</td>
<td>89.86</td>
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<tr>
<td>11</td>
<td>Kabuli</td>
<td></td>
<td>96 (24)</td>
<td>0.00</td>
<td>11.11</td>
<td>25.00</td>
</tr>
<tr>
<td>12</td>
<td>JGK 1</td>
<td></td>
<td>76 (19)</td>
<td>0.00</td>
<td>19.05</td>
<td>49.21</td>
</tr>
<tr>
<td>13</td>
<td>BGD 128</td>
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<td>92 (23)</td>
<td>0.00</td>
<td>27.54</td>
<td>39.13</td>
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<tr>
<td>14</td>
<td>PHULE G 0517</td>
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<td>84 (21)</td>
<td>0.00</td>
<td>28.07</td>
<td>43.86</td>
</tr>
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<td>15</td>
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<td>0.00</td>
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</tr>
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<td>53.62</td>
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<td>84 (21)</td>
<td>0.00</td>
<td>69.84</td>
<td>90.48</td>
</tr>
</tbody>
</table>

Values in parenthesis indicates number seeds germinated out of 25 and percent disease incidence are based on number of seedling germinated at pre-infection stage. S. Em. ± = Standard Error of Mean; C.D. at 5% = Critical differences at 5% (t value); C.V.% = Coefficient of Variance.
the GPX activities as compared to resistance genotypes (WR 315 and KAK 2) under pathogen infestation conditions (Fig. 1B). The APX activity increased in both root and leaf tissues during pre-infection (S1) to post-infection (S3) stage of disease development under compatible interactions. The activity was about 1.34 fold higher in infected root tissue of JG 62 compared with non-infected at S3 stage. Among kabuli genotypes, KAK 2 resistant genotype showed significantly higher activity in their root and leaf tissues under incompatible interaction compared with susceptible genotype L 550 (compatible) at post-infection stage S3 (Fig. 2A).

The desi resistant WR 315 genotype showed 1.34 and 1.23 fold higher CAT activities in their infected root and leaf tissues (compatible interactions), respectively, compared with incompatible interactions at S3 stage.

**Isozymes banding pattern.** The activities of the enzymes involved in ROS scavenging were also analysed by native PAGE in the same extract of root and leaf tissues of inoculated and uninoculated chickpea genotypes. The number of bands was higher with good intensity in leaf tissues and was compared with root in resistant and susceptible chickpea genotypes. Native PAGE electrophoregram revealed unique zymograms with Rf value 0.476 for infected root tissues of desi resistant WR 315 and kabuli resistant KAK 2 at S2 stage (Fig. 3A; Supplementary Table S1). The SOD zymogram profile showed ample multiplicity in roots and leaf tissues extract. Maximum 6 SOD isoforms were detected in S3 stage of infection (Fig. 3B; Supplementary Table S2A). The intensity of SOD isoforms was higher in root tissues followed by leaf tissues of infection stage (S2) compared with S1 and S3 stages. This may be correlated with higher specific activity SOD in root and leaf tissues of S2 stage in chickpea genotypes. The GPX zymograms showed different banding pattern at all three stages. The maximum variation in zymogram pattern was noticed during infection stage S2 (Fig. 4A; Supplementary
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Table S2B). Root tissues showed higher activities in comparison to leaf tissues. Both root and leaf tissues examined had remarkable dark brown isoforms as compared to non-infected, light brown isoforms. The GPX zymogram evoked single band only in infected root of KAK 2 resistant kabuli genotype (Fig. 4A).

The APX showed multiplicity in roots and leaf tissues as SOD. APX zymogram gave monomorphic banding pattern, although no genotypic variation (Fig. 4B; Supplementary Table S3A). In APX electrophoregrams, infected genotype showed more intense isoforms as resulted from non-infected root and leaf tissues. Zymogram of PPO was different in roots as compared with leaf tissues of all four genotypes. This was in good accordance with the spectrophotometrically assayed levels of this enzyme activity. The PPO isoforms of infected roots of susceptible (JG 62 and L550) genotypes showed more intense banding pattern as compared with resistant (WR 315 and KAK 2) genotypes (Fig. 5A; Supplementary Table S3B). The unique PPO isoform was examined for susceptible JG 62 (desi) during pre-infection stage (S1). The same trend was observed in leaf zymograms. The same profile was observed for CAT in root and leaf tissue of resistant and susceptible desi and kabuli genotypes during three disease development stages (Fig. 5B; Supplementary Table S3C). For CAT, the electrophoregram indicated that two isoforms occurred in leaf, a highly predominant form of very low mobility and minor form, while the low mobility bands were found in root.

**DISCUSSION**

This study analyzed the induction of various enzymes of oxidative metabolism during interaction of FOC with resistant and susceptible chickpea genotypes (desi and kabuli). Previous studies have been reported with a limited number of pathosystems referring to localized infections of foliar tissues by obligate biotrophic or necrotrophic...
pathogens for which incompatibility is associated with a rapid development of hypersensitive response. Contrary to that, very little is known for plant pathogen interactions characterized by systemic infection, viz., Fusarium wilt of chickpea, that are produced at root level and apparently could not develop such localized response.

The present study reported induction of antioxidative enzyme system during host-pathogen interaction of desi and kabuli chickpea genotypes with FOC. Results indicated that FOC infection leads to substantial changes in the antioxidant status of chickpea genotypes. There were clear differences between compatible and incompatible interactions as well as between root and leaf tissues of the chickpea in response to FOC infection. The most common responses were the increase of CAT, POX and SOD activities in root and leaf tissues of chickpea by the pathogen under compatible environment. The SOD and CAT activities resulted to be involved in the regulation of H₂O₂ levels in plant tissues (Lamb and Dixon, 1997). Similarly to the present study, Joshi et al. (2012) examined two ‘desi’ chickpea cultivars viz., susceptible JG 62 and resistant WR 315. The activity of SOD maximal increase in JG 62 was comparable with WR 315 in the infected root tissues. A decrease in SOD activity as a result of infection by the pathogen should give rise to an increase in superoxide persistence in the root apoplast of infected plants.

There was a two fold increase in the GPX activity in the root tissues of the infected plants during the pre-infection to post-infection stages. In case of PPO, desi susceptible JG 62 genotype showed 1.56 fold higher activity compared to non-infected root tissue, where kabuli susceptible L 550 genotype showed 1.04 fold highest activities as compared to non-infected root tissue during post-infection stage. This may be due to the fact that the preferred site for wilt infection in chickpea are the root tissues close to the point of seed attachment. Fungal hyphae first colonize the root xylem and then the xylem of shoot. The responses specifically linked to the susceptible compatible interactions where the maximum induction of SOD, GPX and APX was found in roots of susceptible desi JG 62 at S2 stage and GPX and CAT elevated in susceptible kabuli L 550. The pathogen enters the root and stem xylem of susceptible chickpea plants but not that of resistant plants. The activation of antioxidant bursts, particularly, the ASC-glutathione cycle is a response to the H₂O₂ production in the xylem parenchyma due to the presence of the
pathogen in susceptible tissues. Among the antioxidant enzymes, APX and GPX are the two enzymes, which relate to the ASC-glutathione cycle for H$_2$O detoxification in plants (Nakano and Asada, 1981; Stevenson et al., 1994). In response to the biotic stress adopted in the present experiment, the chickpea root and leaves tissues from the infected plants resulted in a 1.34 fold increase in APX activity (Garcia et al., 2008). The GPX-mediated oxidative cross-linking of structural proteins and possibly other polymers makes the cell wall refractory to digestion by microbial protoplasting enzymes (Brisson et al., 1994). These rapid modifications may enhance the effectiveness of the cell wall as a barrier to slow pathogen spread. In our study, the increased GPX activity might have rendered it less efficient in antioxidative activities. The CAT isozyme patterns resulted monomorphic during all three stages of disease development stages, similarly to APX banding pattern, in agreement with Garcia et al. (2002). GPX isoform with Rf 0.508 value were found to be associated with the resistant kabuli KAK-2 genotype rather than with susceptible genotypes. In the case of PPO, intense bands were found in resistant genotypes compared to susceptible genotypes. The isoforms of SOD, APX, CAT could not be distinguished between resistant and susceptible genotypes in agreement with the results of Garcia et al. (2002).

Antioxidative enzymes protect the plant from the damage of oxidative stress. SOD, GPX, APX, and lipoygenase are the major ROS scavenging enzymes of plants (Gajera et al., 2015, 2016). Our previous studies suggested pathogen dependant systemic activation of the defence reaction in groundnut. The results showed that *Aspergillus* infection is necessary for antioxidant enzymes activation in groundnut varieties but it is significantly enhanced in susceptible interactions. SOD is thought to be the key enzyme involved in H$_2$O$_2$ production. GPX plays an important role in the fine regulation of ROS concentration in the cell through activation and deactivation of H$_2$O$_2$. The ascorbic acid is utilized as a specific electron donor by APX and reduces H$_2$O$_2$ to water while GPX oxidized glutathione to form oxidized glutathione (Noctor and Foyer, 1998).

The antioxidant enzyme activity in resistant and susceptible, desi and kabuli chickpea genotypes elevated variably under compatible and incompatible interactions (non-infection) with FOC. *Fusarium* infection is necessary to induce oxidative bursts in chickpea genotypes. Antioxidant bursts in susceptible compatible interactions could be compared with resistant compatible interactions. The native protein bands was unique in resistant genotypes of desi (WR 315) and kabuli (KAK 2) at disease infection stage (S2) which may be novel and correlated with GPX isoforms. The antioxidative responses were found prominent in kabuli compatible interactions compared with desi. Results obtained with induction of antioxidative enzymes activities and isozyme profiles may probably provide more conclusive insights about the production of an oxidative burst and related responses and their role in the pathogenesis of Fusarium wilt of desi and kabuli chickpea genotypes. The SOD, GPX, and PPO isoforms are more diverse and associated with *Fusarium* interactions during disease development stages. The results showed that kabuli genotypes are prominent for induction of an oxidative burst during disease development stages compared with desi chickpea genotypes. Further, unique native protein bands found with resistant genotypes of desi and kabuli might be eluted and characterized with respect to amino acid profiling to find out novel protein responsible for resistant to chickpea genotypes.
REFERENCES


