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Biochemical Basis of Resistance in Chickpea (*Cicer arietinum* L.) against *Fusarium* Wilt

R. SINGH[1*], A. SINDHU[2],
H. R. SINGAL[3] and R. SINGH[3]

[1]Directorate of Wheat Research, Karnal-132 001, India
[2]Department of Biotechnology, CCS Haryana Agricultural University, Hisar-125 001, India
[3]Department of Biochemistry, CCS Haryana Agricultural University, Hisar-125 001, India

Biochemical basis of disease resistance was studied by analysis of total phenols, peroxidase and ß-1,3-glucanase activities in calli of *Fusarium* wilt resistant and susceptible genotypes and one *in vitro* selected resistant cell line of chickpea (*Cicer arietinum* L.). The callus growth of cvs. JG62, C235 and WR315 was completely inhibited at 2% (v/v) concentration of culture filtrate of the fungus, whereas, the callus growth of *in vitro* selected resistant cell line FWR was not inhibited even at 4% (v/v) concentration of culture filtrate, indicating that a cultivar resistant under field conditions could show susceptibility under *in vitro* conditions. Total phenols were more in the resistant cell line than in the susceptible cultivars. However, increase in phenolics was higher in resistant cell line than in susceptible cultivars after treatment with the cultural filtrate, indicating that phenols reach an inhibitory level to the fungus in the resistant cell line. Peroxidase followed a similar pattern. Increase in ß-1,3-glucanase activity, after treatment, was higher in resistant cell line than in susceptible cultivars. Role of total phenols, peroxidase and ß-1,3-glucanase in defense against *Fusarium* wilt disease of chickpea is discussed.

Keywords: *Cicer arietinum,* culture filterate, *Fusarium* wilt, total phenols, peroxidase, ß-1,3-glucanase.

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world, but the susceptibility of the crop to several biotic and abiotic stresses adversely influences the yield. Of the several diseases recorded in chickpea, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* has been reported from several countries (Nene et al., 1984). There are several ways in which plant tissue and cell culture are being utilized in plant pathology research (McCoy, 1988). Screening of disease resistant plants can be effective, if the biochemical pathways of disease resistance in plants are well understood.

The host-pathogen interaction is very complex and diverse in nature. Resistance in higher plants against microbial pathogens is the result of constitutive and inducible defence mechanism. The metabolic changes occurring in diseased plants frequently leads to accumulation of aromatic compounds especially phenolic compounds which are generally more pronounced in resistant varieties (Farkas and Király, 1962; Bashan et al., 1987; Sindhu et al., 1995). Plant resistance to pathogenic fungi involves multiple response pathways including the accumulation of hydrolytic enzymes such as chitinases and ß-1,3-glucanases (Boller, 1985; Nehra et al., 1994). We carried out some biochemical studies on calli of chickpea in relation to its resistance to *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri.*

* Corresponding author

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Materials and Methods

The genotypes of chickpea included in the present study show different degree of tolerance to *Fusarium oxysporum* f. sp. *ciceri* infection in the field. WR315 is wilt resistant and C235 is moderately resistant, while JG62 is wilt susceptible. The seeds of cv. JG62, C235 and WR315 were surface sterilized with 0.1% (w/v) mercuric chloride for 10 min and rinsed twice with sterile distilled H_2O. The seeds were cultured on B_5 medium (Gamborg et al., 1968) supplemented with 0.15% (w/v) 6-benzylaminopurine (BAP) and 0.1% (w/v) 1-naphthaleneacetic acid (NAA). The callus of *in vitro* selected cell line (FWR), resistant to *Fusarium* wilt was also included in the study.

Pure culture of *Fusarium oxysporum* f. sp. *ciceri* was grown on solidified Richard's medium (KNO_3 10 g/l, K_2HPO_4 5 g/l, $MgSO_4$. $7H_2O$ 2.5 g/l, $FeCl_2$ 0.02 g/l, sucrose 50 g/l and agar 20 g/l). Three discs of 8–10 diameter of 25 days old fungal culture were put in 100 ml liquid Richard's medium and shaken continuously on a rotary shaker at 25 °C. After 14 days, the culture filtrate (CF) was separated from the yellowish white mycelial growth and spores by passing through the Whatmann filter paper. The culture filtrate was then filter sterilized through Millipore filter of 0.5 µm pore size and stored at –20 °C in small vials. Twenty pieces of 2–3 mm size of actively growing calli of different genotypes were inoculated in Petri plates containing B_5 medium supplemented with different concentrations (0%, 2%, 4%) of culture filtrate. Total phenols, peroxidase and ß-1,3-glucanase were determined according to the methods of Swain and Hillis (1959), Seevers et al. (1971) and Joosten and DeWit (1989).

Results

Growth kinetics

The different genotypes were compared for their *in vitro* resistance to *Fusarium* culture filtrate. The callus growth in case of WR315, C235, JG62 showed browning after 72 h of application of B_5 medium with CF. However, the callus from FWR grew actively on the medium supplemented with CF without any browning. The callus cultures of WR315, C235 and JG62 were found susceptible to culture filtrate of *F. oxysporum* under *in vitro* conditions, whereas, FWR cell line was found resistant to culture filtrate. The growth of FWR was not inhibited even with the application of 4% (v/v) culture filtrate.

Total phenols

It is seen in *Table 1* that the *in vitro* selected cell line had higher content of total phenols than the susceptible cultivar without the application of CF. However, there was no significant increase in the total phenols with the increase in time of untreated calli of different genotypes. After the application of culture filtrate, the total phenol increased in all of the genotypes with the time and was on maximum at 72 h of treatment. Increase in total phenols was more intensive in calli treated with higher concentration of culture

Table 1

Biochemical characterizations of calli of chickpea genotypes after treatment with CF of *Fusarium oxysporum* f. sp. *ciceri* for total phenols, peroxidase and ß-1,3-glucanase

Varieties/ Cell line	Time of treatment (Hrs)	Total phenols*			Peroxidase**			ß-1,3-glucanase***		
		Concentration of culture filtrate			Concentration of culture filtrate			Concentration of culture filtrate		
		0%	2%	4%	0%	2%	4%	0%	2%	4%
JG62	12	1.31	1.49	1.71	158	176	216	142	168	198
	36	1.33	1.85	2.18	160	182	236	145	193	254
	72	1.35	1.94	2.59	161	184	253	149	212	272
C235	12	1.33	1.58	1.88	174	215	247	137	176	221
	36	1.38	2.00	2.21	175	242	286	141	213	283
	72	1.40	2.28	2.75	176	254	328	143	232	313
WR315	12	1.38	1.57	1.81	188	222	248	153	219	257
	36	1.43	1.97	2.56	188	254	303	166	267	332
	72	1.45	2.28	2.79	190	272	341	169	298	359
FWR	12	2.00	2.21	2.62	210	238	250	172	238	313
	36	2.06	2.62	3.37	216	297	410	178	346	497
	72	2.09	3.85	4.61	214	328	455	183	381	563

* mg g^{-1} fresh wt; ** units= change in O.D. min^{-1} mg^{-1} protein; *** nmoles min^{-1} mg^{-1} protein

filtrate. The increase in total phenols was more pronounced in resistant genotype, as compared to the susceptible genotypes. The increase in total phenols in FWR was 1.8 and 2.2 folds, whereas, in the susceptible cultivars it was 1.4–1.6 and 1.9 folds at 2% and 4% concentration of culture filtrate, respectively after 72 h of CF treatment.

Peroxidase

The healthy calli of FWR had higher peroxidase activity, as compared to JG62, C235 and WR315 *(Table 1).* After treatment with CF, peroxidase activity increased in all genotypes parallel with the increase in time of application and with the increasing concentrations of the culture filtrate. The increase in peroxidase activity was higher in resistant cell line, as compared to the susceptible cultivar and the maximum activity was observed at 72 hrs of treatment. Increase in peroxidase was 1.2–1.4 and 1.5–1.8 folds in susceptible cultivars, whereas, 1.5 and 2.1 folds in resistant cell line after 72 h of treatment with 2 and 4% concentration of culture filtrate, respectively.

ß-1,3-glucanase

Analysis of ß-1,3-glucanase activity showed that untreated calli of the resistant genotype had slightly higher activity, as compared to the susceptible genotype at 12, 36 and 72 h of subculturing on medium without culture filtrate *(Table 1).* However, after treatment with CF of pathogen, activity of the enzyme increased significantly with the time and with the increasing concentration of CF in all the genotypes, but the increase in activity was higher in the resistant genotype, as compared to the susceptible one. Maximum effect of CF was observed after 72 h of treatment. The increase in enzyme activity was 1.4–1.8 and 1.8–2.2 folds in susceptible genotypes (JG62, C235 and WR315) while it was 2.0 and 3.0 folds in the resistant cell line (FWR) at 2%, and 4% concentration of CF, respectively, after 72 h of treatment. The above observations indicate that increase in activity of host ß-1,3-glucanase provides a potential defence mechanism against *F. oxysporum* f. sp. *ciceri* in the resistant genotype.

Discussion

The variation among the callus cultures growing on the medium supplemented with culture filtrate arises from the combined effects of tissue culture conditions and *F. oxysporum* f. sp. *ciceri* CF on different genotypes. The cultivars WR315 and C235 were susceptible under *in vitro* conditions which otherwise were determined as resistant (WR315) and moderately resistant (C325) to *Fusarium oxysporum* f. sp. *ciceri* under field conditions. Physiological and morphological factors may be involved in resistance response of plants to the pathogens (Strange et al., 1992). Selection against pathotoxin should also take into account the biochemical and molecular factors of disease resistance. Thus, resis-

tance against fungal pathogen of existing agronomically superior lines can be enhanced through *in vitro* cellular selection using culture filtrates (Singh et al., 1999).

The results clearly indicated that rate of phenol accumulation was lower in susceptible than in resistant cultivars and hence may account for resistance expression. Studies in maize (Sharma et al., 1983) indicated higher levels of total phenols in maize inbred CM 104 resistant to leaf blight than in the susceptible CM600. The resistant plant showed a tendency to accumulate higher amounts of total phenols than the susceptible ones following infection with fungi. Similar results have been reported in chilli (Bhullar et al., 1972) and rice (Sathiyanathan and Vidhyasekaran, 1981). Relationship between resistance and phenolic content was explained by Farkas and Király (1962) and Rahe et al. (1969) by suggesting that in the susceptible variety the fungus has enough time for its growth before phenol content reaches a level inhibitory to the fungus, whereas, in the resistant variety higher accumulation of phenols in initial stages restricts the growth of the fungus.

Peroxidases are known to be widespread in plant tissues and have been implicated in many physiological processes including phenol metabolism i.e. oxidation of phenolic compounds to quinones which are more toxic to microorganisms and lignin biosynthesis via polymerization of polypropane compounds by an oxidative H_2O_2 dependent system (Vance et al., 1980). The results obtained here indicate that peroxidase may contribute to resistance as postinfectionally since its activity, in general, increased more sharply in calli of resistant genotype following treatment, as compared to the susceptible ones. Similarly, high peroxidase activity in resistant varieties has been observed in other host pathogen interactions (Bashan et al., 1987; Saharan et al., 1999).

Pathogenic attack on plants appears to cause alterations in protein (enzyme) synthesis in the plant that can lead to the development of local resistance or immune layer around infection sites. Resistance or immunity of plants to a pathogen may depend on the speed and extent of protein synthesis induced in the host by the pathogen. After infection, synthesis of pathogenesis related proteins (Van Loon, 1985) and the accumulation of phytoalexins (Bailey and Mansfield, 1982) are especially important reactions. Increase in ß-1,3-glucanase was most conspicuous and drastic in resistant genotypes, as compared to the susceptible ones in pea after infection with powdery mildew (Rakshit et al., 2000). Investigations have revealed that 70 per cent of PR proteins are hydrolases comprising of chitinases and ß-1,3-glucanases (Legrand et al., 1987; Kombrink et al., 1988). Chitinase and ß-1,3-glucanase strongly inhibit fungal growth particularly when the two enzymes act together (Mauch et al., 1988; Tyagi et al., 2001). This antifungal action is probably due to hydrolysis of noncrystalline chitin and glucans present at the apex of the growing fungal hyphae (Wessels, 1986).

In conclusion, phenols, peroxidase and ß-1,3-glucanases are integral components of the response of a plant to various infections and stress conditions, responding in concert with other defence related proteins. These traits could therefore be used as markers for identifying chickpea lines resistant to fungal pathogens.

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