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BIOCHEMICAL CHANGES IN CHICKPEA ROOTS AFTER INOCULATION WITH VIRULENT AND HYPOVIRULENT. ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *CICERIS*

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Total phenolic contents increased in the roots of susceptible and resistant varieties of chickpea after inoculation with the virulent as well as hypovirulent isolates of *Fusarium oxysporum* f. sp. *ciceris* (FOC). Highest increase was observed against the highly virulent isolate 9718 in the roots of Aug - 424 and CM 98. While least increase was found against less virulent isolates viz. 2002 and 2014. Increases in total phenols were more in resistant variety as compared to susceptible one. Inhibition of total phenols was observed in the roots of both varieties nine days after germination by the isolates 2012, 9718 and 2002. No reduction in phenols of both varieties was observed against less virulent isolate 2014. The biautography of the developed TLC by using *Cladosporium cucumerinum* revealed that two inhibition zones were produced by both the varieties against all the FOC isolates. Highest expression of total phenols in chickpea roots was found by the most virulent isolate 9718 followed by 2012 and 2002, reduction was more in CM 98 as compared to Aug - 424. The results suggest that the virulent and hypovirulent isolates produced non-specific elicitors while specific suppressors were produced by the isolates 9718, 2012 and 2002.

Key words**:** *Fusarium oxysporum* f. sp. *ciceris*, Virulent, Phenols, Elicitor, Suppressor.

Introduction

Fusarium oxysporum f. sp. *ciceris* is the most devastating disease resulting in 10 - 50% crop loss every year in Pakistan. The fungus is seed borne as well as soil borne and can survive in the soil for more than five years. Moreover, it has some symptom - less carriers like lentil and peas (Saxena and Singh 1987) and it is impracticable to control the disease by using fungicides and through crop rotation. Use of resistant varieties is the best way to control the disease. But due to absence of true resistance in chickpea against wilt disease and a continuous problem of the occurrence / development of new pathogenic races (Jimenez - Diaz *et al* 1989) it has become difficult to overcome the yield losses.

Isolates of the pathogen induce yellowing or wilt syndromes as a result of vascular infections and both pathotypes showed varying degree of pathogenicity towards chickpea lines. Seven races of FOC have been identified by their differential interactions with chickpea lines (Haware and Nene 1982; Trapero - Cases and Jimenez - Diaz 1985; Jimenez - Diaz *et al* 1989). The underlying mechanism for varying pathogenicity / virulence is still unclear. Several biochemical phenomenons have been reported in plant - microbe interactions where phytotoxins (Yoder 1980; Alam and Iftikhar 1996), fungal enzymes including pectic enzymes, cutinase enzymes etc. Kollattukudy (1985); Artes and Tena (1990) or suppressors (Hiramatsu *et al* 1986) have been found as pathogenicity / virulence factor. Elicitor's production by the pathogen has also been referred as a virulence factor in many cases (Deverall and Deakin 1985; De Wit *et al* 1985). A great deal of work has been done on the production of elicitor / suppressor of *Ascochyta rabiei* causing blight of chickpea (Kessmann and Barz 1986) but no reports are available about FOC.

The objectives of the present studies were to identify the possible mechanisms regarding elicitor / suppressor production by the hypovirulent / virulent isolates of FOC.

Materials and Methods

Chickpea material. Two chickpea varieties Aug - 424 (susceptible) and CM 98 (resistant) were used in this study.

Fungi. The two virulent strain 2012 (virulent) and 9718 (highly virulent) and two hypovirulent isolates 2002 and 2014 of *Fusarium oxysporum* f. sp. *ciceris* (Isolated from the diseased chickpea samples collected from Thal, Punjab, Pakistan, during a survey in 2000), were used in this study.

Estimation of total phenols. Wilt, sick soil was prepared as described by Nene *et al* (1981) and filled in small plastic pots (4″x4″x4″). Ten seeds of each variety were sown in one pot in three replicates. The fresh roots of both varieties were

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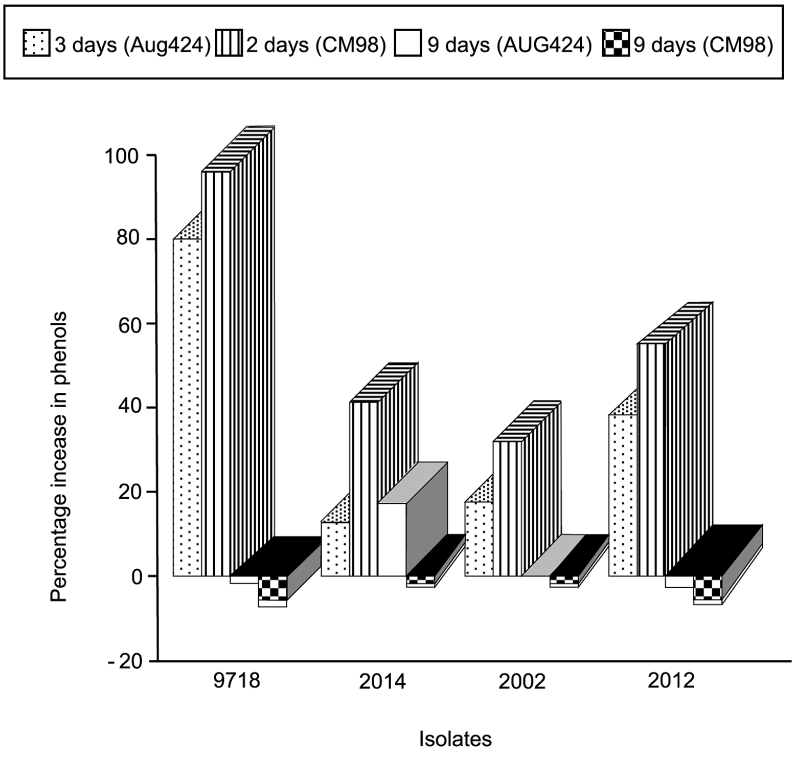

Fig 1. Percentage increase of total phenols in chickpea roots of two varieties at three days and nine days after germination when inoculated with virulent / hypovirulent isolates of *F. oxysporum* f. sp. *ciceris*.

collected after 3 and 9 days of germination in three replicates, washed in distilled water and dried in filter paper. The roots (0.5 gm) were grinded with pestle and mortar in 2.0 ml of acidified methanol (80% with 0.1% HCI) and the material was filtered through buchner funnel using suction pump. The solvent was evaporated on rotary evaporator at 40˚C and finally dissolved in 0.5 ml of methanol. Total phenols were estimated in the fresh roots of these lines by the Folin reagent (Simson and Ross 1971). Total phenols were also estimated in healthy (control) roots of these varieties.

Detection of antifungal compounds. Methanol extract of each sample (50 µl) were spotted on thin layer chromatographic (TLC) plate (0.5 mm thick silica gel 60 GF_{254} plates). The plates were developed in chloroform - methanol (97:3) solvent system. The developed TLC plates were placed in hot incubator at 40˚C for 3 h to evaporate the solvents. The TLC plates were bioautographed against test fungus *Cladosporium cucumerinum* as described by Sibtain *et al* (2002). The developed TLC plates were sprayed with Folin Ciocalteu reagent to identify the nature of these compounds.

Results and Discussion

Total phenolic contents increased in the roots of susceptible and resistant varieties against the virulent as well as hypo virulent FOC isolates as compared to the control (non inoculated plants) three days after germination. Highest increase (80 and 96%) was observed against the highly virulent isolate 9718 in the roots of Aug - 424 and CM 98, respectively. While lowest increase was produced against less virulent isolates viz. 2002 and 2014. The isolate 2012 induced 37.4 and 54.00% increase of total phenols in susceptible and resistant varieties. The percentage increases of total phenols were more in resistant variety as compared to susceptible one Fig 1. The isolated 2012, 9718 and 2002 inhibited total phenols in the roots of both varieties nine days after germination as compared to their control. No reduction in phenols of both varieties was observed against less virulent isolate 2014.

The decrease in total phenols was highest in both varieties against the most virulent isolate 9718 followed by 2012 and 2002. No significant reduction was produced by less virulent isolate 2014. Percentage reduction in phenols was higher in CM 98 as compared to Aug - 424 (Fig 1). Aug - 424 completely wilted at 15 days of germination and the resistant variety CM 98 wilted at 25 - 27 days after germination.

The bioautography of the developed TLC by using *Cladosporium cucumerinum* revealed that two inhibition zones at Rf values 0.85 and 0.88 were produced by both the varieties against all the FOC isolates (Fig 3). Healthy plants (control)

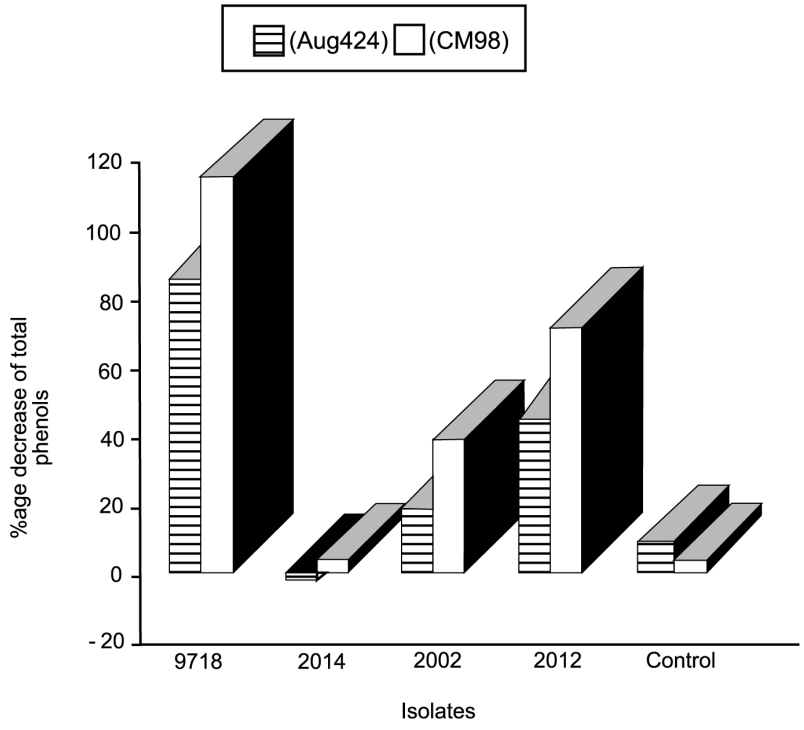

Fig 2. Percentage of total phenols decreased in chickpea roots at 9 days vs. 3 days after germination when inoculated with virulent / hypovirulent isolates of f. *oxysporum* F. sp. *ciceris*.

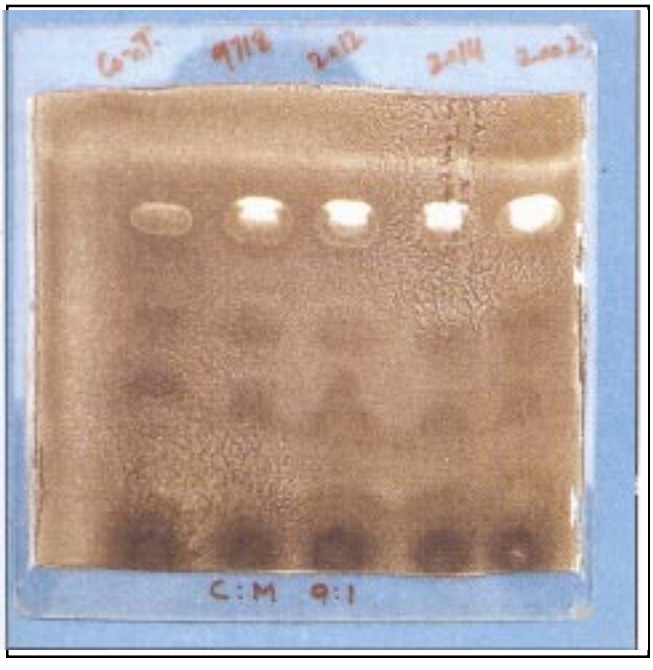

Fig 3. Bioautography of the TLC plates against *Cladosporium cucumerinum* showing antifungal compounds produced by the roots of chickpea.

did not produce these antifungal compounds which revealed that the antifungal compounds were phytoalexins and only produced after fungal attack. No difference was observed in the samples taken at three days and nine days after germination. The antifungal compounds produced blue color after spraying with Folin reagent, which confirmed that the compounds were phenolic in nature.

The results suggested that the phenomenon of elicitor production by the less virulent isolates (production of specific elicitors) might not be operating in FOC isolates because the highly virulent isolates induced higher amount of phenolic contents in both varieties, (three days after germination) as compared to the less virulent isolates. But non - specific elicitor would be produced by all isolates that might be the cause of accumulation of phenolic compounds in chickpea roots. Endopolygalacturonase enzymes and fragments of pectin have been reported to elicit phytoalexins in plants (Stekoll and West 1978; Roberston 1986). The virulent / hypovirulent isolates of FOC produced polygalacturonase and pectin lyase enzymes (Artes and Tena 1990) and in our studies the highly virulent isolate 9718 produced highest polygalacturonase activity (unpublished results). So, the enzyme itself or its degradation products might be eliciting the accumulation of phenols in chickpea roots three days after germination.

The highest percentage decrease of total phenols was produced by the highly virulent isolate 9718 (Fig 2) followed by the virulent isolate 2012 indicating that these isolate would be

producing suppressors. As, there was no difference in the production of anifungal compounds against all the isolates (Fig 3) even during nine days. So, the suppressors produced by the virulent isolates would be specific and might be eliminating or decreasing the effect of non - specific elicitors and resulting compatible interaction. Similar results were observed by Doke and Tomiyama (1980) when they isolated high molecular weight non - specific elicitor and a specific low molecular weight glucan suppressor from *Phytopthora infestans*. The results also indicated that less virulent isolate 2014 is not producing specific suppressor.

Conclusion

The virulent and hypovirulent isolates of *F. oxysporum* produced non - specific elicitors while specific suppressors were only produced by virulent isolates.

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