



Research Article

Mineral Profiling of Chickpea Wilt against *Fusarium oxysporum* f.sp. *ciceris*

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Abstract | Chickpea (*Cicer arietinum* L.) is a rich source of plant protein. A number of diseases attack chickpea crop but wilt disease is the principle one. In mineral contents i.e. nitrogen, phosphorus, potassium, calcium, magnesium, sodium, zinc, iron and copper were decreased in chickpea plants affected with wilt disease. Leaves of three resistant and susceptible (un-inoculated and inoculated) chickpea lines/varieties were tested to find out their ionic status (N, P, K, Ca, Mg, Na, Zn, Fe and Cu). The un-inoculated and inoculated plants of resistant and susceptible groups exhibited significant variation ($p \leq 0.05$) in the mineral contents. Resistant plants group showed 3.75%, 2.93%, 1359, 1667, 1161, 541, 291, 756.6 and 340.26 ppm in “6005” line while susceptible plants group expressed 1.30%, 0.63%, 503 ppm, 441, 515, 152, 70.21, 285.6 and 70 ppm difference in concentration of N, P, K, Ca, Mg, Na, Zn, Fe and Cu in “Thall-2006” variety respectively. Resistant germplasm contained greater concentrations of these minerals related to susceptible lines/varieties. Increased mineral contents in resistant plants build up the physiological and biochemical methods of the host plants which help to prevent the extent of pathogen.

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Keywords | *Cicer arietinum* L., Chickpea, Wilt, *Fusarium oxysporum*, Ionic contents

Introduction

Chickpea (*Cicer arietinum* L.) is cheap and a rich source of plant protein and considered alternate to animal protein. Chickpea is grown under irrigated and rainfed conditions. In Pakistan it can be cultivated in September and November. 2nd week of October is the appropriate time for the cultivation of chickpea crop in Punjab followed by Kyber Pakhtunkhwa and

Sindh **FAO (2009)**. Pakistan is the major producer among chickpea producing countries in the world. Chickpea grain contains protein contents 25.3-28.9%, oil 3%, carbohydrate 38-59%, 0.3% phosphorus, and ash fiber 4.8-5.5% with little kind of ionic contents (**Hulse, 1991; Amjad et al., 2006; Daur et al., 2008**). Various factors responsible in low chickpea grain yields in Pakistan. However, the biological limitations are the most significant factor of low yield in chickpea.

Among these limitations flagging and wilting are responsible for chickpea wilt incited by *F. oxysporum* f.sp. *ciceris* and subsequently loss in yield of the sick plants (Haqqani *et al.*, 2000). Wilt infected seedlings dried quickly, distorted and fall on the ground. Early seedlings mortality (within 25 to 30 days after sowing) and death of adult plants occur due to development of this disease (Haware *et al.*, 1978). Chickpea wilt is the most destructive disease causing 10-50% crop loss annually in Pakistan (Khan *et al.*, 2002).

Disease severity is affected by nutrition which effects totally intermingling constituents. Pathogen and plant were also affected due to nutrients. Contribution of nutrients is vigorous and necessary minerals impact plant diseases interaction. However, a plant is infected which decreases movement, absorption and consumption of mineral nutrients due to a pathogen. Some microorganisms limit the mobility of nutrients in infected tissues or in soil and restrict uptake of nutrients which prompt toxicity or deficiency of minerals. Some others use minerals like N, P, K, Ca, Mg, Zn, Cu and Fe in their body, thus reducing their accessibility to the plant and thereby increasing the plant's susceptibility to disease infection (Timothy and Arnold, 2010; Sahi *et al.*, 2010). A dire need is felt to strengthen the host plant health to manage with the alarming issue of *Fusarium* wilt of chickpea. So, keeping in view the importance of chickpea wilt disease, the recent study was planned to determine the relationship between ionic contents (N, P, K, Ca, Mg, Na, Zn, Fe and Cu) of diseased and healthy plants which may help in understanding disease development mechanism.

Materials and Methods

Collection of diseased samples

Diseased samples were collected from the different farmer's fields of Cholistan desert in Bahawalpur region. These samples were brought to laboratory of Cholistan Institute of Desert studies (CIDS), The Islamia University of Bahawalpur, Pakistan for further study.

Isolation, identification and mass culturing of *Fusarium oxysporum* f.sp. *ciceris*

Chickpea infected roots were cut into 5-8 mm pieces, washed with tap water and surface sterilized by 1% (NaOCl) sodium hypochlorite for one minute. These pieces were given three washings in disinfected

water and placed on sterilized filter paper sheet for drying. These sterilized pieces were transferred in Petri plates contained autoclaved potato dextrose agar and incubated at 26 ± 2 °C for 7 days. The colony of *Fusarium oxysporum* was observed and identified with the help of relevance literature. Pure culture of *Fusarium oxysporum* was kept in autoclaved test tubes in the refrigerator at 4 °C for further investigations.

Determination of ionic status in chickpea cultivars

Seeds of all six advanced lines/variety (6005, 6010, 6015, 7021, 8020 and Thall-2006) were collected from NARC, Islamabad and Ayub Agricultural Research Institute (AARI) Faisalabad for determination of biochemical changes due to attack of wilt disease in chickpea leaves. Seeds of all advanced lines/variety were grown into earthen pots (20×15 cm²) contained 1Kg autoclaved soil in each pot and inoculated with a 1×10^4 spores L⁻¹ suspension of *Fusarium oxysporum* through Haemocytometer at initial flowering stage (Landa *et al.*, 2001). Then pots were kept on glass house bench at 26 ± 2 °C under CRD. Each treatment contained three replications. All recommended agronomic operations were used. Plant samples which were harvested composed of two groups i.e. uninoculated and inoculated; each group was consisted of two types (Susceptible and Resistant). Resistant group contained three advanced lines 6005, 6010, and 6015 while susceptible group contained three lines/variety 7021, 8020 and Thall-2006.

To remove dust leaves of both resistant and susceptible chickpea cultivars washed with 0.2% detergent, to eliminate impurities and again washed in 0.8% HCl. Dried samples were kept in paper bags and dehydrated for three days at 70 °C in oven. The dried grounded samples (100 mg) were boiled in 10 ml of 1.4N HNO₃ on hotplate (TH-550; Advantec, Tokyo, Japan) for half an hour at 100 °C. The mixture was diluted 250 times with distilled water after cooling and then it was investigated for the finding of N, P, K, Ca, Mg, Na, Zn, Fe and Cu following Bhargava and Raghurpathi (1995) procedure. Nitrogen and phosphorous were determined on percent basis, while other elements were noted as parts per million (ppm).

Statistical analysis

The experiment was done under CRD. Collected data was interoperated by statistical analysis. All these data recorded from the experiment were analyzed and means using least significant difference LSD ($P \leq$

0.05) and in SAS/STAT statistical software (Steel *et al.*, 1997; SAS Institute, 1990).

Results and Discussion

Determination of N, P, K, Ca and Mg from inoculated and un-inoculated chickpea plants

The samples of inoculated and un-inoculated plants from both resistant and susceptible plants were evaluated for N, P, K, Ca, and Mg. The un-inoculated and inoculated plants of resistant and susceptible groups were also significantly different from one another ($p \leq 0.05$). Nitrogen content was higher in un-inoculated as well as in inoculated plants of resistant lines compared with that of susceptible lines/variety. The maximum quantity of N was showed by “6005” line at 3.75% in un-inoculated plants and the minimum by “Thall-2006” at 1.30% in inoculated plants (Table 1).

Regarding phosphorus contents, significant variation was observed among un-inoculated and inoculated plants during disease stress at ($p \leq 0.05$). P contents were higher in un-inoculated as well as inoculated plants of resistant ones as compared with that of susceptible lines/variety i.e. 6005 line and Thall-2006 showed maximum and minimum concentration of P to the sort of 2.93% in un-inoculated plants and

0.63% in inoculated plants correspondingly as shown in Table 1.

The potassium content in un-inoculated as well as inoculated plants of susceptible group was lower than that of resistant group. There was a highly significant decrease in K content in the plants of susceptible group than in resistant as an outcome of inoculation ($p \leq 0.05$). The minimum amount of K was detected by “Thall-2006” at 503 ppm in inoculated plants and the maximum by “6005” at 1359 ppm in un-inoculated plants individually as expressed in (Table 1).

Statistically significant difference was recorded regarding Ca in un-inoculated and inoculated plants of resistant and susceptible groups ($p \leq 0.05$). Ca concentration of 1667 ppm was presented by 6005 in un-inoculated plants (Resistant group), while 441ppm concentration in inoculated plants was expressed by Thall-2006 (Susceptible group) separately as presented in Table 1.

Mg contents were found having significant variation in resistant and susceptible groups. Line 6005 (Resistant group) and Thall-2006 (Susceptible group) exhibited 1161 ppm (maximum) in un-inoculated plants and 515 ppm (minimum) concentration in inoculated plants separately (Table 1).

Table 1: Nitrogen, phosphorus, potassium, calcium, magnesium, sodium, zinc, iron and copper contents for the reaction groups and lines/cultivar of chickpea.

Lines/cultivar	Nitrogen (%)												
	6005		6010		6015		7021		8020		Thall-2006		
Type	Resistant group											Susceptible group	
Group	Unin.	Inoc.	Unin.	Inoc.	Unin.	Inoc.	Unin.	Inoc.	Unin.	Inoc.	Unin.	Inoc.	
Phosphorus (%)	3.75a	2.7c	3.11b	2.24d	2.94b	1.96e	2.79a	2.37b	2.14c	1.87d	1.57e	1.30f	
Potassium (ppm)	2.93a	2.11c	2.48b	1.82d	2.04c	1.50e	1.59a	1.09b	1.07b	0.85c	0.81c	0.63d	
Calcium (ppm)	1359a	947c	1031b	887e	917d	635f	823a	624d	700b	529e	648c	503f	
Magnesium (ppm)	1667a	1243d	1421b	1122.33e	1319c	1056f	1167a	771d	960b	534e	845c	441f	
Sodium (ppm)	1161a	985d	1112b	927e	1043c	874f	981a	829c	911b	782d	734e	515f	
Zinc (ppm)	541a	477.23b	491b	419.4d	446c	374e	386a	318b	321.53b	265c	241.8d	152e	
Iron (ppm)	291a	243.11b	236b	170.32d	202c	144.15e	175a	123c	154b	101d	125c	70.27e	
Copper (ppm)	756.6a	624d	714.8b	567.2e	659c	480.9f	601a	559.12b	486c	427d	363e	285.6f	
	340.26a	284c	305.63b	235d	270c	187.23e	207a	142c	176b	114d	102e	70f	

Determination of Na, Zn, Fe and Cu from inoculated and un-inoculated chickpea plants

The inoculated plants of resistant and susceptible groups differed significantly ($p \leq 0.05$) from un-inoculated plants of their respective groups. Na content was significantly higher in resistant group (both in un-inoculated and inoculated plants) than in susceptible group. Na contents 318, 265 and 152 ppm concentrations were observed minimum in inoculated plants of susceptible lines/variety 7021, 8020 and Thall-2006 while 541, 491 and 446 ppm were shown maximum by un-inoculated plants of 6005, 6010 and 6015 (Resistant group) separately as shown in Table 1.

Un-inoculated and inoculated plants of resistant and susceptible groups exhibited significant variation in Zn at ($p \leq 0.05$). Zn concentrations of 291, 236 and 202 ppm (maximum) were found for 6005, 6010 and 6015 in un-inoculated plants (Resistant group), while 123, 101 and 70.27 ppm concentrations in inoculated plants were expressed by the susceptible group 7021, 8020, and Thall-2006, respectively (Table 1).

Significant variation was achieved by resistant and susceptible cultivars in Fe concentrations. Lines 6005 (756.6 ppm), 6010 (714.8 ppm) and 6015 (659 ppm) gave maximum concentrations in un-inoculated plants (Resistant group), while 7021 (559.12 ppm), 8020 (427 ppm), and Thall-2006 (285.6 ppm) at ($p \leq 0.05$) gave minimum concentrations in inoculated plants of susceptible group correspondingly as expressed in Table 1.

Significantly Cu differed in both un-inoculated and in inoculated plants of resistant and susceptible group under disease stress conditions. Resistant group like 6005 (340.26 ppm), 6010 (365.63 ppm) and 6015 (270 ppm) while susceptible group i.e. 7021 (142 ppm), 8020 (114 ppm) and Thall-2006 (70 ppm) expressed their large and least concentrations at ($p \leq 0.05$) individually (Table 1).

Excessive/deficiency amount of nutrients causes different diseases in plants which are affected by amount of elements, type of disease, type of element and environmental factors which effect on disease appearance. Macro and micro nutrients are essential for the growth of plants and completion of their life cycle. Different types of nutrients may affect the resistance status of the host as well as virulence of the pathogen. Plants attaining well balanced nutrition,

with all essential elements easily available in proper amount undergo a smaller amount of disease and get protection from outcomes of new infection and exhibited distinct growth, and yield (Mishra *et al.*, 2005). In recent study a remarkable effect of chickpea wilt disease was observed on the nourishing status of plants.

Nitrogen is an important constituent of many vital biochemicals such as nucleic acids, proteins, coenzymes and amino acids in plant tissues (Lakitan, 2007). Plants need huge amount of nitrogen for normal growth, hence they respond quickly to application of nitrogen (Spann and Schumann, 2010). Some diseases produce due to nitrogen excess in soil while its deficit also favors some disorders in plants (Dordas, 2008; Waller *et al.*, 2007; Hoffland *et al.*, 2000). The inoculated chickpea cultivars contained less nitrogen in susceptible types while the un-inoculated of resistant chickpea lines carried more nitrogen contents. These results are in agreement with the outcomes of Jadon and Shah (2012).

Phosphorus is second essential element for the development of DNA, RNA, phospholipids, ATP, NADP and NAD and some compounds of great energy used by the plants (Huber and Graham, 1999). Phosphorus nutrition improves crop vigour and may decrease severity of diseases through new growth (Buresh, 1997; Smyth and Cassell, 1995). Phosphorous enhanced root development may help the plant to escape fungal pathogens (Prabhu *et al.*, 2007). The un-inoculated and inoculated plants in susceptible group as well as in resistant one differs remarkably with regard to phosphorous concentrations. These findings are also in line with the results of Jadon and Shah (2012).

Potassium has a key role in metabolism of carbohydrates, stomatal opening and photosynthesis (Huber and Graham, 1999; Rice, 2007). In present study, decrease in K amount was noted in resistant group but more pronounced reduction in susceptible group due to chickpea wilt disease. These results are supported by the results of Mishra *et al.* (2005), Dordas (2008), Jadon and Shah (2012) who described similar pattern K contents of diseased and healthy plants.

Calcium is primarily significant part of plant cell wall. It helps in root development, stimulation of leaf, microbial activity and uptake of nutrients. It

produces resistance in host plant because it checks the penetration of pathogens (Devlin and Withman, 1983; Mishra *et al.*, 2005). Significant decrease in Ca contents was found both in susceptible and resistant cultivars which pronounced disease (Marschner, 1995; Mishra *et al.*, 2005). Similar work was reported by Dordas (2008) and Amusa *et al.* (2005).

Magnesium plays a vital role in photosynthesis, chlorophyll contents and carbohydrate metabolism (Spann and Schumann, 2010). Mg is a dynamic element of structural tissues and plays an important role in altered physiological and biochemical processes. In present study increase in magnesium concentrations was observed in resistant cultivars as compared to susceptible lines/variety. These findings are in agreement with the results of Jadon and Shah (2012), Sawant and Gawai (2011) and Amusa *et al.* (2005).

Sodium (Na) is utilized by plants as sodium ions which significantly differed in the existing study. The un-inoculated and inoculated of resistant group contained more sodium as related to susceptible group. Our outcomes are in agreement with the results of Jadon and Shah (2012).

Zinc shows resistance against many diseases but its mechanism in disease resistance is indistinct. It acts as co-factor for various enzymes (Rice, 2007; Cakmak, 2000). A significant dissimilarity in Zn contents was noticed in chickpea lines while low contents of Zn was found in inoculated chickpea lines/varieties as compared to un-inoculated plants. Our results are supported by Reddy and Khare (1984) and Sahi *et al.* (1999).

Iron is the primary constituent of chlorophyll and has significant role in metabolism and nucleic acid. Chlorophyll contents of plants reduce due to its deficiency (Imran and Gurmani, 2011). In current study it was found that Fe contents were reduced due to fungal attack. It may be due to the fact that plant pathogens mostly have greater requisite of Fe and act as virulence factor during the disease development with the help of Fe activate enzymes (Graham and Webb, 1991; Dordas, 2008).

Copper is a significant constituent of lignin and have a vital role in carbohydrate metabolism and protein. It acts as catalyst in altered metabolic activities of

the plant (Imran and Gurmani, 2011). Significant variation was found in Cu concentration of un-inoculated and inoculated plants of both groups i.e. resistant and susceptible upon infection with *Fusarium oxysporum* f.sp. *ciceris* (Foc). Conclusion of the current study is agreed by the work of Mata *et al.* (2001). Cu has direct lethal effects on plant pathogens and its scarcity declines lignification in the xylem (Evans *et al.*, 2007).

Conclusions and Recommendations

Mineral contents i.e. Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sodium, Zinc, Iron and Copper were decreased in chickpea plants affected with wilt disease. Appropriate utilization of these nutrients helps in the host plants in strengthening of its physical and biochemical processes, which finally help in increasing the resistance against wilt disease of chickpea.

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Novelty Statement

In the recent study increased mineral contents in resistant plants build up the physiological and biochemical methods of the host plants which help to prevent the extent of pathogen.

Author's Contribution

Hafiz Tassarwar Abbas: Conducted the research and wrote the manuscript.

Tamoor Khan, Ghulam Khaliq and Muhammad Aqeel Sarwar: Designed the experiment and gave valuable suggestions on data analysis.

Muhammad Rashid, Ghulam Ali Bugti and Intazar Ali: Helped in revision of manuscript.

Muhammad Abuzar Jaffar and Muhammad Waseem: Contributed in final proof reading of manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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