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Full Length Research Paper

Biochemical changes in chickpea caused by *Fusarium oxysporium f.* sp *ciceri*

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Study was conducted to see the changes in ascorbic acid, free amino acids, proline and total phenol content at different stages of infection of wilt disease in chickpea (*Cicer arietinum* L) roots tissues. The results indicated that total phenol content was significantly higher in root of all the cultivars obtained from sick plot. The level of phenol declined from pre infection (S_1) to post infection stage (S_2) and further it increased in all the cultivars among six cultivars tested, JG-62 and GG-1 had lower concentration of total phenol than others. Free amino acid content remarkably decreased with rise in the intensity of wilt disease. Susceptible cultivars had greater reductions in free amino acid content as compared to tolerant cultivars (GG-1 and GG-2) that is (50-52%). Root tissues of different cultivars grown in sick plot possess significantly more amount of free amino acids than the tissues obtained from normal plots at different stages of growth. Ascorbic acids content was significantly higher in chickpea root tissues obtained from normal plot. Susceptible cultivars GG-4 and JG-62 had higher content of ascorbic acid either it was grown in sick soil or normal soil as compared with other cultivars. With the progress of disease from pre infection (S_1) to post infection stage (S_2), a greater reduction was recorded in root tissues received from sick plot (47%) as compared to normal plot (38%). Interaction effect of TxS showed that the percentage reduction in ascorbic acid content was same from pre infection stage to post infection stage (36%) in root tissues from both sick and normal plot.

Key words: Chickpea, wilt, proline, phenol, free amino acid, ascorbic acid.

INTRODUCTION

Chickpea (*C. arietinum L*.) is the second most important pulse crop of the world. India is the world's largest chickpea growing country having a cultivation area of 6.5 Million hectares and an output of 5.77 million tonnes with an average yield of 888 kg/ha (Deshmukh, 2005a) and thus contributes about 63% 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 2000-2001. Productivity of chickpea is, however, restricted due to several abiotic and biotic stresses. The abiotic stresses include drought, high and low temperature, high moisture and soil toxicity. Biotic stresses include the bacterial fungal and viral diseases. Important fungal diseases are wilt and blight caused by *Fusarium oxysporum* and *Ascochyta rabei* respectively. According to Deshmukh (2005b) studied with Fusarium wilt disease. They showed that the altitude level right from 00 to 45° latitude having more Fusarium wilt infection and Geographically Junagadh is situated at 21.5° North altitudes and 70.5° East longitudes with an altitude of 60 m above the mean sea level. Therefore, it is prime important to work on this disease. Wilt of chickpea (*Cicer arietinum*), caused by *F. 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 estimated to range from 10 to 15% but *Fusarium* wilt epidemics can be devastating to individual crops and cause 100% loss under favorable conditions (Halila and Strange, 1996; Chaube and Pundhir, 2005). In the present study, biochemical changes in susceptible and resistant cultivars of chickpea were estimated on the basis of important biochemical compounds like free amino acids, total phenol, ascorbic acids. Susceptible and resistant cultivars infected with wilt disease were used for understanding biochemical

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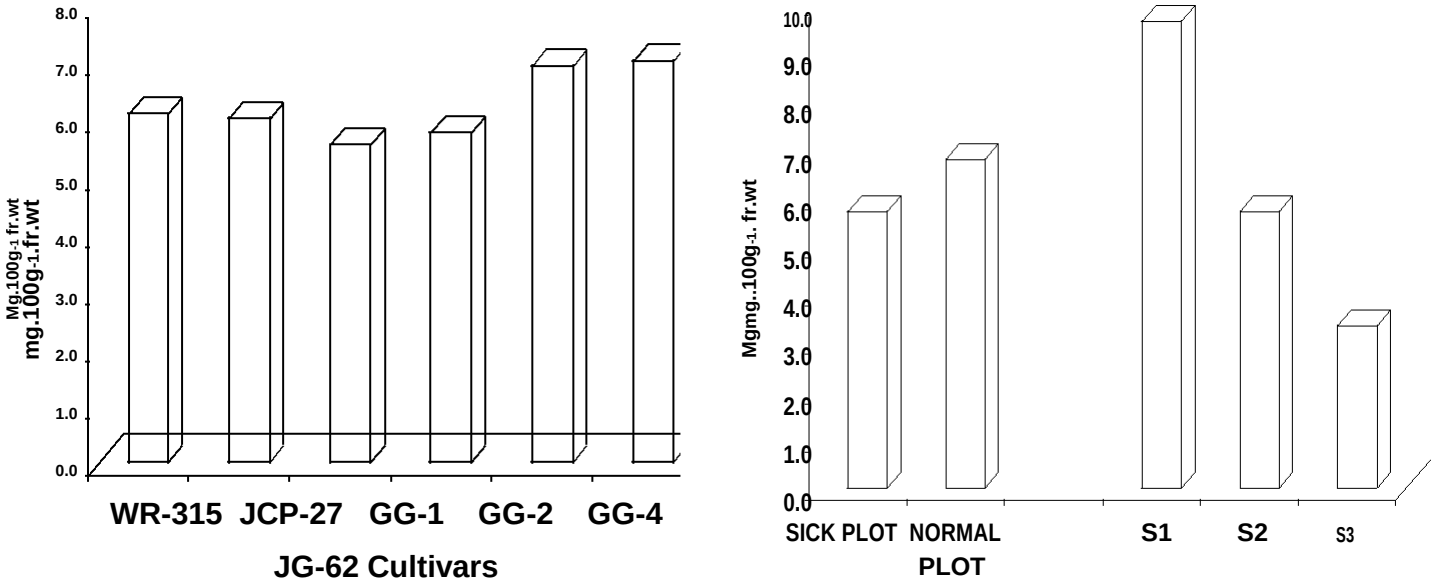

Figure 1. Mean effect of cultivars, treatments and stages on ascorbic acid content in chickpea root tissues. S1-pre infection stage; S2-infection stage; S3-post infection stage. S.E.M± 0.04 (V), 0.01 (T), 0.03 (S); C.D. at 5%, 0.05 (V), 0.11 (T), 0.08 (S).

mechanism of disease resistance.

MATERIALS AND METHODS

Seeds of six cultivars viz. WR-315 (Resistant) JCP-27 (Resistant), GG-1(Tolerant), GG-2 (Tolerant) GG-4 (Susceptible) JG-62 (Highly Susceptible) was obtained from the Pulse research station Junagadh India were used for the study. Plants were raised in chickpea sick-plot maintained since 1998 in Pulse research Farm, GAU, Junagadh, containing heavy load of soil borne of *F. oxysporum* f. spp. *ciceri*. Disease free (control) plants of all the cultivars were raised in normal plot. The crop was fertilized as per package of practices in normal plot. No insecticides or herbicides were applied in sick plot or normal plot. Split plot design were used for the experiment design in which First factor as plot, (two treatment), 2nd factor as cultivars (Six) and 3rd factor as at three different stages, viz., pre infection (12 DAS-S_1), infection (21 DAS –S_2) and post infection stages (26 DAS- S_3).

Extraction and estimation of biochemical compounds

The root tissues were separated and cut into small uniform pieces. From this, representative samples of 500 mg were taken from each plot at three different stages, viz., pre infection (12 DAS-S_1), infection (21 DAS –S_2) and post infection stages (26 DAS- S_3). Roots were cleaned with tap water followed by distilled water and water soaked with filter paper. Than after roots were cut below two cm hypocotyls and subsequently weigh according to biochemical parameters. Total phenols were analyzed by adopting methods according to Bray and Thorpe (1954), free amino acid content was estimated as described by Lee and Takahashi (1966), Free proline was determined using the method suggested by Bates et al. (1973) and Ascorbic acid content was measured as method described by Malik and Singh (1980). All the estimations were done in triplicate and the results on fresh weight basis are statistically analyzed and reported.

RESULTS

Ascorbic acid

Chickpea cultivars grown in normal and sick plots have shown that the root tissues obtained from sick plot contained lower ascorbic acid level (5.70 mg.100 g^{-1}.fr.wt) as compared to the tissues received from the normal plot (6.77 mg.100 g^{-1}.fr.wt) (Figure 1). Cultivar differences were found to be significant in their ascorbic acid contents. Among the cultivars, JG-62, and GG-4 showed the highest ascorbic acid content (Figure 1). The ascorbic acid content was significantly decreased from 9.64 to 3.36mg.100 g^{-1}.fr.wt. With the advancement of disease and growth of plants at different infection stages that is, pre-infection stage (S_1) to post infection stage (S_3) the Ascorbic acid content drastically decreased (41%) at infection stage (S_2) as compared to S_1 and it declined further upto post infection stage (S_3). Interaction effect of T × V were significantly differed for ascorbic acid content as is evident from the data observed from diseased and healthy plants (Table 1). Plants grown in sick plot, the ascorbic acid content in diseased root tissues was varied between 5.06 to 6.69 mg.100 g^{-1}.fr.wt. and cultivar GG-4 hold the significantly highest content. Both resistant and tolerant cultivars revealed significantly lower values than that of in susceptible cultivars that is, JG-62 and GG-4. The same trend was observed in plants from normal plot but the content was little higher in all cultivars as compared to the plants received from sick plot. Combined effect of cultivars Vs stage was found to be significant (Table 1). At pre-infection stage (S_1), resistant cultivars contained significantly lower content of ascorbic

Table 1. Combined effect of cultivars, treatments and stages on ascorbic acid content (mg. 100g^{-1}.fr.wt) in root tissues of chickpea.

Treatments and stages	WR-315 (V_1)	JCP-27 (V_2)	GG-1 (V_3)	GG-2 (V_4)	GG-4 (V_5)	JG-62 (V_6)
Sick plot (T_1)	5.33	5.28	5.06	5.07	6.69	6.76
Normal plot (T_2)	6.85	6.73	6.09	6.48	7.22	7.28
Pre infection stage (S_1)	9.41	9.29	9.12	9.66	10.00	10.35
Infection stage (S_2)	5.92	5.93	5.67	5.72	5.47	5.54
Post infection stage (S_3)	2.94	2.8	1.94	1.95	5.40	5.16
	S_1	**S_2**	**S_3**			
Sick plot (T_1)	9.11	4.85	3.14			
Normal plot (T_2)	10.16	6.57	3.59			
	V×T	**V×S**	**T×S**			
S.E m	0.07	0.04	0.05			
C.D. at 5%	0.19	0.11	0.15			

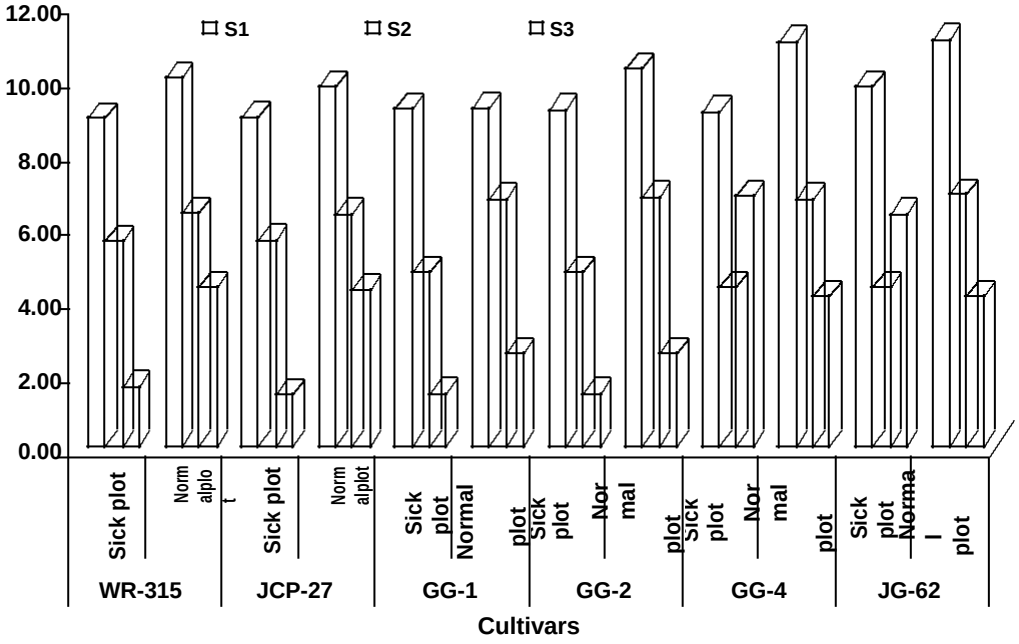

Figure 2. Interaction effect of T×V×S on ascorbic acid content in root tissues of chickpea cultivars. S_1- pre infection stage; S_2-infection stage; S_3-post infection stage. S.E M ± 0.09, C.D. at 5%, 0.26.

acid than the susceptible (JG-62 and GG-4) and tolerant cultivars (GG-1 and GG-2). Generally the content was significantly lowered with the advancement of growth or disease in all the cultivars. In case of susceptible cultivars greater reduction in ascorbic acid was observed (that is, 45-48%) from S_1 stage to S_2 stage and it did not change much from S_2 and S_3. However, on the basis of percent reduction, pre infection to infection stage the reduction was between 37-41% which was further increased from S_2 to S_3 stage (64-80%). Interaction effect of treatment X stage for ascorbic acid content was found to be significant (Table 1). Chickpea cultivars grown in sick plot showed significantly lower ascorbic acid content (3.14 to 9.11 mg.100g^{-1}.fr.wt) as compared to the healthy tissues obtained from normal plot (3.59 to 10.16 mg.100g^{-1}.fr.wt). In both sick and normal plots the content was found to decrease with growth of the plants from S_1 stage to S_3 stage and same pattern was observed in sick plot also. However higher ascorbic acid content was recorded in plants from normal plot. Interaction effects of T×V×S for ascorbic acid content revealed significant changes in root tissues (Figure 2). Ascorbic acid content varied between

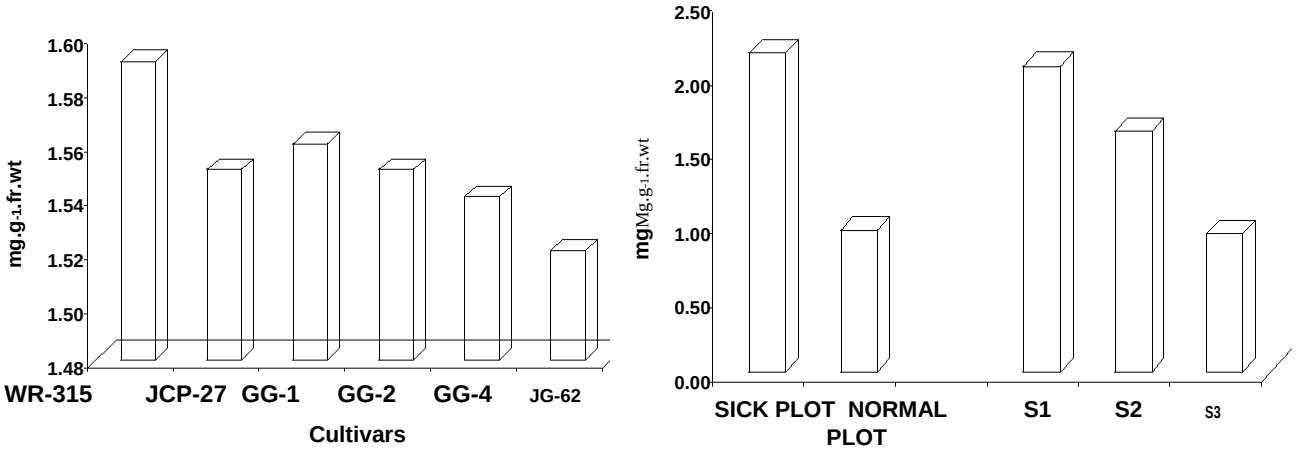

Figure 3. Mean effect of cultivars, treatments and stages on amino acids content in chickpea root tissues. S_1-preinfection stage; S_2-infection stage; S_3-post infection stage. S.E M ± 0.01 (V), 0.01 (T), 0.01 (S), C.D. at 5%, 0.03V), 0.02 (T), 0.02 (S).

Table 2. Combined effect of cultivars, treatments and stages on free amino acids (mg.g^{-1}.fr.wt) in root tissues of chickpea**.**

Treatments and stages	WR-315 (V_1)	JCP-27 (V_2)	GG-1 (V_3)	GG-2 (V_4)	GG-4 (V_5)	JG-62 (V_6)
Sick plot (T_1)	2.24	2.16	2.17	2.16	2.09	2.09
Normal plot (T_2)	0.93	0.93	0.94	0.94	0.99	0.96
Pre infection stage (S_1)	2.10	2.01	2.03	2.04	2.07	2.13
Infection stage (S_2)	1.63	1.62	1.64	1.63	1.62	1.58
Post infection stage (S_3)	1.04	1.01	1.00	0.98	0.93	0.87
	S_1	**S_2**	**S_3**			
Sick plot (T_1)	2.87	2.39	1.21			
Normal plot (T_2)	1.26	0.85	0.74			
	V×T	**V×S**	**T×S**			
S.E m	0.02	0.01	0.02			
C.D. at 5%	0.04	0.03	0.04			

8.86 to 9.71 mg.100 g^{-1}.fr.wt at pre-infection stage in root tissues obtained from sick plot.

Free amino acids

Tissues obtained from sick plot showed higher free aminoacids content (2.15 mg.g^{-1}.fr.wt) as compared to the tissues received from normal plot (Figure 3). Cultivars differences were found to be significant in their free amino acids contents. Among the cultivars, WR-315 hold significantly higher amount of free amino acids content and it was at par with cultivar GG-1. Susceptible cultivar JG-62 contained the lowest level of free amino acids. Cultivars JCP-27, GG-1, GG-2 and GG-4 were at par (Figure 3). At different infection stages, the amino acids content decreased significantly from 2.69 to 0.93 mg.g^{-1}.fr.wt. When the advancement of disease in sick plot grown plant or advancement of tissue growth in normal plants. The free amino acids content declined by 21% at infection stage (S_2). The contents drastically reduced from infection (S_2) to post infection stage (S_3) and it was about 43%. Combined effect T × V revealed significant difference in free amino acids content (Table 2). Plants grown in sick plot had higher content of free amino acids (2.09 to 2.24mg.g^{-1}.fr.wt) as compared to the plants from normal plot (0.99-0.93 mg.g^{-1}.fr.wt). Interaction effect of cultivars and stages found to be significant as seen in Table 2. At pre-infection stage (S_1), the susceptible cultivars JG-62 hold significantly higher contents of free amino acids than the resistant (WR-315 and JCP-27) and tolerant cultivars. The content was significantly declined with the advancement of growth. Resistant and tolerant cultivars had less reduction of free amino acids 50-52% while susceptible cultivars had greater reduction in free amino acids (that is, 55-59%) from pre infection (S_1) to post infection stage (S_3). Combined effect of treatment X stage for free amino acids content was found to be

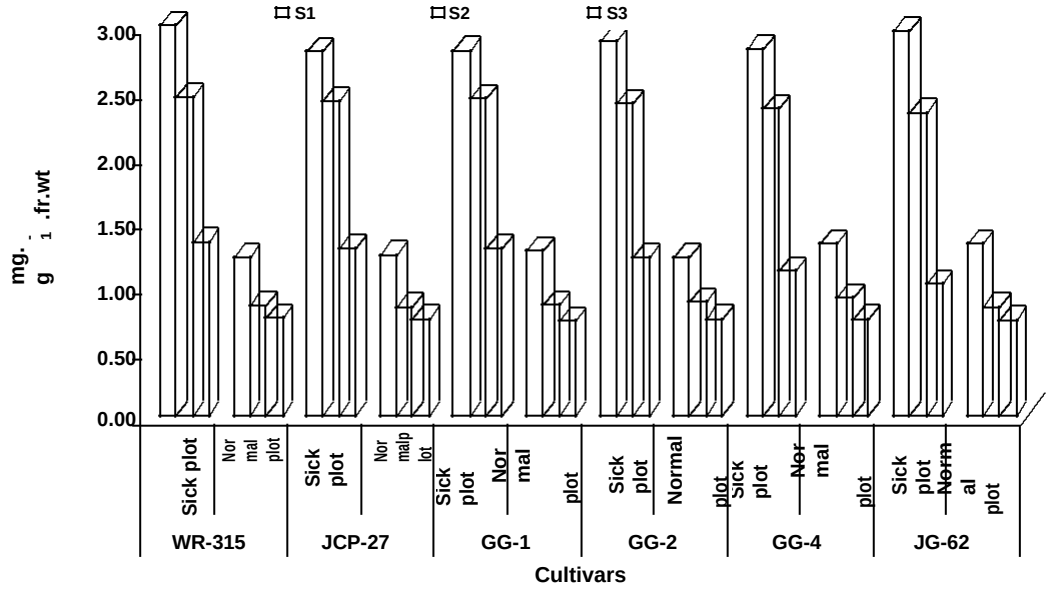

Figure 4. Interaction effect of T×V×S on free amino acids contents in root tissues of chickpea cultivars. S_1- pre-infection stage; S_2-infection stage; S_3-post infection stage. S.E M ± 0.03, C.D. at 5%, 0.08.

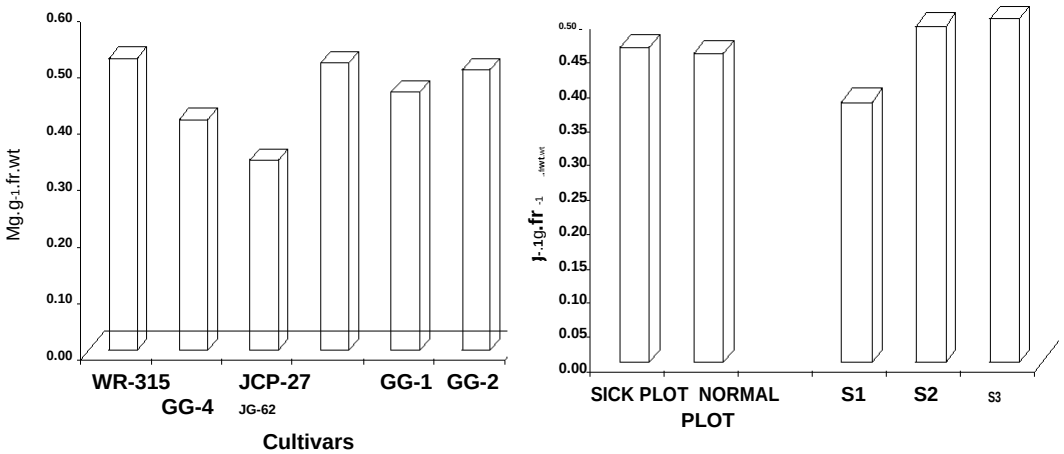

Figure 5. Mean effect of cultivars, treatments and stages on proline content in chickpea root tissues. S1- pre infection stage; S2-infection stage; S3-post infection stage. S.E M ± 0.01 (V), 0.01 (T), 0.01 (S); C.D. at 5%, NS (V), 0.02 (T), 0.02 (S).

significant (Table 2). Chickpea cultivars grown in sick plot (diseased root tissues) showed significantly higher free amino acids content (1.21 to 2.87mg.g^{-1}.fr.wt) as compared to the healthy tissues obtained from normal plot (0.74 to 1.26 mg.g^{-1}.fr.wt). In both normal and sick plots, free amino acids content of root tissues was decreased as growth of the plants from pre infection (S_1) to post infection (S_3). Free amino acids content reduced in both the plots (both normal and sick) but the reduction was more (33% at infection stage) at the time of infection. However, the advancement of growth from infection to post infection stage the reverse trend was recorded. Interaction effects of T×V×S of free amino acids content revealed significant difference in root tissues (Figure 4).

Plant grown in sick plot resulted variation in free amino acids and values were in the range of 2.79 to 2.99 mg.g^{-1}.fr.wt. in all cultivars at pre infection stage. All the cultivars showed reduction in their free amino acids content at infection stage (S_2). At all stages, susceptible cultivars (GG-4 and JG-62) contained lower amino acids as compared to resistant cultivars (WR-315 and JCP-27).

Proline

Mean treatment effect for proline content did not show any significant difference (Figure 5). The root tissues obtained from sick plot had same value of proline content

Table 3. Combined effect of cultivars, treatments and stages on proline content (mg.g^{-1}.fr.wt) in root tissues of chickpea.

Treatments and stages	WR-315 (V_1)	JCP-27 (V_2)	GG-1 (V_3)	GG-2 (V_4)	GG-4 (V_5)	JG-62 (V_6)
Sick plot (T_1)	0.53	0.43	0.34	0.50	0.51	0.47
Normal plot (T_2)	0.51	0.4	0.34	0.32	0.42	0.5
Pre infection stage (S_1)	0.46	0.36	0.31	0.42	0.36	0.42
Infection stage (S_2)	0.52	0.43	0.38	0.59	0.51	0.51
Post infection stage (S_3)	0.58	0.48	0.33	0.52	0.53	0.57
	S_1	**S_2**	**S_3**			
Sick plot (T_1)	0.35	0.48	0.56			
Normal plot (T_2)	0.2	0.50	0.44			
	V×T	**V×S**	**T×S**			
S.E m	0.01	0.01	0.01			
C.D. at 5%	0.02	0.02	0.02			

as compared to the tissues received from normal plot that is, 0.46 and 0.45 mg.g^{-1}.fr.wt. respectively. Cultivars differences were found to be significant in their proline contents. Among the cultivars, WR-315 showed higher amount of proline content as compared to other cultivars. Cultivar GG-1, JCP-27 and GG-4 contained significantly lower amount of proline than the WR-315, GG-2 and JG-62 (Figure 5). Among the different infection stages, the proline content increased from 0.38 to 0.50 mg.g^{-1}.fr.wt. with the progress of disease and growth of plants that is, pre-infection stage (S_1) to post infection stage (S_3). The content was significantly increased from pre infection stage (S_1) to infection stage (S_2) and there after the content did not change much from infection stage (S_2) to post infection stage (S_3). Irrespective of the stages, the interactions effect T × V significantly differed in proline content as observed from the diseased and healthy plants (Table 3). In plants grown in sick plot, the content in root tissues varied between 0.34 and 0.53 mg.g^{-1}.fr.wt. Resistant cultivar WR-315 hold the highest content of proline that is, 0.0.53 mg.g^{-1}fr.wt. Cultivars JG-62 and GG-1 JCP-27 showed significantly lower amount of proline than that of cultivars in WR-315, GG-4 and GG-2. While in case of normal plot, the proline content was higher in susceptible cultivar JG-62 as compared to JCP-27, GG-4 and GG-1. Irrespective of plots (treatments), interaction effect of cultivars vs stage was found to be significant (Table 3). At pre-infection stage (S_1), cultivars WR-315, GG-2 and GG-4 contained significantly higher proline content than JCP-27, GG-1 and JG-62 with the advancement of disease or growth at infection stage. The content was increased in all the cultivars. Thus, the proline was accumulated in all the cultivars as growth of plants from S_1 to S_3 stage except in tolerant cultivars GG-1 and GG-2, where it decreased at post infection stage (S_3) as compared to the infection stage. Combined effect of treatment X stage proline content was found to be significant (Table 3). Root tissues obtained from sick plot that is, inoculated with *F. oxysporum* showed significantly higher proline content as compared to the plants from normal plot at pre infection (S_1) the content increased with the advancement of the disease from pre infection (S_1) to post infection stage (S_3). Similar pattern of data was observed in plant obtained from normal plot, though the values were little lower than the sick plot except at stage S_2 (Table 3). Interaction effects of T×V×S of proline content revealed significant differences in root tissues (Figure 6). Plant grown in sick plot resulted increasing trend in proline content in response to disease infection in root tissues of all the six cultivars. All the cultivars showed rise in proline content from pre infection (S_1) to infection stage (S_2) and continued to increase at post infection stage (S_3). Cultivar GG-2 and GG-4 visualized significantly higher proline content at infection (S_2) and post infection stage (S_3). However, cultivar GG-1 showed significantly lower level of proline as compared to other cultivars grown in sick plots.

In case of normal plot, healthy plants showed increasing trend of proline content as progress or growth of the plants from S_1 stage to S_3 stage only in cultivars (JCP-27 and WR-315 and JG-62), while the proline content drastically reduced in cultivar GG-1, GG-2 and GG-4.

Total phenol

The root tissues obtained from sick plot (T_1) revealed higher amount of total phenol (0.83 mg.g^{-1}.fr.wt) as compared to the tissue received from (T_2) normal plot (0.65. mg.g^{-1}.fr.wt) (Figure 7). Cultivars difference was found to be non significant in their total phenol contents. Among the cultivars, Cultivar GG-2 showed maximum amount of total phenol content (0.88 mg.g^{-1}.fr.wt), while susceptible cultivars JG-62 contained the lowest value of total phenol. Among the different infection stages, phenol

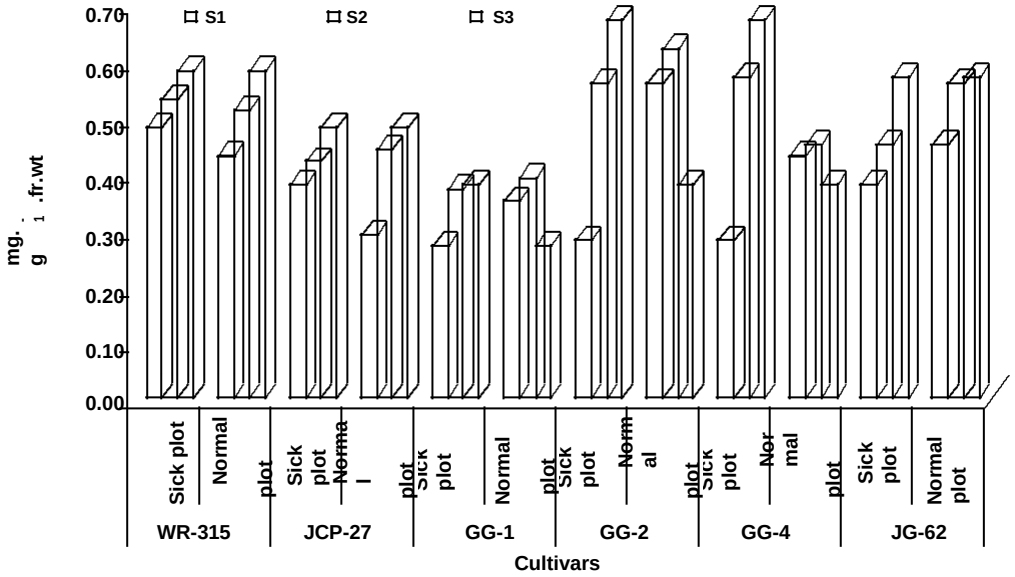

Figure 6. Interaction effect of T×V×S on proline content in root tissues of chickpea cultivars. S_1- pre infection stage; S_2-infection stage; S_3-post infection stage. S.E M ± 0.01, C.D. at 5% 0.04.

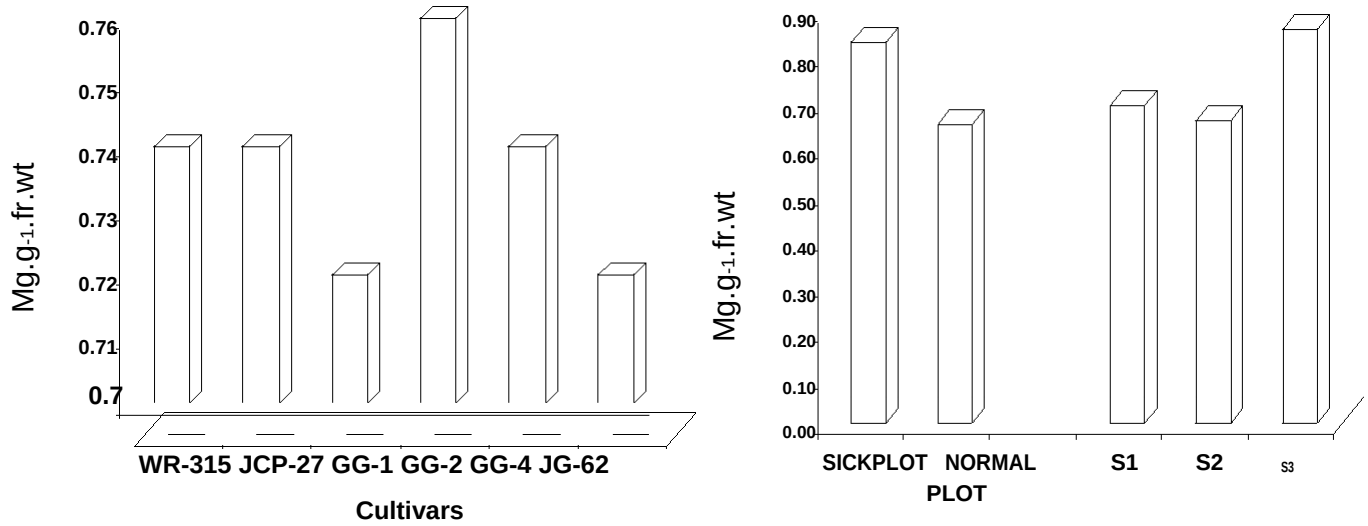

Figure 7. Mean effect of cultivars, treatments and stages on total phenol content in chickpea root tissues. S_1-pre infection stage; S_2-infection stage; S_3-post infection stage. S.E M ± 0.02 (V), 0.01 (T), 0.02 (S); C.D. at 5%, NS (V), 0.04 (T), 0.05 (S).

content significantly varied between 0.69 and 0.86 mg.g^{-1}.fr.wt. The total phenol content decreased at infection stage (S_2) but the difference was non significant. The content significantly increased from 0.66 mg.g^{-1}.fr.wt infection stage (S_2) to 0.86 mg.g^{-1}.fr.wt post infection stage (S_3). Irrespective of stages, the interaction effect T x V were significantly differed is evident from the data presented in Table 4. In plants grown in sick plot, the total phenol content was varied from 0.80 to 0.88 mg.g^{-1}.fr.wt. Cultivar GG-2 holds the highest content of total phenol. A reverse trend was recorded when plant grown in normal plot. The phenol contents were higher in resistant cultivars as compared to the susceptible cultivars. In general, plants grown in normal plot showed significantly lower level of phenol content (0.63-0.68 mg.g^{-1}.fr.wt) as compared to plants obtained from sick plot. Irrespective of plots (treatments), interaction effect of cultivars v/s stage was found to be non significant (Table 4). At pre-infection stage (S_1), the phenol content varied between 0.67-0.71 mg.g^{-1}.fr.wt. The resistant cultivars revealed little lower content of total phenol than the susceptible cultivars whereas at infection stage, the phenol contents decreased as compared to pre infection stage. The content again increased at post infection stage (S_3) in all

Table 4. Combined effect of cultivars, treatments and stages on total phenol content (mg.g^{-1}.fr.wt) in root tissues of chickpea.

Treatments and stages	WR-315 (V_1)	JCP-27 (V_2)	GG-1 (V_3)	GG-2 (V_4)	GG-4 (V_5)	JG-62 (V_6)
Sick plot (T_1)	0.80	0.80	0.80	0.88	0.85	0.82
Normal plot (T_2)	0.67	0.68	0.64	0.64	0.63	0.63
Pre infection stage (S_1)	0.67	0.67	0.68	0.71	0.71	0.69
Infection stage (S_2)	0.65	0.65	0.65	0.68	0.67	0.64
Post infection stage (S_3)	0.89	0.89	0.83	0.89	0.84	0.84
	S_1	**S_2**	**S_3**			
Sick plot (T_1)	0.84	0.73	0.91			
Normal plot (T_2)	0.54	0.59	0.82			
	V×T	**V×S**	**T×S**			
S.E m	0.03	0.89	0.03			
C.D. at 5%	0.08	NS	0.08			

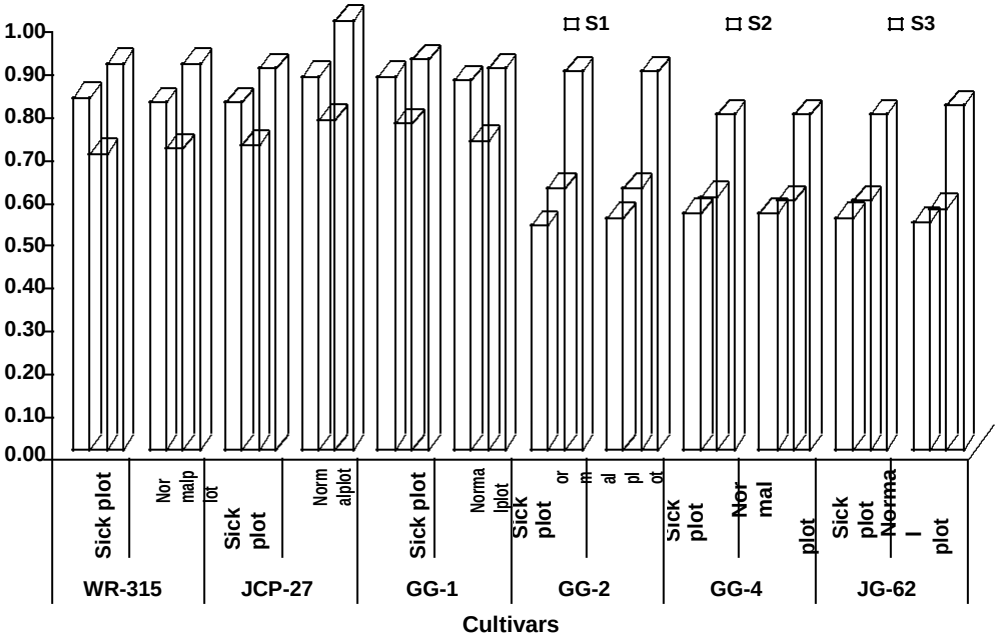

Figure 8. Interaction effect of TxVxS on phenol content in root tissues of chickpea cultivars. S_1- pre infection stage; S_2-infection stage; S_3-post infection stage. S.E.M±0.17, C.D. at 5% NS.

the cultivars. However, the content was higher (0.89 mg.g^{-1}.fr.wt) in resistant cultivars as compared to susceptible cultivars (0.84 mg.g^{-1}.fr.wt) though the differences were found to be non significant.

Combined effect of treatment X stage for phenol content was found to be significant. (Table.4) Chickpea cultivars grown in sick plot that is, inoculated with *F. oxysporum* (diseased root tissues) showed significantly higher level of total phenol content (0.73 to 0.91mg.g^{-1}.fr.wt) as compared to the healthy tissues obtained from normal plot (0.54 to 0.82 mg.g^{-1}.fr.wt). In sick plots, total phenol content in root tissues was significantly decreased from pre infection (S_1) to (S_2) infection stage and reverse trend was found from S_2 to S_3. In normal plot, phenol content increased from 0.54 to 0.82 mg.g^{-1}.fr.wt with the advancement of crop growth however, the values were significantly lower than the sick plot at all the stages. Interaction effects of TxVxS of phenol content revealed non significant differences in root tissues (Figure 8). Plant grown in sick plot resulted little changed in response to disease infection in root tissues of all the six cultivars. Susceptible cultivars JG-62 and GG-4 contained significantly higher amount of total phenol content as compared to resistant cultivars at pre infection stage. All

cultivars showed decrease in their content at infection stage but the trend remains same at post infection stage. The phenol content was again increased in all the cultivars. All the cultivars showed almost similar level of phenol content except GG-2 where the value was little higher.

DISCUSSION

Ascorbic acid content significantly declined from pre infection (S_1) to infection stage (S_2) in all the cultivars. The same continued to decrease at post infection stage (S_3) also except in cultivar JG-62 and GG-4 where it increased. In case of normal plot similar trend was recorded for ascorbic acid content as observed in sick plot. Overall data recorded for ascorbic acid content were lower in resistant cultivars than the susceptible cultivars. These results are in agreement with findings suggested by (Chhabra et al., 2000; Rebenko et al., 1972; Wang-JianMing et al., 2002). They have observed low level of ascorbic content in different crop plant parts at different stages of diseases development. In case of normal plot, healthy plants showed increasing trend of total phenol content as progress of disease or growth of the plants from S_1 to S_3. The greater accumulation of phenol content was recorded between S_2 stage to S_3 stage. However, the accumulation of total phenol content was more in resistant cultivars JCP-27 and WR-315 as compared to susceptible cultivars. The reason behind that the rich amount of secondary metabolites in the host plant inhibits the growth and development of the pathogen. Overall data recorded for total phenol content was higher in resistant cultivars than the susceptible cultivars of chickpea infected with *F. oxysporum, f.sp. ciceris* (FOC) these results are in agreement with the findings suggested by (Khan et al., 2005; Singh et al., 2003), they reported that the phenolic content was increased in the roots of susceptible and resistant cultivars of chickpea after inoculation with the virulent and hypovirulent isolates of *F. oxysporum*, f.sp. *ciceris* (FOC) in fact the susceptibility or resistantence of host appear to follow common pathways involving the preexisting and induced expression of defense component s activated by a number of fungal and plant metabolites. They found the highest increase in phenol content against the highly virulent isolate in the roots of both cultivars, whereas least increase was found in less virulent isolates. There was no reduction in phenols of both cultivars against the less virulent isolate. Saikia et al. (2006), reported increased level of content in chickpea plant parts and showed enhanced synthesis of phenolic compounds and finding suggested that it systemically induced resistance in chickpea seedling exposed CWPs elicitors. Resistant cultivars recorded with the highest free amino acids while susceptible cultivar showed the lowest amount (2.09 $mg.g^{-1}$.fr.wt). Similar results were obtained in chickpea plants infected with Fusarium oxysporium f.sp. ciceris using two cultivars that is, resistant and susceptible (Mandavia et al., 1990). If data expressed on the basis of percent reduction, a greater loss of free amino acids was also recorded in susceptible cultivars from infection (S_2) to post infection stages (S_3). In case of normal plot, healthy plants showed similar pattern of free amino acids as progress or growth of the plants from S_1 to S_3. However, percentage reduction data showed less reduction in free amino acids (43-45 %) as compared to sick plot data (60-65%). In general, free amino acids content reduced, as disease infection in the susceptible cultivars and the resistant cultivars at all infection stage. These results are in agreement with the findings suggested by Mandavia et al. (1990), Gowily et al. (1995), Shukla (2001) and Bhut (2005) who have reported in both normal and sick plots, free amino acids content in root tissues decreased as growth of the plants from pre infection (S_1) to post infection (S_3) in chickpea plant parts at different stages of disease development or growth of the plants. Because of amino acids utilized by the fungus for their development in disease plants and general evidence leads us to believe that the free amino acid increase in infected plants may results in large part from increased aerobic oxidation directly related to carbon dioxide liberation as well as role of TCA and other intermediary metabolic pathways. Resistant JCP-27 displayed higher levels of free amino acids at all stages except at post-infection stage (S_3). JCP-27 also holds higher levels of cysteine, glutamic acid, proline, phenylalanine and leucine at the infection stage (Mandavia et al., 1990). In the present experiment the data recorded for proline content was higher under stress conditions. Proline is used as indicator for stress. These results are in agreement with findings suggested by Mandavia et al. (1990) reported in chickpea infection with wilt fungus, Jiang-YuRong et al. (2005) in cotton infected with wilt. Kannaiyan et al. (1973) suggested that the presence of specific aminoacids is linked with resistance to a particular pathogen (*Claviceps microcephala*) in bajra and also reported increased level of proline during diseases development. This result signifies that due to varying nature of plants and pathogen, biochemical changes occurred at different stages of infection as well as growth of the plant. Many important issues that remain to be resolved include (*i*) interaction effect of proline and ascorbic acid, free amino acids and total phenol together such information, together with the provision of molecular tools, will allow manipulating and identifying traits that contribute to the disease resistance and host pathogen interaction.

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