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Biochemical Effects of Phytotoxins on Chickpea and its Possible Role in Wilt Disease

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Abstract: *Fusarium oxysporum* f. sp. *ciceris* and its phytotoxins induced the leakage of electrolytes from chickpea leaflets, A direct correlation was found between the conductivity of the leaching solution and susceptibility to chickpea wilt. The resistant varieties (CM88 and ILC-3279) released lesser amount of electrolytes as compared to the susceptible varieties (Aug-424 and ILC-1929). The losses of most of the electrolytes such as phosphates, total phenols, protein, carbohydrates, K^+ and Ca^{+2} were significantly higher in toxin treated tissues than in control of both the cultivars. There was a little effect of toxin on leakage of Na^+ ion in resistant or in susceptible cultivars. The susceptible cultivars released higher amount of phosphates, carbohydrates and K^+/Ca^{+2} ions from toxin treated tissues than the resistant cultivars.

Key words: Electrolytes, conductivity, phytotoxins, *Fusarium oxysporum* f. sp. *ciceris,* Resistance, susceptibility

Introduction

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is a major problem in Thal area where most of the chickpea crop is cultivated. This disease is reported to cause 10% annual yield losses to the crop (Halila *et al.,* 1984). The fungus is seed borne as well as soil borne in nature; it is impracticable to control the disease by using fungicides and through crop rotation. Use of resistant varieties is the best way to combat the disease, For this purpose it is necessary to have a full knowledge about the fungus, disease, mode of resistance in host and screening techniques.

Toxins are known to be one of the significant causal factors in the development of a number of destructive diseases of plants (Scheffer, 1983). Most of them are low molecular weight compounds, which may cause necrosis, chlorosis, wilting or combination of these symptoms (Scheffer, 1983). The strawberry pathotype of *Alternana alternata* produced AF toxin, which consist of three related compounds (Akamatsu *et al.,* 1997). In all cases, the sensitivity of the host plant to the toxin was correlated with the pathogenicity of the isolates. These three toxins showed similar biological effect of leaf necrosis and K^+ leakage. *Fusarium oxysporum* f. sp. *ciceris* was reported to produce a Pterocarpan toxic compound nyolved in the wilt of chickpea (Alam *et al*., 2000). The phytotoxins are reported to be involved in the electrolytes leakage from the plant tissues (Lepoivre, 1982). Cristinzio and Lannini (1998) recommended the electrolytes leakage assay as a rapid and reliable technique for the identification of fungal disease resistance in plants. Field screening method is usually employed for the identification of resistance which is time consuming and frequently unrepeatable. In plant breeding the efficiency of the selection depends on the speed and accuracy of the screening method.

The purpose of this work was the standardization of a very simple and rapid technique (electrolyte leakage assay) as a first step in a stepwise selection for wilt resistance in chickpea. The paper also describes the nature of electrolytes leaked from the chickpea leaflets induced by the wilt toxins.

Materials and Methods

Isolation of Phytotoxins: The culture filtrate of the fungus was produced as described by Alam *et al.* (2000). The culture filtrate was partitioned into ethyl acetate at pH 3. The concentrated ethyl acetate phase was eluted on flash column using 200 ml of each solvent respectively, n-Hexane, Benzene, Toluene, Chloroform. Ethyl acetate, Acetonitrile, Acetone and Methanol. The fractions obtained were rotavapoured to dryness and the toxicity of these fractions were measured by cut seedlings method (Huang and Hartman, 1998). Semi purified toxic fractions obtained were combined and diluted to 10.00 ml with ethanol. This served as the stock solution for use in electrolytes leakage assay.

Electrolytes Leakage assay:

Effect of phytotoxins on different chickpea varieties: The chickpea varieties ILC-3279, CM88 (resistant) and ILC-1929, Aug-424 (susceptible) were grown in plastic pots under fluorescent lights (3310 lux) for 15 days. Chickpea leaflets (0.1 g) were removed from these plants and taken into scintillation vials. Toxins (75 μl) was added in 5.00 ml distilled water in two replicates. The toxins solutions were transferred to the scintillation vials containing chickpea tissues. Ethanol (75 μl) without toxins was used as control. The vials were agitated on shaker for 1 hr. The toxin or the water was decanted from the vials and the leaflets were washed five times in 10 ml of distilled water, then 5.00 ml of distilled water was added. The vials were agitated on a shaker at room temperature. The conductivity ($\mu S\ cm^{-1}$) was measured at 1, 4, 21 and 26 hour using conductivity meter.

Pathogen induced electrolytes leakages: It was done by two methods:

Water culture method: The fungus was grown on Czapek dox liquid medium (Oxoid) at 25°C in dark for 14 days. The culture filtrate was centrifuged at 1000xg for 5 minutes. The supernatant was removed and residues (micro conidia and macro conidia) were dissolved in sterilized distilled water. The concentration of the spores was measured by hemacytometer and adjusted to 6.5×10^5 spores mL^{-1}. Spore suspension (50 ml) was taken in each 50 ml conical flasks. Two weeks old seedlings of Aug-424 were removed from pots, the roots were washed with tap water and then with sterilized distilled water. These seedlings were transplanted into the conical flasks containing the spore suspension. Sterilized distilled water was used. Three replicates were used for each treatments. Electrolytes leakages were measured from the tissues of these plants after 7 days as mentioned earlier.

Table 1: Pathogen induced electrolytes leakages from the tissues of chickpea

Methods	Varieties	Net leakage µS/cm (C-T)	%age increase
Pot method	CM88	4.00	73%
7 days	Aug-424	15.0	
Pot method	CM88	23.0	74%
14 days	Aug-424	90.46	
Water culture	Aug-424	59.67	------
	Control	25.56	

Pot Method: The wilt sick soil was prepared (Nene *et al.,* 1981) and filled in small plastic pots (4 × 4"). Seeds of the two varieties Aug-424 and CM88 were sown in these pots and were placed under 3000 lux fluorescent lights at 30 ± 3°C. After 7 and 14 days of germination the electrolytes leakage study was performed.

Analysis of the materials leached from tissue of two chickpea varieties named CM88 and Aug-424 were conducted by the following methods: total carbohydrates by Anthrone method; total phosphorus by the method of Fiske and Subbaow (1925); protein by the 2601260 spectrophotometer method (Layne, 1957) and total phenols by Folin ciocalteu reagent. Flame photometric methods were used to determine sodium, potassium and calcium.

Results and Discussion

The toxic activities were obtained in chloroform and ethyl acetate fractions, which caused initially yellowing, burning of leaves and finally caused the wilting to chickpea cuttings. These fractions were intermediate polar and were free from non-polar and highly polar non-toxic impurities. It is always better to use purified or even semi purified phytotoxins in bioassays for the judgment of their role in diseases because other metabolites produced by the fungus (elicitors, suppressors and etc) could either effect the activity of the toxins or could be responsible for the induction of some biochemical changes in the host. That is the reason we used semi-purified toxins in these assay.

The wilt toxins were found to be responsible for the induction of electrolytes from chickpea tissues. Increase in the concentration of phytotoxins caused an increase in the leakage of the electrolytes from chickpea tissues. The conductivity of the leached solution was directly proportional to the susceptibility of the host. Tissues from susceptible chickpea cultivars (Aug-424 and ILC-1929) released higher amounts of electrolytes than the resistant ones (CM88 and ILC-3279). CM88 released least amounts of electrolytes and Aug-424 released highest amount of electrolytes (Fig. 1). The data confirmed the better resistance in CM88 as compared to the other three varieties. The assay could be a useful method for the identification of qualitative and quantitative resistance against wilt disease in chickpea. If Aug-424 was considered 100% susceptible to wilt disease then it might be stated that toxin/pathogen inoculated tissues of CM88 released 72% less electrolytes than Aug-424 so CM88 has 72% more resistance as compared to Aug-424, the similar results were also obtained in the pot method in which CM88 was found to be 73%/74% (Table 1) more resistant. Similarly ILC-3279 was 52% and ILC-1929 was 9% more wilt resistant than Aug-424.

Tissues from the diseased chickpea plants (both pot method and water culture method) also released higher amounts of electrolytes (Table 1) than the healthy ones in both the varieties (CM88 and Aug-424). This might be the indication

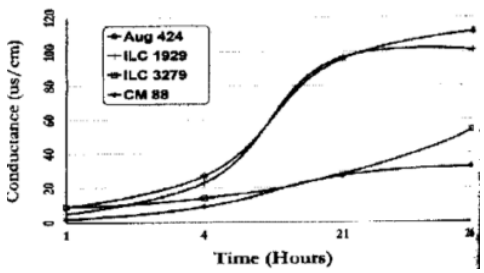

Fig. 1: Rates of electrolytes loss from chickpea leaflets four varieties, induced by *Fusarium oxysporumrs* f.sp ciceris toxins

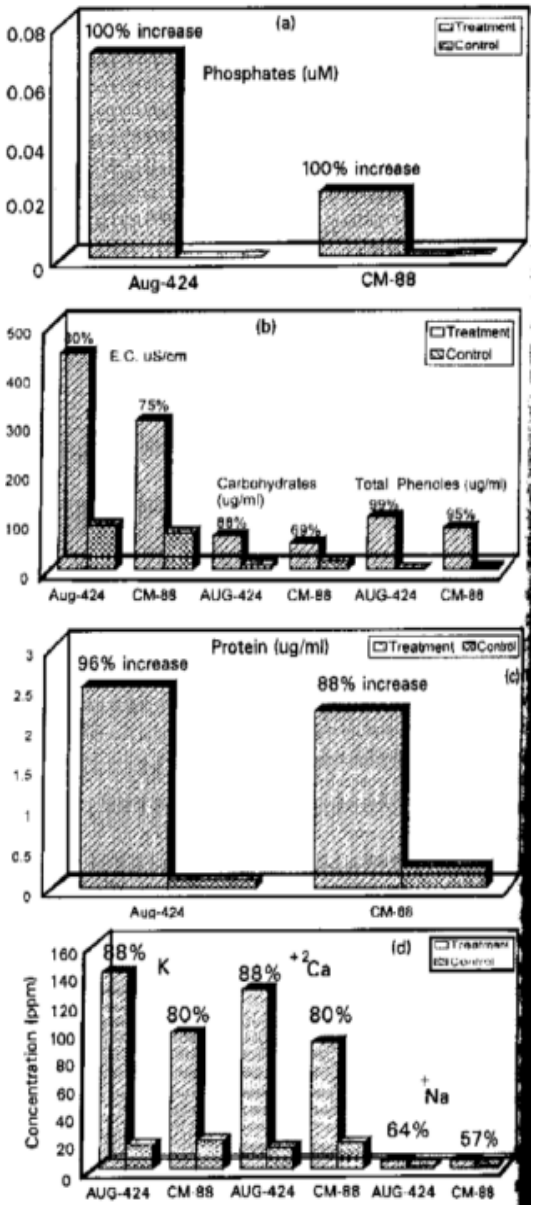

Fig. 2a, b, c, d: Electrolytes from two Chickpea varieties induced by the phytosins of *Fusarium oxysporumrs* f. sp. ciceris

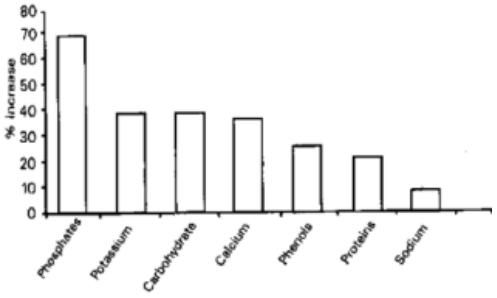

Fig. 3: Percent increaes of toxin induced leachers from Aug-424 as compared to CM-88

that the fungus produced the phytotoxins within the plant tissues, which might be carried to the leaves by the conducting vessels and damaged the cells. In pot method both the data taken at 7 and 14 days showed that Aug-424 released 73 and 74% increased electrolytes leakages as compared to CM88.

Leachers such as phosphates, total phenols, protein, carbohydrates, K^+ and Ca were significantly higher in toxin treated tissues in both the varieties. The phosphates showed the highest leakages (100%) in Aug-424 (Fig. 2a) as compared to its control followed by phenols 199%), protein (96%) carbohydrates (88%), potassium (88%) and Ca^{+2} (88%) with 64% of Na ion (Fig. 2b-d). Phosphates were released in higher amount in both the varieties when treated with toxins. The release of potassium, carbohydrate and calcium was almost the next highest leachers (36.39%) in the Aug-424. Phenols, which were the next higher leachers against control in both the cultivars, showed 27% increased leaches against CM88. Proteins and sodium had 20 and 7% leakages (Fig. 3). Changes in the permeability are the characteristic of the diseased plants but there are a few reports about the materials released from the diseased plants. Sadasivan and Kalyanasundara (1956) reported large decrease in the potassium contents of the cotton plants infected with *Fusarium yenisfectum,* potassium was reported as the chief inorganic on released from tomato cuttings treated with fusaric acid. Gaumann (1958) and Black and Wheeler (1966) also reported the significant losses of potassium, inorganic phosphates and total nitrogen from victorin treated oat tissues. Our data also suggested that the phosphates and the potassium are the major contents released from the toxin treated chickpea tissues, which might be responsible for the elevation of respiration.

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