

Original Research Article

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Inbuilt Characteristics of Hydrolytic Enzymes Activities in Root Tissues of Chickpea (*Cicer arietinum L*) Cultivars of Gujarat against Fusarium Wilt (*Fusarium oxysporium f.spp ciceri*) Disease Infection

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ABSTRACT

A field experiment was conducted In Sick plot of Pulse research station, JAU., Junagadh. To find out the genetic makeup of hydrolytic enzymes of six chickpea cultivars that was grown in normal (healthy soil) and sick plot (Diseased soil) in response to wilt disease. The pattern of these enzymes showed The β -1,3-glucanase revealed that the significantly higher activity as the growth of plants and as the disease development, Plants grown in sick plot, the β -1, 3-glucanase activity in the different cultivars were varied between 89.02 to 368.42 μ mole glucose released.h-1.g-1.fr.wt. Susceptible cultivar JG-62 and GG-4 had significantly higher activity, than resistant and tolerant cultivars. Similarly In case of chitinase activity resulted significant differences in our experiments. The root tissues obtained from sick plot visualized higher chitinase activity as compared to the tissues received from normal plot. Among the cultivars, susceptible cultivars have similar trend as observed in β -1, 3- glucanase. Lower level of the enzyme activity in root tissues was observed in tolerant cultivars. Thus hypothesis on the basis of fungal invasion is also proved by hydrolytic enzymes and its can be classified as GG-1(tolerant), GG-2 (Tolerant), JG-62 (Highly susceptible), JCP-27, WR-315, (Highly Resistant) and GG-4 (Susceptible) to fusarium. It can be concluded that both β -1, 3-glucanase and chitinase activity having defensive role thus it can be used as biochemical marker for identification of fungal resistant cultivars.

Keywords

β -1,3- glucanase,
Chitinase, Chickpea

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Introduction

Chickpea (*Cicer arietinum L.*) is the second most important pulse crop of the world. India is the world's largest chickpea growing country having contributes about 63 percent

to the global production of chickpea. Gujarat having cultivation area of 0.17 lakh hectares and an output of 0.09 metric tonnes with yield 530 kg/ha in 2000-01(Anon, 2003). Now it is not much change in yield.

Chick pea flour (besan) is an ingredient in various types of sweets and bhajiya. Chick pea is also having medicinal effects for blood purification. Its nutritional composition varies with varieties but averages are 21.1-22.8 per cent protein, 55-61.5 per cent carbohydrates, 3-4.5 per cent fat. Chickpea is rich in calcium, iron and niacin. (Rathod and Vakhariya, 2008) so far as In a data of the re-sequencing of 429 chickpea accessions from 45 countries identified key candidate genes that were under selection and those associated with agronomically important traits (Varshney *et al.*, 2019). Numerous approaches are taken from world scientist on crop improvements from genomics, proteomics, transcriptomics, as well as in recently suggested novel approach called “super-pangenome”, which includes the development of pangenome or pangenomes of different species in a given genus, provides an opportunity to identify genus-level genomic variation (Khan *et al.*, 2020). But without the Fundamental knowledge of enzymes works on pathogen and activities differ in different condition are very crucial to give most imperative wrapping up in given experiments. So Author has tried to justify the activities of both enzymes during inflectional, and normal condition in chickpea.

Wilt of chickpea (*Cicer arietinum*), caused by *Fusarium oxysporum* f. sp. *ciceris* is a major limiting factor of chickpea production in the Mediterranean Basin and the Indian Subcontinent (Jalali and Chand, 1992). Annual yield losses due to *Fusarium oxysporum* f.sp. *ciceri* have been epidemics and devastating to individual crops and cause 100% loss under favorable conditions (Halila and Strange, 1996; Chaube and Punthir, 2005). Defense reaction of the plant that is related to the mechanisms of cell wall modification is the rapid formation of papillae-localized appositions of dense material between the plasmalemma and the

cell wall at the penetration site of the pathogen. They are composed of cross-linked proteins, phenolic compounds and callose, (Heitefuss, 1997). β -1,3-glucanase enzymes that act on other substrates present in the cell wall include invertase, peroxidase, phosphatase and various dehydrogenases.

Enzymes with potential activity against fungal pathogens include chitinases and β -1, 3-glucanases (Cosgrove, 1997). Since the accumulation of PR-proteins and cell wall enforcement by oxidative cross-linking of structural proteins and the formation of papillae have been documented in the interaction between *F. graminearum* and wheat (Pritsch *et al.*, 2000; Pritsch *et al.*, 2001; El-gendy *et al.*, 2001; Kang and Buchenauer, 2003), fungal proteases are almost certainly part of the interaction between the pathogen and the host. Therefore the present enzymes β -1, 3-glucanases and chitinase were examined biochemically to elucidate the changes in enzyme activities in various growth stages as well as to prove hypothesis of design of experiments in various cultivars for better used in predicted off climate situations.

Materials and Methods

Field experiments was conducted with the experimental design of split plot at Pulse research station, Junagadh Agricultural University Junagadh., Gujarat India. Two different separate plots as main factors, Three different stages were (preinfectional, inflectional and post inflectional stages), Varieties as split factors in both plots, in which the Chickpea cultivars GG-1 (tolerant), GG-2 (Tolerant), JG-62 (Highly susceptible), JCP-27, WR-315, (Highly Resistant) and GG-4 (Susceptible), were grown under field condition in two plots. One plot was *i.e* normal plot (Helathy) without diseased while other was kept free for infection of wilt

disease in chickpea plants i.e, Sick plot (Diseased plot) that is maintaining, since 28 years for *F.oxysporium f.sp.ciceri* with appropriate inoculation and tested for diseased infection in the seasons as well as use for AICRP trial too. Root tissues were harvested with preconditioning of tap water and cleaning effects at pre infectional (12 Days after sowing), infectional (21 days after sowing) and post infectional (26 Days after sowing) stage from both plots. Sample was preserve in -80°C until analysis is over.

Extraction and assay of activity β -1-3 glucanase (EC 3.2.1.16)

The enzymes β -1-3 glucanase were estimated using the method suggested by Malik and Singh (1980). Kauffman *et al.*, (1987) and Nelson (1944). Weigh one gram root tissue grind with 10ml of 0.1M sodium Acetate pH 4.8 addition with 15mM Mercapto ethanol, 1mM PMSF. Centrifuged to 10000 rpm, 15 min, -4°C . As a substrate laminarin was used. Assay mixture consists of 0.4ml 0.1M sodium Acetate buffer pH 4.8 with 2.5 mg laminarin. To initiate reaction 0.5ml Enzyme with assay mixture incubates temperature at 37°C for 2hour. Glucose releases per hour which was measured Spectrophotometrical at 660 nm. Enzyme activity expressed in μmole glucose released/h/g.

Extraction and Assay of activity for Chitinase assay (EC 3.2.1.14)

Determination of N- acetylglucosamine: (for Chitinase assay): The enzymes chitinase were estimated using the method suggested by (Boller and Mauch ,1988; Reissig *et al.*,1955). Suitable aliquot (0.5ml) after incubation was taken in to test tubes and 0.1 ml 0.12 M potassium borate buffer pH 8.9 was added. The tubes were kept in boiling water bath exactly for 3 min and cooled in tap

water. Three ml DMAB (10 g DMAB was dissolved in 1000 ml glacial acetic acid (AR) which contains 12.5 % v/v 10 N HCl (AR). It was stored at 2°C as a stock prior to use before it was diluted with nine volume of glacial acetic acid.) was added in each tube and incubated at 38°C for 20 minutes. Tubes were cooled and absorbance was measured at 544 nm in spectrophotometer. Standard N-acetylglucosamine in the range of 0.05 to 0.30 μmole was prepared in borate buffer and was calibrated by following the above procedure.

Results and Discussion

β -1,3-glucanases

Chickpea cultivars grown in normal and sick plots showed significant difference in root β - 1, 3- glucanase activity. The root tissues were obtained from sick plot contained lower β -1,3 glucanase activity as compared to the tissues received from normal plot (Fig.1). Cultivars differed significantly in their B-1-3 glucanase activities. Among the cultivars, susceptible cultivars JG-62 and GG-4 showed significantly higher β -1, 3-glucanase activity as compared to the resistant cultivars (WR-315 and JCP-27) and tolerant cultivars (GG-1 and GG-2). However tolerant cultivars V₃ contained significantly lower level of enzyme activity in root tissue. (Fig.1) Among the different infectional stages, the β -1, 3-glucanase activity increased from 86.55 to 225.84 μmole glucose release. $\text{h}^{-1}.\text{g}^{-1}.\text{fr.wt.}$ with the advancement of disease and growth of plants. The enzyme activity drastically increased at infectional stage (S2) and it was more pronounced at post infectional stage (S3). Data showed increasing trend of activity of the enzyme in root tissues from S₁ to S₃ stage, in general.

Plants grown in sick plot, the β -1, 3-glucanase activity in the different cultivars were varied between 89.02 to 368.42 μ mole

glucose released. $\text{h}^{-1} \cdot \text{g}^{-1} \cdot \text{fr. wt.}$ Susceptible cultivar JG-62 and GG-4 had significantly higher activity, resistant and tolerant cultivars had lower activity as compared to susceptible cultivars grown in sick plot. Plants grown in normal plot, similar trend as recorded in sick plot. Healthy plant tissues from normal plot displayed significantly higher enzyme activity in JG-62 as compared to cultivars grown in sick plot. Cultivars contained almost similar values in enzyme activity recorded for resistant and tolerant cultivars grown in normal plots but wide variation in the enzyme activity was seen in sick plot. In general, plants grown in normal plot showed higher activity of enzyme as compared to the plants from sick plot and it was varied between 200.9 to 382.19 $\mu \text{mole glucose released. h}^{-1} \cdot \text{g}^{-1} \cdot \text{fr. wt.}$ Our data received for β -1, 3-glucase activity are in agreement with Naik *et al.*, (2005) who revealed that the increase in activity of β -1, 3-glucase in both susceptible and resistant lines against fusarium wilt.

Irrespective of plots (treatments), at pre-infectious stage (S_1), resistant (WR-315 and JCP-27) and tolerant (GG-1 and GG-2) cultivars resulted significantly higher β -1, 3 glucanase activity than the susceptible cultivars JG-62 and GG-4. The tolerant cultivar GG-2 contained the significantly highest activity i.e 96.26 $\mu \text{mole glucose release. h}^{-1} \cdot \text{g}^{-1} \cdot \text{fr. wt.}$ at pre infectious stage (S_1) the activity was continued to rise in all the cultivars from pre infectious (S_1) to post infectious stage (S_3). However by this stage, the susceptible cultivars JG-62 and GG-4 showed significantly remarkably higher activity than the resistant and tolerant cultivars. In general, the trend of enzyme activity increased as progress of plant growth and development of tissues.

Interaction effect of TxVxS of β -1, 3-glucanase activity revealed significant difference in root tissues. (Fig. 2). Plant

grown in sick plot resulted significant change in response to disease infection in root tissues of all the six cultivars. Susceptible cultivars JG-62 and GG-4 showed the highest β -1-3 glucanase activity at all stages of infection in tolerant and resistant cultivars. At infectious stages, all the cultivars had remarkably higher activity. Susceptible cultivars JG-62 and GG-4 visualized appreciable change in β -1-3 glucanase from infectious (S_2) to post infectious stage (S_3).

The activity of enzyme was declined in resistant and tolerant cultivars at post infectious stage except in JCP-27, where the activity was increased. Changes among the severity of diseases development and activity correlate at this stage showed significant cultivars differences in root tissues except in JCP-27. Susceptible cultivar had the significantly highest β -1,3-glucanase activity as compared to tolerant cultivars and, resistant cultivars (WR-315 and JCP-27) at infectious stage (S_2) with the advancement of diseases. At post infectious stage (S_3) JG-62 and GG-4 had the significantly highest β -1-3 glucanase activity as compared to other cultivars grown in sick plots.

In case of plants from normal plot, cultivars grown in healthy soil increasing trend of β -1-3 glucanase activity as progress or growth of the plants from S_1 to S_3 . The activity drastically increased from S_1 to S_3 in all the cultivars. However, differences were greater in susceptible cultivars JG-62 and GG-4. Overall data recorded for β -1, 3-glucanase activity are supported by Thangavelu *et al.*, (2003), Saika *et al.*, (2005). Ramamoorthy *et al.*, (2002) revealed that the activity of beta -1,3 glucanase and chitinase were induced to accumulate at higher levels at 3-5 days of challenge inoculation in bacterized plants of banana with *Pseudomonas fluorescens* isolate PF1.

Chitinase

The fungal chitinases was consist of five different domains: (a) N-terminal signal peptide region, (b) catalytic domain, (c) chitin-binding domain, (d) serine/theonine rich-region, and (e) C-terminal, (Dean et al,2012) It also possess specific affinity towards polymer chitin to degrade it into low-molecular-weight COS (chitooligosaccharides) and GlcNAc (N-acetylglucosamine). Chickpea cultivars grown in normal and sick plots had resulted significant difference in root chitinase activity. The root tissues obtained from sick plot visualized higher chitinase activity (0.72 n.mole N-acetyl glucosamine.h⁻¹.g⁻¹.fr.wt) as compared to the tissues received from normal plot (0.57n.mole acetyl glucosamine.h⁻¹.g⁻¹.fr.wt). Fig.3. Among the cultivars, susceptible cultivars JG-62 and GG-4 showed significantly higher chitinase activity as compared to the resistant cultivars JCP-27 and WR-315 and tolerant cultivars GG-1 and GG-2. Lower level of the enzyme activity in root tissues was observed in tolerant cultivars (Fig.3). Chitinases have been implicated more

in the plant defense mechanism (Vanloon, 1985; Schlumbaum *et al.*,1986;Linthrost *et al.*, 1991). It is known that chitinases degrade chitin in fungal cell wall (Schlumbaum *et al.*,1986) and also act synergistically with the β-1,3 glucanase for inhibiting fungal growth (Mauch *et al.*,1984). Among the different infectional stages, the activity increased from 0.63 to 0.87 n.mole N-acetyl glucosamine.h⁻¹.g⁻¹.fr.wt. with the advancement of disease and growth of plants i.e. pre-infectional stage (S₁) to post infectional stage(S₃). However the levels of chitinase activity significantly decreased at infectional stage (S₂) (0.42 n.mole N-acetyl glucosamine.h⁻¹.g⁻¹.fr.wt) and increased remarkably at post infectional stage (S₃) (0.87n.mole N-acetyl glucosamine.h⁻¹.g⁻¹.fr.wt).

Chitinase activity in chickpea plants either grown in sick plot or normal plot did not show any significant change. In general plants grown in sick plot had little higher activity in all the cultivars as compared to the plants grown in normal plot. At pre infectional stage all the cultivars did not show any significant differences in their activity.

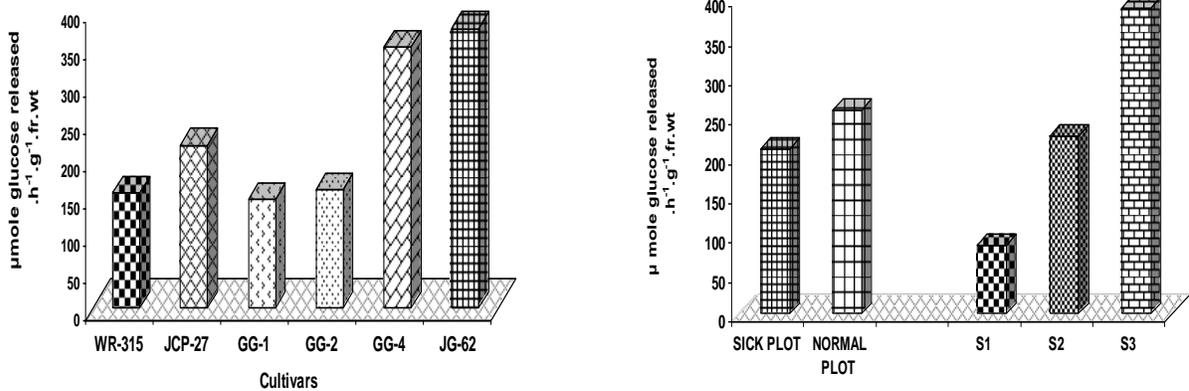


Fig.1 Mean effect of cultivars, treatments and stages on β-1,3-glucanase activity in chickpea root tissues. S₁-pre infectional stage; S₂-infectional stage; S₃-post infectional stage. S.Em± 0.71 (V), 0.41 (T), 0.68 (S); C.D. at 5%, 2.09 (V), 1.21 (T), 1.93 (S)

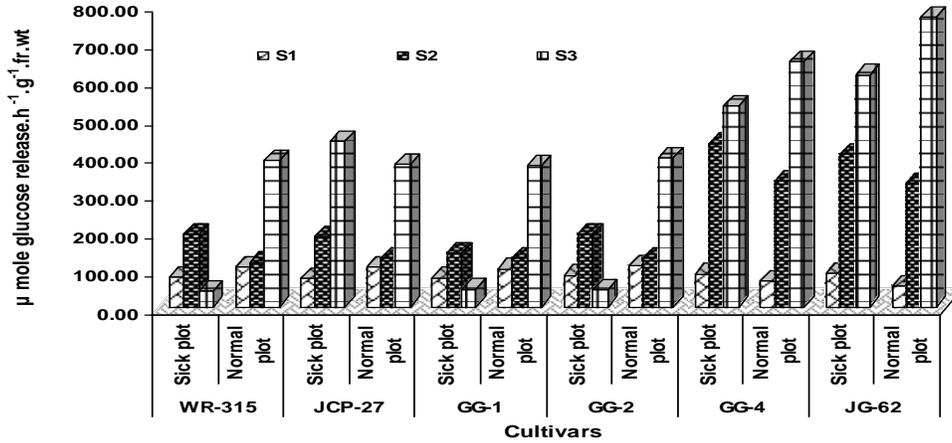


Fig.2 Interaction effect of TxVxS on β -1,3-glucanase activity in root tissues of chickpea cultivars. S₁- pre infectious stage; S₂-infectious stage; S₃-post infectious stage. S.Em \pm 2.34, C.D. at 5% 6.67

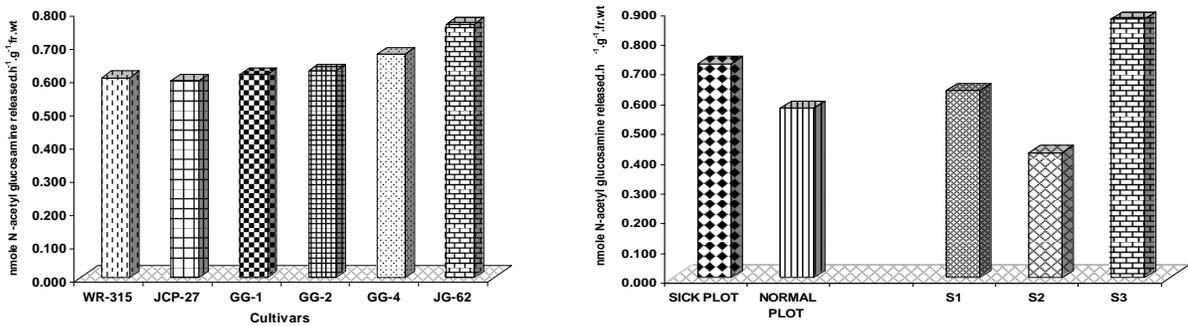


Fig.3 Mean effect of cultivars, treatments and stages on chitinase activity in chickpea root tissues. S₁-pre infectious stage; S₂-infectious stage; S₃-post infectious stage. S.Em \pm 0.04 (V), 0.03 (T), 0.04 (S); C.D. at 5%, NS (V), 0.08 (T), 0.11 (S)

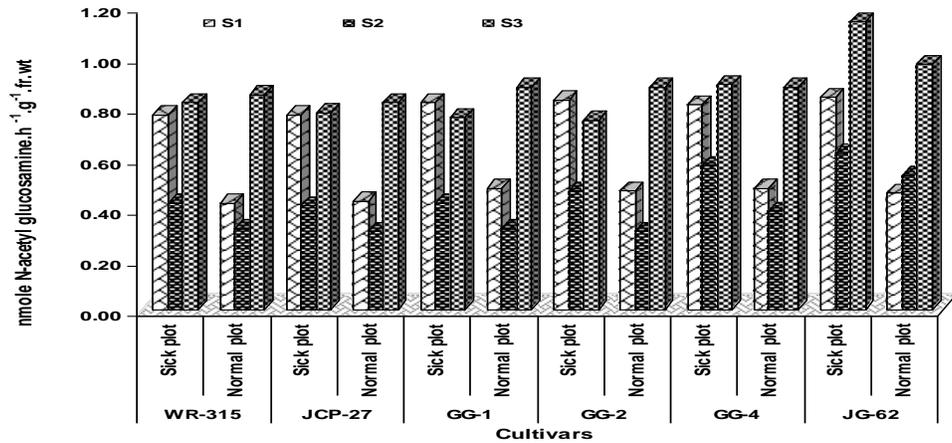


Fig.4 Interaction effect of TxVxS on chitinase activity in root tissues of chickpea cultivars. S₁-pre infectious stage; S₂-infectious stage; S₃-post infectious stage. S.Em \pm 0.13, C.D. at 5% NS

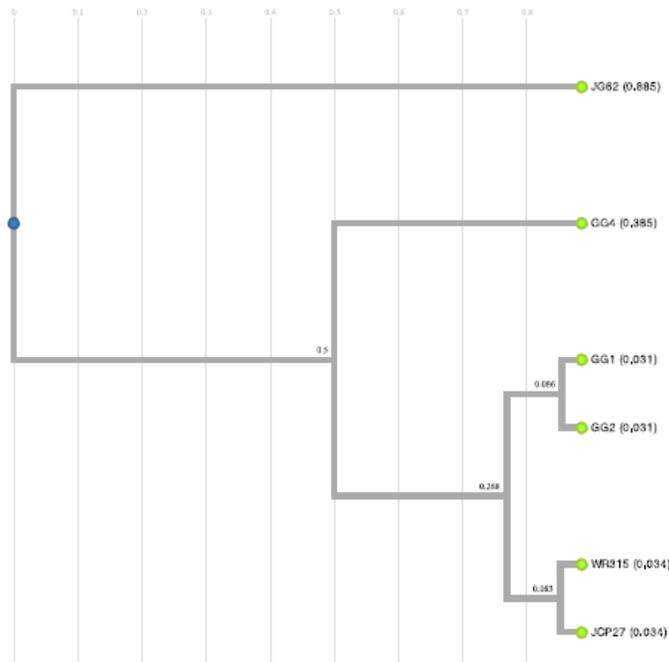


Figure.5 Chitinase dendrogramme of varietal differences among the varieties

The enzyme activity drastically declined at infectional stage (S_2). However the reduction in chitinase activity was less in susceptible cultivars. At post infectional stage (S_3), again increased in all the cultivars. Saikia *et al.*, (2005) revealed that the maximum activity of chitinase was recorded after three days of inoculation in all induced plant of chickpea. Thereafter, the activity decreased progressively. Two chitinases detected in induced chickpea plants infected with *Fusarium oxysporum f. sp. ciceris*.

Interaction effects of TxVxS did not revealed any significant differences in chitinase activity (Fig.4). Irrespective of plant grown in sick plot and normal plot, susceptible cultivars JG-62 and GG-4 had higher chitinase activity as compared to resistant cultivars at all the stages. The enzyme activity at infectional stage (S_2) though the plants from normal plot had little higher value.

In general, chitinase activity in root tissues at infectional stage in sick plot (diseased plant) and normal plot (healthy plant) are in

agreement with the published literature. In some plant species, resistant tissues accumulate chitinase more rapidly and at higher concentration than susceptible tissues (Benhamou *et al.*, 1990; Hedrick *et al.*, 1988; Irving and Kuc, 1990; Joosten *et al.*, 1990; Rasumussen *et al.*, 1992; Samac *et al.*, 1990; Wyatt *et al.*, 1990). In many of these tissues the resistance response was initially hypersensitive reaction with very rapid localized cell death (Hahlbrock *et al.*, 1989; Vogeli *et al.*, 1988; Voisey and Slusarenko, 1989).

Overall data reported for chitinase activity are in agreement with the findings suggested by Shukla (2001), Shukla and Suthar (2017). He examined chitinase activity in root tissues of resistant and susceptible cultivars of chickpea at different stages of infection in inoculated and uninoculated pot experiments. The results obtained in the field experiments are in agreement with data obtained by Cachinero *et al.*, (2002) studied on plant defense reactions against fusarium wilt in chickpea induced by incompatible race 0 of *Fusarium oxysporum*

f.sp. ciceri and nonhost isolates of *F. oxysporum*. This defense-related response was induced more consistently and intensely by non-host isolates of *F. oxysporum* than by incompatible FOC race 0 and the antifungal hydrolases,

Chitinase enzyme in healthy plants may be involved in elicitation reaction to activate plant defense mechanism. In case of disease infection, chitinase may also accumulate in response to fungal elicitors and participate in response to the elicitors and may take part in defense reaction by preventing further development of fungal pathogen. (Cachinero *et al*, 2002, Saika *et al.*, 2005). Chitinase and β 1-3, glucanase have been examined in present study but other enzymes capable of degrading hyphal cell wall are known to be present in higher plants. The possibility exists that host polysaccharides operating under specific conditions could provide an explanation for the lysis of vascular pathogens. The enzyme can either induce by infection with pathogens or treatment with the elicitors/chemicals (Bowles, 1990).

β -1,3-glucanase is another hydrolytic enzyme involved in degradation of fungal cell wall. The major products formed due to hydrolysis are oligomer β -1,3- glucans. There are large numbers of factors responsible for accumulation of this enzyme and lack of high degree of pathogen specificity in their induction implies that these are part of a general response of the plant stress. However their induction has correlated with greater resistance to subsequent pathogen attacks. Pattern of rising β -1,3-glucanase from infection to post infection stage same was true for healthy plants also with growth of the plants. β -1,3-glucanase is actively associated with during infection process as a part of disease resistance mechanism. As the fungi could not progress further the high level of activity was no longer persist in root tissues

of resistant plants grown in sick plot. In infected plants there is continuous progress of fungi hence the high level of β -1,3-glucanase is always beneficial as a part of defense reaction to hydrolyse the fungal cell wall. The results of present investigation are supported to the findings of Naik *et al.*, (2005); Thangavelu *et al.*, (2003); Saika *et al.*, (2005) and Ramamoorthy *et al.*, (2002), (Rathod, 2008).

In conclusions, the results obtained in the field experiments are in agreement with previous findings and Interaction effect of TxVxS of β -1, 3-glucanase and chitinase activity revealed significant difference in root tissues infected with *Fusarium oxysporum f.sp. ciceri* grown in sick plot as well as in diseased tissues of all the six cultivars. It exhibits defensive reaction in susceptible and diseased plots and cultivars grown in healthy plot showed resistant and higher activities of the both enzymes under experimental condition thus its prove hypothesis of experimental design. It can be concluded that both β -1, 3-glucanase and chitinase activity having role in disease resistance and it can be biochemical marker for identification of fungal resistant cultivars.

Conflict of interest

There is no conflict of interest, it is part of Ph.D research of Junagadh Agricultural University thesis submitted and published some parts in some journals and books too.

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