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Activity Profile of Defense Related Enzymes in Chickpea (*Cicer arietinum* L.) against *Fusarium* Wilt (*Fusarium oxysporum* f. sp. *ciceris*) at Different Growth Stages

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Twenty four promising chickpea genotypes with known wilt resistant and susceptible checks were evaluated for phenol content and activity profile of ß-1,3 glucanase, chitinase, phenylalanine ammonia lyase and polyphenol oxidase at pre and post infection stages under wilt sick soil with simultaneous recording of wilt incidence score . The activity of all these enzymes was significantly increased at post infection stage of growth in wilt resistant chickpea genotypes as compared with wilt susceptible genotypes suggesting induction of the defense response. Amongst twenty four chickpea genotypes when screened in wilt sick soil along with wilt resistant and susceptible checks , three genotypes *viz.,* GL29285(83.3%), PBG5(26.8%) and GL27014 (81.7%) were categorized as wilt susceptible, while twenty one genotypes were wilt tolerant having higher activity profile of defense related enzymes with less than 10% wilt incidence. Among the twenty one chickpea genotypes screened under wilt sick soil BCP 2010-1, JG24 and JG2001-4 recorded significantly higher increase in activity profile of all the four enzymes and total phenolics content. The same three genotypes also recorded low wilt incidence in the field.

Key words: Wilt, Chickpea, Genotypes, Enzyme, Defense.

Among the pulses chickpea (*Cicer arietinum* L.) is the third important pulse after dry bean (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.)[1]. Chickpea is high protein legume grown in India. It is grown on about 9.21 million ha with production of 8.88 million ton with productivity of 995 kg/ha[2]. The chief constraints to chickpea production are biotic stresses such as *Ascochyta* blight (*Ascochyta rabiei*), *Fusarium* wilt (*Fusarium oxysporum* f. sp.*ciceri*), and pod borer (*Helicoverpa armigera*) which causes yield losses of about 4.8 million tonnes [3]. Several diseases are known to limit worldwide production of chickpea, of which *Fusarium oxysporum* f. sp. *ciceris* (*Fusarium* wilt) is one of the most important. Management of *Fusarium* wilt has been primarily through development of resistant cultivars as part of an integrated management approach. However, the high pathogenic variability in populations of *F. oxysporum* f. *sp. ciceris* presents problems for sustainability of resistant cultivars. Two pathotypes and eight races of the wilt pathogen have been identified. The reliance on resistant cultivars for disease management of *Fusarium* wilt therefore places significant importance on the confident and efficient identification of pathogenic races of *F. oxysporum* f. *sp. ciceris*.

In interactions with invading pathogens, plants frequently activate defense-related genes that lead to expression of pathogenesis-related (PR) proteins [4]. PR proteins are one of the important non-specific defense mechanisms of plants against pathogen. The hypersensitive reaction (HR), one of the most efficient and visible parts of the defense

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mechanisms in nature against invading pathogens, is associated with a coordinated and integrated set of metabolic alterations which are instrumental in impeding further pathogen ingress or alleviating stress. It includes a variety of novel proteins and secondary metabolites. Active research on various aspects of defense/stress response in various crops has resulted in the identification of a variety of response proteins and antifungal secondary metabolites induced in response to pathogen attack[5]. In this context we aimed a basic study in chickpea regarding changes of several defense related enzymes and phenolics upon *Fusarium* wilt attack.

Plants are equipped with a variety of defense mechanism against such pathogen or biotic stress to protect against the attack, some of these are constitutive while others are induced upon pathogen attack like accumulation of phenolics or induction of antioxidant and its related enzymatic system [6]. Plant resistance to biotic and abiotic stresses is often regulated by the metabolism of phenolics. Phenolics are known as hydrophilic antioxidants and play an important role in defending injured plants against pathogens [7]. Among the PR proteins chitinases and â-1,3 glucanases are two important hydrolytic enzymes that are abundant in many plant species after infection by different type of pathogens. The amount of them significantly increase and play main role of defense reaction against fungal pathogen by degrading cell wall, because chitin and â-1, 3 glucan is also a major structural components of the cell walls of many pathogenic fungi but they also release elicitors from the walls of fungi, which in turn stimulate various defense responses in plants [8].

In the present investigation attempts were made to find differences in the degree of resistance against *Fusarium* wilt (*Fusarium oxysporum* f. sp.*ciceri*) among 31 genotypes of chickpea based on the activity of defense related enzymes and phenolics.

MATERIALS AND METHODS

Seeds of twenty four promising chickpea genotypes which are often used in chickpea breeding along with seven checks *viz.*, Vijay, Digvijay, SAKI 9516, Vikas, JG-62, WR 315 and ICC 4958, were obtained from All India Co-ordinated Pulses Improvement Project, MPKV, Rahuri. The seeds were surface sterilized with 0.1% (v/v) sodium hypochlorite solution for 15 min, washed thoroughly with sterile distilled water and sown in wilt sick plot having inoculum load of $2x10^7$ cfu/g soil with completely randomized block design. The activities of enzyme from roots at pre infection (10-12 DAS), post infection (22-26 DAS) and flowering stage (55-60DAS) were evaluated with simultaneous recording of wilt incidence score of all chickpea genotypes.

Estimation of total phenols was carried out with Folin-Ciocalteu reagent by method of [9]. Exactly 0.250 g of root sample was weighed and macerated with 5-time volume of 80% ethanol in pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was collected and residue was re-extracted two more times with 80% ethanol. The combined supernatant was evaporated to dryness. The residue concentrate was dissolved in known volume of distilled water and aliquot was used to estimate the total phenol. A 0.1 ml aliquot was pipetted into test tubes and made up the 3 ml with water. To this 0.5 ml Folin-ciocalteu reagent was added and after three min, 2 ml of 20% sodium carbonate solution was added to each tube, mixed thoroughly and tubes were placed in boiling water bath for exactly 1 min. The tubes were cooled and absorbance was measured at 650 nm against reagent blank. The concentration of phenols in the test sample was expressed as mg g^{-1} fresh weight from standard curve prepared using different concentration of catechol.

The assay of â-1, 3-glucanase was carried out as per the method described by Rakshit *et al.*, [10]. The fresh root samples 0.2 g were washed in sterile distilled water and blotted with filter paper and macerated with chilled 2ml of 0.2M Tris-HCl buffer (pH 7.5) in pre-chilled mortar and pestle. The homogenate was centrifuged at 11,000 x g at 4^0C for 30 min and the supernatant was used as the crude enzyme source. One ml reaction mixture contained 950 µl of laminarin and 50 µl of crude enzyme extract was incubated at 37^0C for 30 min and the reducing sugar released in to the solution at the end of reaction was estimated by Nelson-Somogyi method and the absorbance was read at 520 nm. The â-1, 3 glucanase activity was

expressed as nmoles of glucose released mg^{-1} protein min^{-1}. The protein contained in the crude enzyme extract was estimated according to the method of Lowry *et al*.[11]. The chitinase activity was estimated as per to the method described by Giri *et al*. [12]. The fresh root samples of 0.2 g were washed in sterile distilled water and blotted with filter paper and macerated with chilled 2ml of 0.1M sodium citrate buffer (pH 5.0) in pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000 x g at 4^0C for 20 min and the supernatant was used as the crude enzyme source for chitinase. The protein content in the crude enzyme extract was estimated as described earlier. For the chitinase assay, 1 ml of supernatant, 4 ml of chitin suspension containing 15 mg BSA were incubated in water bath at 37°C for 3 hr. One ml water and 1 ml of reaction mixture was boiled in glass marble-covered centrifuge tube for 10 min and was centrifuged. An aliquot of 0.5 ml was taken for the estimation of N- acetyl glucosamine as per the method of Nelson–Somogyi method [13]. The chitinase activity was expressed as nmoles of N acetyl glucosamine released mg^{-1} protein $min^{-1.}$ from standard curve prepared using different concentration of N-acetyl glucosamine. The PAL activity was assayed by the method of Campos *et al.*[14]. 0.2 g root tissues were macerated with 2ml of 50mM borate buffer (pH 8.5) containing 5mM of 2-mercaptoethanol and 0.4 g insoluble polyvinyl polypyrrolidone. The homogenate was centrifuged at 20000 g at 4^0C for 20 min. The supernatant was used as an enzyme source. The protein content in the crude enzyme extract was estimated as described earlier. The assay mixture containing 0.1 ml aliquots of the supernatant, 0.9 ml of distilled water and 1 ml of 100mM L-phenylalanine were incubated at 40^0C for 30 min. Then, 1 ml of 4% TCA was added in it to terminate the reaction. Similarly the TCA was added in one of the test tube at 0 min to serve as a blank. The assay mixture was incubated with TCA for 5 min at room temperature and centrifuged at 10000g for 5 min and the absorbance of the supernatant was read at 290 nm. PAL activity was calculated as nmoles of trans-cinnamic acid formed mg^{-1} protein min^{-1} using standard curve prepared form different concentrations of trans-cinnamic acid. The polyphenol oxidase activity was assayed by the method of Kumar and Khan [15]. All chickpea genotypes with wilt resistant and susceptible checks used for enzyme analysis were scored in the wilt sick plot for wilt incidence and percentage of wilt was recorded as per the scale.

All the biochemical parameters were analyzed in three replications. The data obtained for biochemical constituents and enzymes determination were subjected to simple completely randomized block design for significance of various data [16].

RESULTS AND DISCUSSION

Phenolics play an important role in plant metabolism and protect the plant against stresses. The total phenolics content did not vary significantly at pre-infection stage in wilt susceptible and resistant checks while at post infection stage a significant increase was recorded (Table 1). At post infection stage mean total phenolics content in wilt tolerant check genotypes was 2.59 mg g^{-1} fresh wt. and while in wilt susceptible checks it was 1.82 mg g^{-1} fresh wt. Among the twenty four chickpea genotypes, JG 24 recorded maximum increase in phenolics content at post infection stage from 0.59 to 2.76 mg g^{-1} fresh wt. recording 4.68 fold increase. Similarly chickpea genotypes BCP 2010-1 and JG 2001-4 recorded significant increase in total phenolics content. The field wilt incidence of these three genotypes when recorded was below 10% supporting involvement of phenolics in *Fusarium* wilt resistance. Similar increase in total phenolics in wheat plant roots after infection with *Ustilago tritici* to the extent of 161 % in diseased roots as compared to healthy roots has been reported [17]. Total root phenolics content of inoculated chickpea genotype WR 315 showed about 1.92 fold increase at wilt infection stage while only 1.2 fold increase was recorded in wilt susceptible genotype JG 62 [18].Pigeon pea wilt resistant genotype BDN 2 showed 30% increase in root total phenolics content over non inoculated plants than wilt susceptible genotype ICP 2376 with 20% increase [19].

At the post infection stage significant increase in the enzyme activity was recorded in all the wilt resistant check genotypes with a mean ß-1, 3 glucanase activity of 598.4 nmoles of glucose released mg^{-1} protein min^{-1} (Table 2). The ß-1,3

glucanase activity in susceptible checks at post infection stage varied in the range from 141.2 to 323.5 nmoles of glucose released mg^{-1} protein min^{-1} with a mean activity of 261.2 nmoles of glucose released mg^{-1} protein min^{-1}. The fold increase in enzyme activity from preinfection to post infection

Table 1. Root total phenolics content of wilt resistant and susceptible chickpea genotypes at different growth stages gown in wilt sick soil

Sr.No.	Genotype	Total phenolics (mg g^{-1} fresh wt.) Pre infection stage	Post infection stage	Flowering stage	Fold increase (Preinfection to post infection stage)
1	Vijay (R)	0.62	2.45	2.75	4.10
2	Digvijay (R)	0.54	2.54	2.84	4.52
3	WR 315 (I)	0.67	2.79	2.9	4.77
4	ICC4958 (R)	0.56	2.92	3.02	4.50
	Range	0.54-0.67	2.45-2.92	2.75-3.02	4.10-4.77
	Mean	0.58	2.59	2.88	4.47
5	SAKI9516(S)	0.58	1.82	1.92	3.14
6	Vikas(S)	0.6	1.76	1.84	2.93
7	JG 62(S)	0.63	1.88	1.94	2.98
	Range	0.56-0.63	1.76-1.88	1.92-1.94	2.93-3.14
	Mean	0.60	1.82	1.90	3.02
8	GJG 0920	0.56	2.45	2.6	4.38
9	GJG0919	0.67	2.4	2.55	3.58
10	IPC 08-11	0.58	2.56	2.68	4.41
11	IPC 05-74	0.64	2.48	2.54	3.88
12	GJG 0814	0.6	2.66	2.7	4.43
13	IPC1048	0.66	2.25	2.38	3.41
14	GJG0922	0.59	2.38	2.48	4.03
15	GL23094	0.64	2.72	2.86	4.25
16	GJG0904	0.68	2.42	2.48	3.56
17	IPCK09-40	0.7	2.38	2.56	3.40
18	GL29285	0.66	1.8	1.88	2.73
19	PBG5	0.58	1.78	1.9	3.07
20	GL27014	0.65	1.7	1.8	2.62
21	IPC10-73	0.55	2.64	2.72	4.51
22	BCP49	0.6	2.6	2.7	4.33
23	HC 1	0.62	2.54	2.64	4.10
24	JG 2-14-110	0.59	2.44	2.5	4.14
25	JG 552476	0.68	2.78	2.86	4.09
26	BCP26	0.6	2.58	2.72	4.30
27	BCP2010-1	0.58	2.86	2.96	4.66
28	BCP60	0.64	2.56	2.66	4.00
29	JG2001-4	0.6	2.8	2.9	4.65
30	JG24	0.59	2.76	2.86	4.68
31	JG 2000-7	0.62	2.5	2.62	4.03
	Range	0.55-0.68	1.70-2.86	1.80-2.96	2.62-4.68
	Mean	0.61	2.66	2.76	4.34
	Overall Mean	0.62	2.43	2.54	3.87
	Comparison	SE±	CD at 5%		
1.	Genotype	0.003	0.05		
2.	Stage	0.005	0.04		
3.	Genotype x stage	0.014	0.18		

stage was 4.24 to 5.30 in wilt resistant checks as against a fold increase of 1.27 to 2.90 in wilt susceptible checks. Among the twenty four chickpea genotypes, BCP 2010-1 recorded maximum induction as evident from increase in ß-1,3 glucanase activity from 100.7 to 533.9 nmoles

Table 2. Root β-1, 3 glucanase activity of wilt resistant and susceptible chickpea genotypes at different growth stages gown in wilt sick soil

		β-1, 3 glucanase activity (nmoles of glucose released mg-1 protein min-1)			
Sr.No.	Genotype	Pre infection stage	Post infection stage	Flowering stage	Fold increase (Preinfection to post infection stage)
1	Vijay (R)	119.3	607.9	1428.6	5.10
2	Digvijay (R)	135.9	576.5	1358.0	4.24
3	WR 315 (I)	123.5	655.1	1598.9	5.30
4	ICC4958 (R)	128.6	554.0	1372.6	4.31
	Range	119.3-135.9	554.0-655.1	1358.0-1598.9	4.24-5.30
	Mean	126.8	598.4	1439.6	4.74
5	SAKI9516(S)	128.3	323.5	556.0	2.52
6	Vikas(S)	109.8	318.9	584.3	2.90
7	JG 62(S)	111.6	141.2	0.00	1.27
	Range	109.8-128.3	141.2-323.5	556.0-725.1	1.27-2.90
	Mean	116.6	261.2	640.6	2.23
8	GJG 0920	113.5	537.1	1029.4	4.73
9	GJG0919	126.6	544.6	1244.9	4.30
10	IPC 08-11	101.5	528.0	1225.5	5.20
11	IPC 05-74	112.4	494.1	1179.6	4.40
12	GJG 0814	133.5	562.3	1314.2	4.21
13	IPC1048	137.9	549.0	1292.8	3.98
14	GJG0922	122.7	544.8	1235.3	4.44
15	GL23094	101.1	534.5	915.1	5.28
16	GJG0904	124.9	536.6	1260.5	4.30
17	IPCK09-40	139.1	542.0	1181.9	3.90
18	GL29285	122.3	322.3	354.6	2.64
19	PBG5	131.8	398.6	482.2	3.03
20	GL27014	131.2	324.2	364.3	2.47
21	IPC10-73	136.1	490.2	1243.9	3.60
22	BCP49	136.8	410.8	1104.8	3.00
23	HC 1	140.2	524.7	1372.6	3.74
24	JG 2-14-110	139.6	539.5	1391.1	3.87
25	JG 552476	119.7	599.4	1299.1	5.01
26	BCP26	138.6	549.7	1353.0	3.97
27	BCP2010-1	100.7	533.9	1286.8	5.30
28	BCP60	131.9	439.6	1111.8	3.33
29	JG2001-4	100.9	533.3	1365.1	5.28
30	JG24	108.2	562.9	1403.8	5.20
31	JG 2000-7	111.1	511.3	1316.7	4.60
	Range	100.7-140.2	322.3-599.4	354.6-1403.8	2.47-5.30
	Mean	123.4	504.7	1138.7	4.26
	Overall Mean	123.2	493.2	1108.7	4.05
	Comparison	SE±	CD at 5%		
1.	Genotype	2.84	7.96		
2.	Stage	1.80	5.03		
3.	Genotype x stage	9.84	27.56		

of glucose released mg^{-1} protein min^{-1} (5.30 fold). JG 2001-4 genotype recorded 5.28 fold increase from pre infection to post infection, whereas GL 27014 recorded comparatively less induction as evident from ß-1,3 glucanase activity which increased from 131.2 to 324.2 nmoles of glucose

Table 3. Root chitinase activity of wilt resistant and susceptible chickpea genotypes at different growth stages in wilt sick soil

Sr.No.	Genotype	Chitinase activity (nmoles of N acetyl glucosamine released mg-1 protein min-1) Pre infection stage	Post infection stage	Flowering stage	Fold increase (Preinfection to post infection stage)
1	Vijay (R)	51.7	114.2	131.9	2.21
2	Digvijay (R)	52.5	117.8	131.5	2.24
3	WR 315 (I)	48.8	136.3	169.4	2.79
4	ICC4958 (R)	50.7	115.8	134.7	2.29
	Range	48.8-52.5	114.2-136.3	131.5-169.4	2.21-2.79
	Mean	50.9	121.0	141.9	2.38
5	SAKI9516(S)	38.9	64.2	70.3	1.65
6	Vikas(S)	37.5	63.1	68.6	1.68
7	JG 62(S)	41.1	53.0	0.00	1.29
	Range	37.5-41.1	53.0-64.2	68.6-70.3	1.29-1.68
	Mean	39.2	60.1	69.4	1.54
8	GJG 0920	56.8	98.7	114.5	1.74
9	GJG0919	55.5	113.2	130.7	2.04
10	IPC 08-11	43.5	83.1	98.1	1.91
11	IPC 05-74	43.7	80.6	89.8	1.84
12	GJG 0814	54.7	103.2	105.6	1.89
13	IPC1048	56.2	112.4	128.5	2.00
14	GJG0922	57.4	109.5	118.0	1.91
15	GL23094	52.6	91.8	98.9	1.74
16	GJG0904	56.2	113.6	114.7	2.02
17	IPCK09-40	54.1	98.8	105.8	1.83
18	GL29285	56.7	82.0	87.4	1.44
19	PBG5	48.7	80.7	87.8	1.66
20	GL27014	55.9	83.3	85.4	1.49
21	IPC10-73	52.1	91.0	113.2	1.75
22	BCP49	47.6	85.0	114.2	1.78
23	HC 1	57.6	101.3	135.7	1.76
24	JG 2-14-110	44.5	90.9	131.7	2.04
25	JG 552476	45.2	113.1	129.4	2.50
26	BCP26	49.0	92.8	112.4	1.89
27	BCP2010-1	44.0	103.2	138.2	2.34
28	BCP60	41.0	80.0	110.5	1.95
29	JG2001-4	49.6	109.2	123.2	2.20
30	JG24	40.7	103.1	129.9	2.54
31	JG 2000-7	47.6	101.5	128.5	2.13
	Range	40.7-57.6	80.0-113.6	85.4-138.2	1.44-2.54
	Mean	50.46	96.74	113.8	1.93
	Overall Mean	49.43	96.33	111.7	1.95
	Comparison	SE±	CD at 5%		
1.	Genotype	0.22	0.60		
2.	Stage	0.34	0.96		
3.	Genotype x stage	1.19	3.33		

released mg^{-1} protein min^{-1} at post infection stage (2.47 fold). Similar results were recorded by some researchers like [20], [21]earlier in different crops.

Twenty four chickpea genotypes were evaluated for root chitinase activity profile with simultaneous recording of wilt incidence at

Table 4. Root phenylalanine ammonia lyase activity of wilt resistant and susceptible chickpea genotypes at different growth stages in wilt sick soil

Sr.No.	Genotype	Phenyl alanine ammonia lyase activity (nmoles of trans-cinnamic acid formed mg-1 protein min-1) Pre infection stage	Post infection stage	Flowering stage	Fold increase (Preinfection to post infection stage)
1	Vijay (R)	6.60	22.19	26.44	3.36
2	Digvijay (R)	6.96	24.51	28.49	3.52
3	WR 315 (I)	7.91	31.62	33.30	4.00
4	ICC4958 (R)	7.84	23.40	28.99	2.98
	Range	6.60-7.91	22.19-31.62	26.44-33.30	2.98-4.00
	Mean	7.32	25.43	29.31	3.47
5	SAKI9516(S)	5.69	11.4	11.88	2.00
6	Vikas(S)	6.67	12.20	14.26	1.83
7	JG 62(S)	6.17	12.26	0	1.99
	Range	5.69-6.67	11.4-12.26	11.88-14.26	1.83-2.00
	Mean	6.18	11.40	12.94	1.94
8	GJG 0920	7.64	16.79	19.32	2.20
9	GJG0919	9.02	23.85	24.67	2.64
10	IPC 08-11	9.63	22.44	24.84	2.33
11	IPC 05-74	8.70	17.62	21.74	2.03
12	GJG 0814	9.40	18.54	23.68	1.97
13	IPC1048	7.83	19.17	20.83	2.45
14	GJG0922	7.81	19.00	25.04	2.43
15	GL23094	7.32	15.76	20.61	2.15
16	GJG0904	6.67	15.55	22.01	2.33
17	IPCK09-40	7.24	12.40	18.36	1.71
18	GL29285	9.34	11.59	12.28	1.24
19	PBG5	9.04	12.32	15.85	1.36
20	GL27014	6.81	8.76	12.02	1.29
21	IPC10-73	9.83	17.89	21.74	1.82
22	BCP49	10.02	16.56	22.48	1.65
23	HC 1	10.03	18.40	24.53	1.83
24	JG 2-14-110	8.47	23.05	30.55	2.72
25	JG 552476	8.56	24.90	29.81	2.91
26	BCP26	9.36	18.91	24.84	2.02
27	BCP2010-1	5.52	18.13	21.74	3.28
28	BCP60	8.42	17.22	17.04	2.04
29	JG2001-4	6.17	20.26	23.82	3.28
30	JG24	6.71	21.54	27.27	3.21
31	JG 2000-7	5.80	17.10	21.03	2.95
	Range	5.52-10.03	8.76-24.90	12.02-30.55	1.24-3.28
	Mean	8.14	17.82	21.92	2.24
	Overall Mean	7.85	17.74	21.84	2.32
	Comparison	SE±	CD at 5%		
1.	Genotype	0.12	0.36		
2.	Stage	0.18	0.50		
3.	Genotype x stage	0.38	1.10		

different growth stages. Analysis of chitinase activity in these chickpea genotypes revealed maximum increase in chitinase activity from preinfection to post infection stage in JG 552476, BCP 2010-1 and JG 24 genotypes. In genotype JG 24, maximum increase in chitinase activity was

Table 5. Root polyphenol oxidase activity of wilt resistant and susceptible chickpea genotypes at different growth stages in wilt sick soil

Sr. No.	Genotype	Polyphenol oxidase activity (δA mg^{-1} protein min^{-1}) Pre infection stage	Post infection stage	Flowering stage	Fold increase (Preinfection to post infection stage)
1	Vijay (R)	0.24	0.75	0.81	3.14
2	Digvijay (R)	0.23	0.71	0.79	3.12
3	WR 315 (I)	0.25	1.06	1.12	4.20
4	ICC4958 (R)	0.23	0.72	0.77	3.10
	Range	0.23-0.25	0.71-1.06	0.77-1.12	3.10-4.20
	Mean	0.24	0.81	0.87	3.39
5	SAKI9516(S)	0.25	0.56	0.65	2.29
6	Vikas(S)	0.24	0.46	0.53	1.89
7	JG 62(S)	0.23	0.28	0.000	1.20
	Range	0.23-0.25	0.28-0.56	0.53-0.65	1.20-2.29
	Mean	0.24	0.43	0.59	1.71
8	GJG 0920	0.21	0.45	0.52	2.12
9	GJG0919	0.20	0.61	0.71	3.10
10	IPC 08-11	0.24	0.55	0.70	2.27
11	IPC 05-74	0.28	0.61	0.70	2.15
12	GJG 0814	0.27	0.62	0.74	2.29
13	IPC1048	0.17	0.64	0.70	3.70
14	GJG0922	0.16	0.62	0.77	3.95
15	GL23094	0.20	0.41	0.49	2.07
16	GJG0904	0.14	0.54	0.61	3.84
17	IPCK09-40	0.20	0.45	0.48	2.19
18	GL29285	0.22	0.40	0.44	1.84
19	PBG5	0.23	0.46	0.52	2.00
20	GL27014	0.22	0.39	0.41	1.76
21	IPC10-73	0.25	0.66	0.67	2.64
22	BCP49	0.21	0.49	0.53	2.33
23	HC 1	0.26	0.67	0.72	2.55
24	JG 2-14-110	0.20	0.64	0.75	3.24
25	JG 552476	0.25	0.99	1.00	3.97
26	BCP26	0.21	0.48	0.62	2.27
27	BCP2010-1	0.21	0.81	0.84	3.92
28	BCP60	0.21	0.60	0.66	2.81
29	JG2001-4	0.19	0.69	0.76	3.70
30	JG24	0.19	0.67	0.71	3.51
31	JG 2000-7	0.22	0.69	0.79	3.20
	Range	0.14-0.28	0.39-0.99	0.48-1.00	1.76-3.92
	Mean	0.21	0.60	0.67	2.81
	Overall Mean	0.22	0.61	0.66	2.78
	Comparison	SE±	CD at 5%		
1.	Genotype	0.002	0.008		
2.	Stage	0.003	0.009		
3.	Genotype x stage	0.004	0.012		

recorded from 40.7 to 103.1 nmoles of N acetyl glucosamine released mg^{-1} protein min^{-1} from preinfection stage to post infection stage with an increase of 2.54 fold. GL29285 genotype showed minimum increase in chitinase activity from preinfection to post infection stage from 56.7 to 82.0 nmoles of N acetyl glucosamine released mg^{-1} protein min^{-1} an increase of 1.44 fold showing maximum wilt incidence of 83.3% and categorized as a highly susceptible genotype for wilt. The root chitinase activity in these chickpea genotypes ranged from 40.7 to 57.6 nmoles of N acetyl glucosamine released mg^{-1} protein min^{-1} at preinfection stage with mean of 50.46 nmoles of N acetyl glucosamine released mg^{-1} protein min^{-1} while activity ranged from 80.0 to 113.6 nmoles of N acetyl glucosamine released mg^{-1} protein min^{-1} at post infection stage of growth with a mean of 96.74 nmoles of N acetyl glucosamine released mg^{-1} protein min^{-1} (Table 3). The chitinase activity in smut-resistant (Yacheng05-179) and smut-susceptible (Liucheng03-182) sugarcane cultivars inoculated with *Sporisorium scitamineum* showed 2 fold increase in smut resistant cultivar Yacheng05-179 over smut susceptible cultivar Liucheng03-182 after 72 hr inoculation which suggesting positive correlation between sugarcane chitinase activity and smut resistance [22].

Time-course increase in activity of root phenylalanine ammonia lyase was observed in wilt resistant and susceptible check genotypes of chickpea from preinfection to flowering stage but activity increased more in wilt tolerant checks as

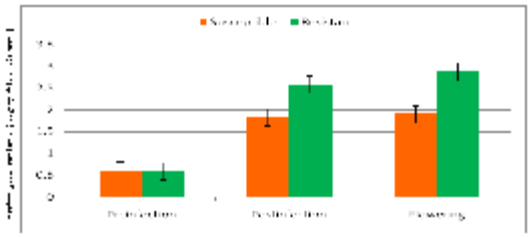

Fig. 1. Mean root total phenolics content of wilt resistant and susceptible check chickpea genotypes at different growth stages in wilt sick soil

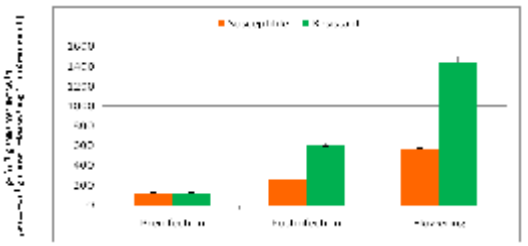

Fig. 2. Mean root β-1, 3 glucanase activity of wilt resistant and susceptible check chickpea genotypes at different growth stages in wilt sick soil

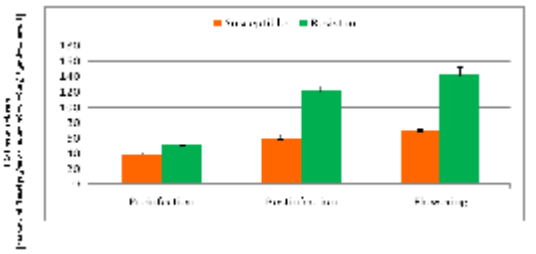

Fig. 3. Mean root chitinase activity of wilt resistant and susceptible check chickpea genotypes at different growth stages in wilt sick soil

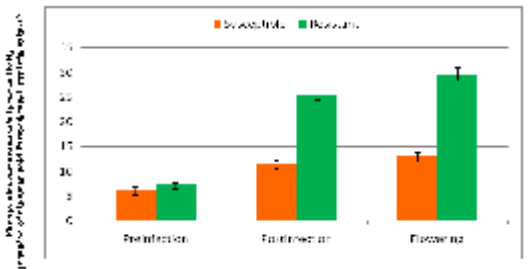

Fig. 4. Mean root phenylalanine ammonia lyase activity of wilt resistant and susceptible check chickpea genotypes at different growth stages in wilt sick soil

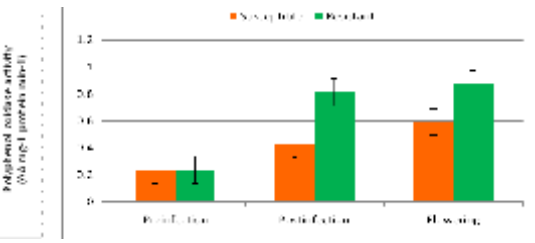

Fig. 5. Mean root polyphenol oxidase activity of wilt resistant and susceptible check chickpea genotypes at different growth stages in wilt sick soil

compared to susceptible at post infection stage. The mean PAL activity was significantly higher at post infection stage in resistant checks with 25.43 nmoles of trans-cinnamic acid formed mg^{-1} protein min^{-1} as against 11.40 nmoles of trans-cinnamic acid formed mg^{-1} protein min^{-1} in wilt susceptible checks. The maximum fourfold increase in PAL activity was recorded in WR 315 a wilt immune chickpea genotype from preinfection to post infection stage (Table 4). It was observed that among the twenty four chickpea genotypes evaluated for PAL activity genotypes JG 2001-4, BCP 2010-1 and JG 24 recorded maximum increase in PAL activity from preinfection to post infection stage. In genotype JG 2001-4, PAL activity increased from 6.17 to 20.26 nmoles of trans-cinnamic acid formed mg^{-1} protein min^{-1} from preinfection to post infection stage (3.28 fold) with minimum wilt incidence and genotype GL 29285 showed minimum increase in PAL activity from preinfection stage to post infection stage from 9.34 to 11.59 nmoles of trans-cinnamic acid formed mg^{-1} protein min^{-1} with 1.24 fold increase with maximum wilt incidence. Similar results were recorded by some researchers as in wilt tolerant genotype of pigeon pea, BDN-1 showed 22.61% increase in PAL activity when inoculated with *F. udum* and *H. cajani* while susceptible genotype GS-1 showed minimum increase in PAL activity after inoculation [23]. PAL activity was increased maximum in roots of wilt resistant pigeon pea genotype ICPL 87119 from 185.90 to 288.15 ìg trans-cinnamic acid h^{-1} mg^{-1} protein over control at 7DAI of wilt pathogen while in wilt susceptible genotype ICP 2376, PAL activity in the roots was increased from 110.29 to 143.21 ìg trans- cinnamic acid h^{-1} mg^{-1} protein over control at 7 DAI [19].

Table 6. Confirmation of resistance against wilt in chickpea genotypes under wilt sick soil

Sr. No.	Genotype	Percent wilt incidence
1	Vijay	6.18
2	Digvijay	2.71
3	WR 315	0.00
4	ICC4958	5.84
5	SAKI9516	42.6
6	Vikas	30.0
7	JG 62	100.00
8	GJG 0920	1.60
9	GJG0919	0.0
10	IPC 08-11	0.0
11	IPC 05-74	1.4
12	GJG 0814	2.3
13	IPC1048	1.4
14	GJG0922	0.0
15	GL23094	18.1
16	GJG0904	0.0
17	IPCK09-40	7.7
18	GL29285	83.3
19	PBG5	26.8
20	GL27014	81.7
21	IPC10-73	6.7
22	BCP49	2.3
23	HC 1	0.0
24	JG 2-14-110	1.5
25	JG 552476	2.4
26	BCP26	0.0
27	BCP2010-1	1.5
28	BCP60	0.0
29	JG2001-4	0.0
30	JG24	3.8
31	JG 2000-7	2.4

Scale: 1. 0.0 to 10.0% Wilt resistant2. 10.1 to 30.0% Moderately resistant, 3. 30.1 to 50.0 % Susceptible 4. Above 50.1% Highly susceptible

Among the twenty four chickpea genotypes screened in wilt sick soil at three growth stages the root PPO activity increased maximum in genotype JG 552476 and GJG 0922 with 3.97 and 3.95 fold respectively as compared to wilt resistant check WR 315 with 4.20 fold increase. The PPO activity in genotype JG 552476 was increased from 0.25 to 0.99 ÄA mg^{-1} protein min^{-1} from preinfection to post infection stage. These genotypes also recorded low wilt incidence in field. Genotype GL27014 recorded minimum increase in PPO activity from 0.22- 0.39ÄA mg^{-1} protein min^{-1} with 1.76 fold increase and was also highly susceptible as per the wilt incidence scale recorded in the field (Table 5). The results of the present investigation are in agreement with the previous results recorded in chickpea.

Wilt incidence of three genotypes *viz.*, GL29285 (83.3%), PBG5 (26.8%) and GL27014 (81.7%) was confirmed with low enzyme activities as wilt susceptible genotypes, while twenty one genotypes were wilt resistant having below 10% wilt incidence with higher defense responsive enzyme activities. Amongst the twenty one chickpea wilt resistant genotypes screened under wilt sick soil four chickpea genotypes *viz*; JG24, BCP 2010-1, JG2001-4 and JG 552476 recorded

overall increase in four enzymes studied under this experiment which were at par with the wilt resistant checks and also confirmed with low wilt incidence under field observations as wilt resistant genotypes (Table 6).

CONCLUSIONS

Based on the levels of phenolics and induction levels of enzymes involved in plant defense three genotypes *viz*., BCP 2010-1, JG 24 and JG 2001-4 appeared to be wilt resistant. Also increase in phenolics and these enzymes were significantly increased at post infection stage as compared to preinfection stage of plant growth. Wilt resistant checks recorded significant increase in defense responsive enzymes and phenolics than wilt susceptible chickpea check genotypes at post infection stage. The field performance of these three genotypes in the wilt sick soil recorded wilt incidence of less than 10% at all the three growth stages. The induction level of phenolics and defense related enzymes from pre to post infection stage was in the range and even more than that recorded in wilt resistant checks.

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