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Research Article

Variation in individual phenolics pattern in wilt infected chickpea

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ABSTRACT

Phenolic compounds have been most renowned secondary products in determination of resistance in plants. The study with six different cultivars suggests that increase of phenolics are resistant molecules of plant with preformed as well as due to the pathogen or biotic involvement in plant growth. In present experiment over all data of individual phenolics did not show any consistent prototype in all the chickpea cultivars. Doubtless, a single individual phenolic may have limited role in wilt disease resistance. Among the individual phenolics, only hydroquinone showed reverse trend except in cultivar JCP-27 and GG-4 where the contents were increased from infectional (S_2)to post infectional (S_3) stage. Some cultivars did not show any change in their phenolic content from S_2 to S_3 in root tissue obtained from normal plot. The levels of ferulic and salicylic acids remained same from S_2 to S_3 stage, and cultivar WR-315 for umbeliferon and vanillic acid for cultivar GG-1.However, it visualized from the data that instead of single individual phenolic. It may have synergistic effect of all individual phenolics in controlling the inflectional process in all the cultivars under investigation.

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INTRODUCTION

Chickpea crop is very important crop Junagadh district of Gujarat and it suffers due to infection of wilt which is most destructive in field condition. The wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is an important disease of chickpea (*Cicer arietinum* L.) worldwide (Trapero-Casas and Jimenez-Diaz, 1985). Most of the cultivars are susceptible to this disease. Since little is known about the phenolics content in root tissue of varying degrees of susceptibility. In many experiments, it has been reported that correlation exists between degree of resistance and phenol level in healthy plants induced significant increase in the activity of several defense-related enzymes such as peroxidases and polyphenoloxidases and in the accumulation of phenolic compounds (Arfaoui *et al*., 2005). Penetration of infected hyphae and spore germination is also inhibited by phenolic acids. So, attempts were made to generate information in phenolics constituent of healthy roots well as diseased roots excavated from normal plot grown plants and sick plot grown plant.

Phenolic acid metabolism is activated through phenyl propanoid pathway during infection which gives rise to suberin, lignin and wall bound phenolics as described below (Hahlbrock and Scheel, 1989). Amongst the secondary plant products, phenolic compounds are the most important group implicated in both constitutive and induced resistance. Presence of phenols and their oxidation products in plant tissues is considered to be potentially toxic to the growth and development of pathogens.

MATERIALS AND METHODS

Chickpea (*Cicer arietinum L*) seeds of six cultivars *viz.,* WR-315 (resistant) JCP-27 (resistant), GG-1(tolerant), GG-2 (tolerant) GG-4 (susceptible) and JG-62 (highly susceptible) were grown in two plots using split plot design and cultivars taken as 2nd factor where as 1st factor was plot *i.e.* one was healthy plot (normal plot) and other one was sick plot where soil was inoculated with *Fusarium oxysporium* f.sp. *ciceris* race-2 . Recommended package of practices were followed to raise plants in normal plot. Each cultivar grown in five raws with one yard stick of JG-62 for bordering each cultivar. Roots

were excavated from each plot at three different stages, *viz.,* pre-infectional (12 DAS), infectional (21 DAS) and post infectional stages (26 DAS). Roots were cleaned with tap water followed by distilled water and socked with filter paper and roots were separated below two cm hypocotyls and subsequently weighed according to biochemical parameter under ice cold condition from excavation to sampling process. Phenolics extraction was carried out according methods developed by Sharma and Singh (2002) and total 11 phenolics were identified using chromatography of High Performance Liquid Chromatography (HPLC).

Sample preparation:

One gram root tissue was quick frozen in liquid nitrogen extracted with 10 mL methanol: H_2O (80:20, v/v) for 24 hours at -80C^0 and fresh root tissue grinded with mortal pastel in 80 per cent methanol and centrifuged at 10000rpm, extract pore into 1.5 ml eppendorf tube then filtrated through 1cc cartridge on guard R-II-RP. Extracts were passed through a non-sterile 15 mm syringe filter with a 0.45 micron PFTE membrane (VWR International, Bridgeport, NJ, USA) and combined with 90 per cent methanol (1:4, v/v) for injection samples. Twenty µL of each injection was injected by manual sampler into the HPLC and separated on a Keystone Beta Basic C18 column (1 x 150 mm) using a mobile phase based on a isocratic gradient program which included solvents *i.e.* 1 per cent acetic acid, H_2O, and methanol (1:4:5) mixed previously in single (Eluent) bottle samples were run at a flow rate of 0.750 mL/min and visualized with a UV visible detector (Shimandzuliquid chromatography Ver -3) and software was SPINCHRON for the analyzing data with slight modification in Sharma and Singh (2002) and chemicals were used of HPLC grade and water used in the preparation of solvents was purified to 18.2 M.-cm using Millipore (SAS-67120) water system Molischam, FRANCE.and phenolics standard prepared as per standard protocol was injected at a volume of 15 µL.

RESULTS AND DISCUSSION

The results obtained from the present investigation have been discussed in the following points :

Individual phenolics:

All six cultivars grown in normal and sick plot differed in their phenolics content but in general, higher amount of phenolics was found in normal plot with some exceptions (Fig. 1 to 5).

Changes at pre-infectional to infectional stage:

A comparison was made between plants grown in normal and sick plots. Plants grown in normal plot resulted higher amount of all the phenolics present in cultivar WR-315 at pre-infectional stage. Greater amount of caffeic acid was present in cultivar JCP-27, GG-1 and JG-62 (Fig. 2), catechol in cultivar JCP-27 and GG-1 (Fig. 4), coumaric in JCP-27, GG-1 and GG-4, hydroquinone in cultivar JCP-27 and GG-2, pyrocatechol in GG-2 and GG-4 (Fig. 2), ferulic acids in cultivar GG-1 , salicylic acids in GG-4 whereas umbeliferon was higher in cultivar GG-2 and GG-4. However, vanillic was remained absent in all the cultivars (Fig. 4). With the advancement of growth stage (S_2), the hydroquinone, coumaric acid, pyrocatechol and salicylic acid were declined in all the cultivars from S_1 to S_2 stage when plants grown in normal plot. Incase of cultivar GG-2 and JG-62 did not show any reduction in salicylic acid (Fig. 5). However, vanillic acid was detected and it was observed in cultivar JCP-27, GG-1 and JG-62 at S_2 stage. Plants grown in sick plot revealed that the hydroquinone level reduced in all the cultivars from S_1 to S_2 stage. In contrast to this, umbeliferon and catechol increased from S_1 to S_2 stage in all the cultivars. However the chlorogenic acid, caffeic acid, catechol, cinnamic acids (Fig.6) and ferulic acid did not change from S_1 to S_2 stage in cultivar WR-315. Presence of vanillic acids was recorded in cultivars GG-1 and JG-62. Fluctuating pattern of data were recorded for chlorogenic, catechol, ferulic and vanillic acids in all the cultivars grown in normal plot. Similarly variations were observed for coumaric, ferulic, salicylic and vanillic acid in

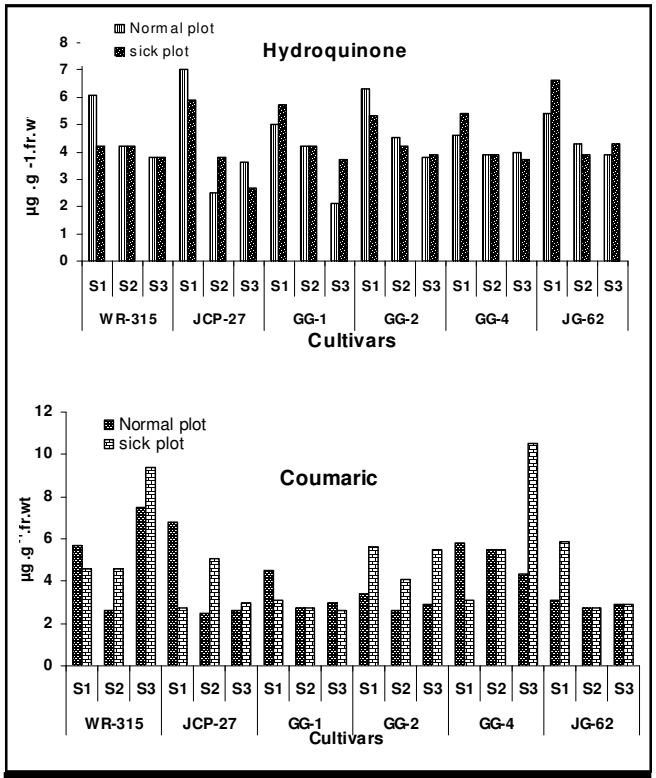

Fig. 1 : Effect of cultivars, treatments and stages on hydroquinone and coumaric acid content in chickpea root tissues

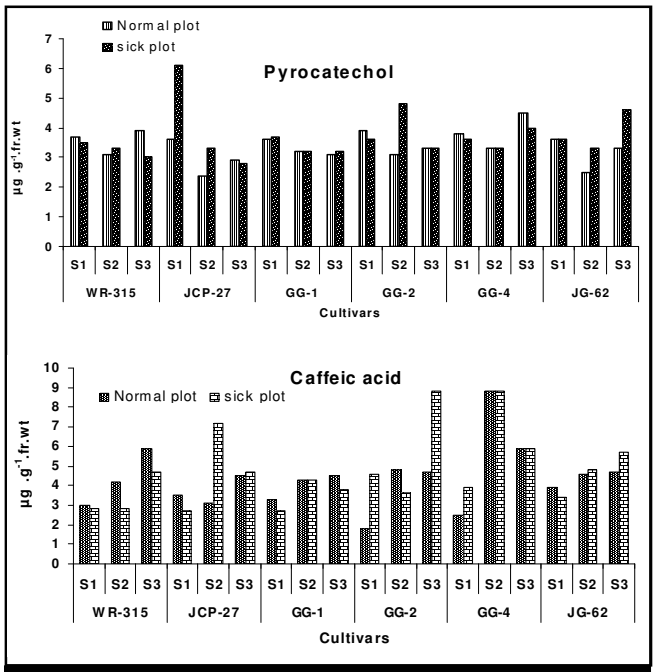

Fig. 2 : Effect of cultivars, treatments and stages on pyrocatechol and caffeic acid content in chickpea root tissues

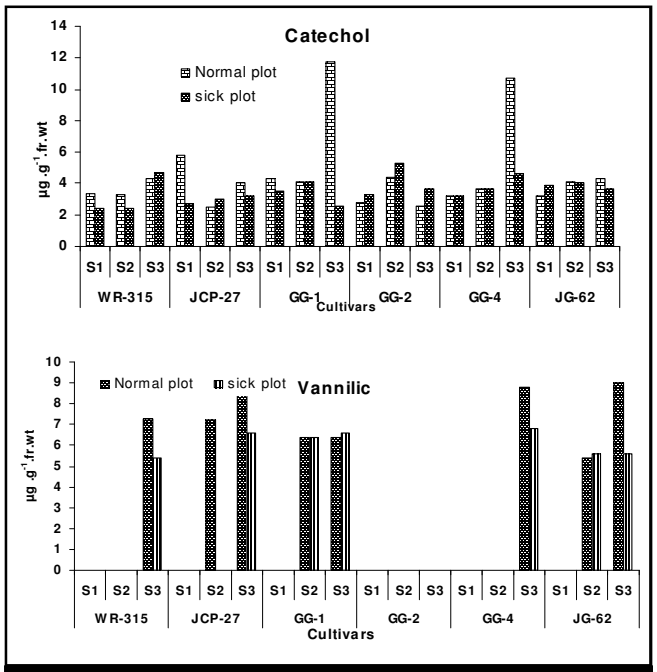

Fig. 5 : Effect of cultivars, treatments and stages on catechol and vannilic acid content in chickpea root tissues

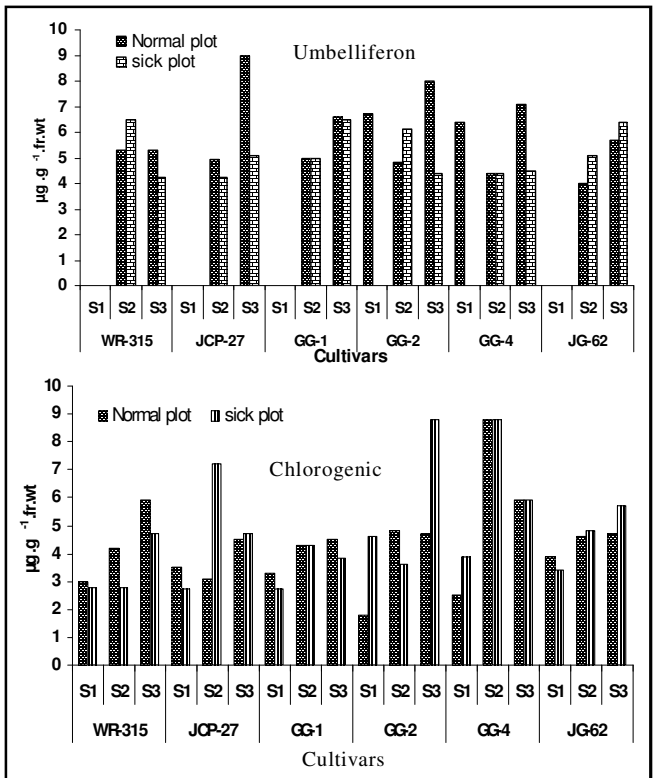

Fig. 3 : Effect of cultivars, treatments and stages on umbeliferon and chlorogenic acid content in chickpea root tissues

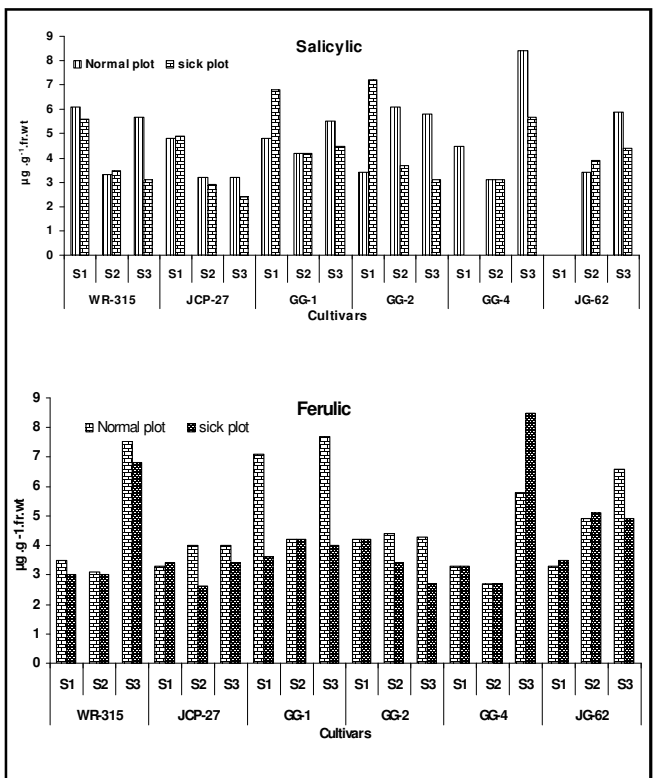

Fig. 5 : Effect of cultivars, treatments and stages on salicylic and ferulic acid content in chickpea root tissues

plants grown in sick plot.

Changes at infectional to post infectional stage:

Incase of plants grown in normal plot resulted higher amount of individual phenolics from S_2 to S_3 stage in all the cultivars with some exceptions. The level of individual phenolics revealed that chlorogenic acid was more in cultivar JCP-27 and GG-1 (Fig. 3), caffeic acid in GG-2 and GG-4, catechol in JCP-27 and GG-2 (Fig. 4). Among the individual phenolics, only hydroquinone showed reverse trend except in cultivar JCP-27 and GG-4 where the contents were increased from infectional (S_2) to post infectional (S_3) stage (Fig.1). Some cultivars did not show any change in their phenolic content from S2 to S3 in root tissue obtained from normal plot. The levels of ferulic and salicylic acids remained same from S_2 to S_3 stage, and cultivar WR-315 for umbeliferon and vanillic acid for cultivar GG-1. Chickpea plants grown in sick plot revealed very low level of hydroquinone in all the cultivars except in JG-62. In contrast to this, chlorogenic acids content increased from S_2 to S_3 stage except in cultivar JCP-27 and GG-1. In present experiment over all data of individual phenolics did not show any consistent pattern in all the chickpea cultivars. Probably, a single individual phenolic may have limited role in disease resistance. However, it visualized from the data that instead of single individual phenolic it may have synergistic effect of all individual phenolics in controlling the infectional process in all the cultivars under investigation. Overall data obtained from experiment are in agreement with the Chakrabarti and Mishra, (2002) who studied to evaluate the resistance of 10 chickpea cultivars infected to *Meloidogyne incognita* and found that at post-infectional stage rise in phenolic level was significantly correlated to the tolerance mechanism of the cultivars. Mandavia *et al*. (2002) observed relationship between wilt susceptibility and total phenol content in root exudates of chickpea seedlings. Sharma and Singh (2002) analyzed individual phenolic compounds using HPLC method from the leaves, collars and roots of the PGPR-treated and untreated (control) plants. They showed the presence of gallic, ferulic, chlorogenic and cinnamic acids

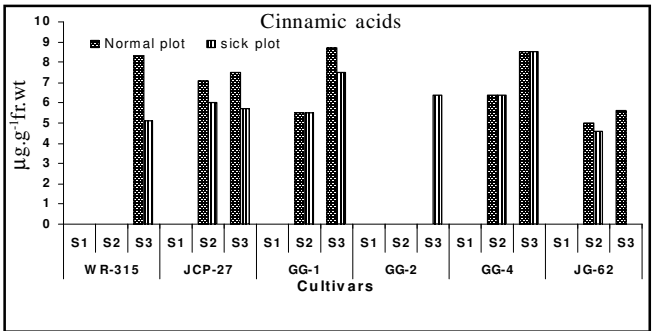

Fig. 6 : Effect of cultivars, treatments and stages on cinnamic acid content in chickpea root tissues

with varied amounts in the untreated (control) plants of chickpea. Same researchers studied the status of phenolic compounds in seedlings of same crop infected with *Sclerotium rolfsii*. They observed three major peaks of gallic, vanillic and ferulic acids. Higher amounts of phenolics were found in the stems and leaves of *S. rolfsii* infected seedlings in comparison to the healthy ones. Matern and Kneusel (1988) reported that synthesis of phenolics and their polymerization in the cell wall is generally regulated by p-coumaric hydroxylase which is extremely pH dependent and not by de novo synthesis of enzyme. Membrane damage leads to decrease in cytoplasmic pH. Cohen *et al*. (1990) reported that phenolic compounds are fluorescent and appearance of fluorescent materials in diseased plant tissues is considered to be due to the presence of phenolic materials that accumulate in the tissues as the host attempts to limit the development of pathogen.

Summary:

Chickpea plants grown in sick plot revealed very low level of hydroquinone in all the cultivars except in JG-62. In contrast to this, chlorogenic acids content increased from S_2 to S_3 stage except in cultivar JCP-27 and GG-1. In present experiment over all data of individual phenolics did not show any consistent pattern in all the chickpea cultivars. Probably, a single individual phenolic may have limited role in disease resistance. However, it visualized from the data that instead of single individual phenolic it may have synergistic effect of all individual phenolics in controlling the infectional process in all the cultivars under investigation. Data obtained from the present experiment are in agreement with the various research workers Chakrabarti and Mishra, (2002) and Mandavia *et al*. (2002). Overall data recorded for total phenol content were 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) studied the total phenolic contents and reported that the 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).

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