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INFLUENCE OF INOCULATION WITH *ASCOCHYTA LENTIS* ON MINERAL CONTENTS (NA, CA, MG, ZN, CU AND FE) OF SUSCEPTIBLE AND RESISTANT LINES OF LENTIL (*LENS CULINARIS* MEDIK.)

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Abstract

An experiment was conducted to determine the mineral contents of the healthy and inoculated plant of lentil and their relationship toward the *Ascochyta lentis* disease. The results revealed that magnesium, copper and zinc contents of un-inoculated lentil lines, included in susceptible group were higher than those included in resistant group whereas, sodium, calcium and iron contents were more in the resistant as compared to the susceptible group. Upon inoculation with *Ascochyta lentis,* the cause of lentil blight disease, sodium, calcium, zinc, copper and iron contents increased invariably in both the susceptible and resistant groups of lentil lines. On the other hand, magnesium contents increased in susceptible group but decreased in resistant group. The over all results proved that considerable variation exists in micromineral contents of resistant and susceptible lines of lentil.

Introduction

Lentil (*Lens culinaris* Medik) is nutritious pulse crop as it contains 28.6% protein, 3.1% ash, 4.6% crude fiber, 44.3% starch, 36.1% amylose, 63.1% total carbohydrates and 420 Cal. gross energy 100 g^{-1} (Bhatty & Wu, 1974). Moreover, lentil is lower in anti-nutritional factors such as haemagglutinins, oligosaccharides and favogens as compared with most of other legumes. Although they contain tannins in the seed coat, yet not in the cotyledons (Vaillancourt *et al*., 1986). In case of the red cotyledons of *microsperma* (small-seeded lentil) even this is not important, since the seed coat, or testa, is removed before this type of lentil is used in food preparation in most of the cases. The *macrosperma* (large-seeded lentil) contain tannins, which can cause digestive disorders. The high level of protein together with a lower level of anti-nutritional factors and a shorter cooking time than most of other pulses, make lentil very suitable for human consumption (Williams *et al*., 1974).

Lentil is an important dietary component in Afghanistan, Bangladesh, India, Nepal, Pakistan, Ethiopia, Morocco, Tunisia, Sudan, Iran, Syria, Turkey, Egypt and Iraq. Many of these countries are also major producers. Countries in Southern Europe, Central Asia and the Caucasus and in Latin America grow and consume lentil to a lesser extent. During the last two decades, the crop has been grown in developed countries such as Australia, Canada and the USA, and has become an important agricultural export commodity. Canada is now the second largest producer after India, with an area of about 700 000 ha (Tullu *et al*., 2005). However, world lentil production has tripled in the last three decades from 1.05 million MT in 1971 to 3.8 million MT in 2004, through a 124% increase in sown area and a 58% increase in average national yield from 611 to 966 kg/ha (Anon., 2004). Lentil is the second most important pulse crop of Pakistan after chickpea. In 2004-05, lentil was grown over an area of 43.4 thousand hectares with an average yield of 599 Kg ha^{-1} only (Anon., 2005).

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The low lentil yield in Pakistan is attributed to continuous cultivation of low yield potential cultivars with and excessive vegetative growth, narrow adaptability, low stability of yield, and susceptibility to stress conditions (Rajput & Sarwar, 1989) and inadequate nitrogen nutrition. One of the most important stresses is damage by diseases. The common diseases of lentil in Pakistan are blight and rust. Blight is caused by *Ascochyta lentis* and may cause considerable losses in yield especially under cool and moist conditions. Lentil blight can be managed through a number of means, the cheapest and most practicable being the use of resistant varieties. Although resistance to lentil blight is not scarce in the existing lentil cultivars yet the phenomenon of resistance is not clearly known, due to lack of studies on the morphological and biochemical basis of resistance.

Minerals, apart from being a vital part of the plant nutrition, may manifest certain maladies in the plants either through disturbing normal metabolism and physiology of the plants or by favouring or discouraging the plant pathogens, if in excess or otherwise deficient. Lentil varieties susceptible to rust contain higher amounts of nitrogen, which increased initially in inoculated plants but decreased when the disease became severe. While healthy plants of resistant varieties contain a higher percentage of phosphorus compared with healthy plants of susceptible varieties. Phosphorus content decreased in inoculated plants more than it did in un-inoculated plants. Resistant varieties contained a higher percentage of potassium compared with susceptible varieties. During initial stages of infection, potassium content decreased while it increased during advanced stages of infection. Significant increase in calcium and magnesium was found in inoculated plants (Reddy & Khare, 1984).

In case of chickpea, the amounts of N, P, Zn and Fe did not vary much in healthy plants of resistant and susceptible cultivars. The amounts of K and S were more in susceptible than in resistant cultivars. Barring the recovery of Cu and Fe, the amounts of all other elements were enhanced upon inoculation on overall basis in resistant, moderately resistant, moderately susceptible and susceptible cultivars. There was a noticeable increase in the amount of K in the resistant cultivars but the reverse was true for the P, S and Mg content after inoculation (Randhawa, 1994).The present studies were under taken to ascertain the role of Na, Ca, Mg, Zn, Cu and Fe of different lentil lines showing resistance and susceptibility to *Ascochyta lentis* the cause of lentil blight.

Materials and Methods

Isolation of a pathogenic isolate of *Ascochyta lentis* was made from diseased lentil pods. The fungus was purified by spore streak method (Ricker & Ricker, 1936). The pure culture was grown on 6 percent Lentil Seed Meal Agar (LSMA) in the test tubes (30 ml cap.) and stored at 5 ± 1 C° in a refrigerator for further studies. *A. lentis* was mass cultured on boiled and subsequently sterilized dried chickpea grains for inoculation of lentil cultivars.

Seven advanced lines 91549, 93523, 93536, 93564, 93566, 95560 and 96504 of the susceptible group and seven advance lines 94503, 94506, 95509, 95513, 95515, 95527 and Masoor-93 of the resistant groups were sown in the field. The lines were sown in single row subplots, 3 meter long with 30 cm and 3-cm row to row and plant to plant distance respectively with four replications, in group balanced block design. At early flowering stage, the plants of both susceptible and resistant were inoculated with *A. lentis* spore suspension (2×10^3 spore ml^{-1}) to develop blight disease on them.

When the blight disease was fully developed on susceptible lentil lines, the plant samples (comprising of shoots) of inoculated and un-inoculated, resistant and susceptible lines, were collected from the field on 22nd of March (30 days after inoculation). These were washed in 0.2 % detergent solution in order to remove any dirt, followed by washing in 0.8 % HCl (to remove metallic contaminants) and deionized water (to remove the previous two solutions). The samples were air-dried in the shade on paper towels and then placed in paper bags. The samples were dried in an oven at 70°C for 72 hours, to constant weight. These samples were ground with the help of Buhler Sample Grinder and afterwards processed for the determination of Na, Ca, Mg, Zn, Cu, and Fe contents in (ppm) following Bhargava & Raghupathi (1995).The data collected were analyzed following Gomez & Gomez 1984; Petersen (1989a, 1989b); Steel *et al*., (1996) and using computer programme MstatC. To elucidate the data, graphs, bar diagrams etc. were prepared using computer programme HG4.

Results

The Na, Ca, Mg, Zn, Cu and Fe contents of the healthy and susceptible lines of lentil is given as under:

Sodium: The sodium content in un-inoculated plants of susceptible group was lower than that of resistant group. Inoculation with the pathogen increased the sodium content in both the groups, but this was more pronounced in the plants of susceptible lines as compared to resistant ones (Table 1). There was a highly significant increase in sodium content in the plants of susceptible group than in resistant as a result of inoculation with the pathogen. The range of sodium content in un-inoculated and inoculated plants of susceptible group was 262.7 to 479.3 (average 335.0) and 356.3 to 617.7 (average 440.4), respectively. On the other hand, the range of sodium content in un-inoculated and inoculated plants of resistant group was 263.7 to 397.3 (average 345.4) and 308.3 to 441.3 (average 372.5), respectively.

Calcium: The inoculated plants of susceptible and resistant groups differed significantly (p=0.01) from un-inoculated plants of their respective groups. Calcium content was significantly higher in resistant group (both in un-inoculated and inoculated plants) than in susceptible group. The range of calcium content in un-inoculated and inoculated plants of susceptible lines was 8448 to 8971 (average 8722) and 8543 to 9235 (average 8827), respectively. However, in case of resistant lines/cultivars, the range of calcium content in un-inoculated and inoculated plants was 8421 to 9089 (average 8805) and 8488 to 9665 (average 8948), respectively (Table 1).

Magnesium: As a result of inoculation there was highly significant increase in magnesium content of resistant lines of lentil but on the contrary there was highly significant decrease in the magnesium content of susceptible lines. The range of magnesium content in un-inoculated and inoculated plants of susceptible lines was 1080.0 to 1240.0 (average 1169.9) and 807.7 to 1157.0 (average 1033.7), respectively. On the other hand, range of magnesium content of un-inoculated and inoculated plants of resistant entries was 985.0 to 1025.0 (average 1005.2) and 1025.0 to 1143.0 (average 1085.6), respectively (Table 1).

Table 1. Sodium, calcium and magnesium contents (ppm of dry weight) for the reaction groups and lines/cultivars of lentil.

Lines/ cultivar	Sodium (ppm)		Calcium (ppm)		Magnesium (ppm)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Susceptible group						
91549	350.7 e*	356.3 e	8502 k	8543 j	1080.0 ef	1157.0 bcd
93523	345.0 e	397.0 d	8971 c	8779 f	1212.0 ab	1116.0 de
93536	302.7 f	402.7 d	8448 l	8646 h	1174.0 bc	1077.0 ef
93564	262.7 g	442.0 c	8862 e	8906 d	1240.0 a	1058.0 f
93566	479.3 b	617.7 a	8849 e	9235 a	1191.0 ab	1026.0 fg
95560	299.3 f	412.7 d	8696 g	8588 i	1130.0 cde	993.3 g
96504	305.0 f	454.3 c	8722 g	9092 b	1162.0 bcd	807.7 h
LSD	21.24		29.35		55.41	
Mean	**335.0 b**	**440.4 a**	**8722 b**	**8827 a**	**1169.9 a**	**1033.7 b**
Resistant group						
94503	263.7 e	308.3 d	8579 i	8899 e	996.3 ef	1065.0 c
94506	306.3 d	354.3 c	8820 g	8900 e	1006.0 def	1025.0 d
95509	304.3 d	441.3 a	8421 k	9126 b	1011.0 de	1060.0 c
95513	397.3 b	392.7 b	8964 d	9665 a	1016.0 de	1081.0 d
95515	353.0 c	355.3 c	9089 c	8687 h	996.7 ef	1143.0 a
95527	395.7 b	354.7 c	8803 g	8488 j	1025.0 d	1106.0 b
Masoor-93	397.3 b	401.0 b	8962 d	8873 f	985.0 f	1120.0 ab
LSD	15.20		25.14		24.18	
Mean	**345.4 b**	**372.5 a**	**8805 b**	**8948 a**	**1005.0 a**	**1085.6 b**

*Means sharing similar letters in the same column do not differ from each other at p=0.05

Zinc: The un-inoculated and inoculated plants of susceptible and resistant groups were also significantly different from one another (p=0.01). Zinc content was higher in un-inoculated as well as inoculated plants of susceptible lines as compared with that of resistant ones (Table 2). The range of zinc content of un-inoculated and inoculated plants of susceptible lines was 88.33 to 117.70 (average 98.00) and 107.00 to 126.00 (average 116.00), respectively, while in case of resistant lines/cultivars the range of zinc content of un-inoculated and inoculated plants was 75.33 to 107.70 (average 91.19) and 72.33 to 118.00 (average 98.19), respectively.

Copper: The inoculation with the *A. lentis* increased the copper content of lines/cultivars of both the groups but this increase was more pronounced in susceptible lines than in resistant lines/cultivars. The range of copper content of un-inoculated and inoculated plant within the susceptible group was 19.67 to 103.70 (average 67.76) and 50.67 to 130.30 (average 86.48), respectively, but the range of copper content within the resistant group was 30.33 to 60.67 (average 43.19) for un-inoculated and 34.67 to 53.67 (average 46.95) for inoculated plants (Table 2)

Iron: Increase in the iron content upon inoculation with the pathogen, in both the susceptible and resistant groups was highly significant, being more pronounced in susceptible group. Mean iron content in un-inoculated and inoculated plants of susceptible lines was 657.2 within the range of 596.0 to 872.0 and 1005.8 within the range of 801.7 to 1099.0), respectively, while mean iron content in un-inoculated and inoculated plants of resistant lines/cultivars was 715.3 (509.7 to 899.0) and 883.1 (721.3 to 1115.0), respectively (Table 2).

Table 2. Zinc, Copper and iron contents (ppm of dry weight) for the reaction groups and lines/cultivars of lentil.

Lines/ cultivar	Sodium (ppm)		Calcium (ppm)		Magnesium (ppm)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Susceptible group						
91549	104.33 ef*	116.33 bc	19.67 i	110.70 b	601.0 f	1005.0 b
93523	86.00 I	113.33 cd	30.00 h	70.00 e	596.0 f	801.7 d
93536	96.67 g	115.33 bc	103.70 c	130.30 a	608.0 f	1099.0 a
93564	93.67 gh	112.00 cd	81.00 d	80.33 d	600.7 f	1010.0 b
93566	88.33 hi	107.00 de	70.33 e	100.33 c	600.3 f	1013.0 b
95560	117.70 bc	126.00 a	100.00 c	50.67 g	872.0 c	1005.0 b
96504	99.33 fg	122.00 ab	69.67 e	63.00 f	722.7 e	1106.0 a
LSD	7.315		6.478		43.38	
Mean	**98.00 b**	**116.00 a**	**67.76 b**	**86.48 a**	**657.2 b**	**1005.8 a**
Resistant group						
94503	107.70 bc	115.30 ab	50.00 b	53.67 b	597.7 g	922.3 bc
94506	93.33 de	118.00 a	30.33 e	42.00 c	604.0 g	823.0 d
95509	99.00 cd	110.00 ab	50.33 b	52.67 b	899.0 c	1115.0 a
95513	92.00 de	92.33 de	40.33 c	34.67 d	898.0 c	827.3 d
95515	99.00 cd	89.00 e	40.33 c	50.67 b	509.7 h	721.3 f
95527	75.33 f	90.33 de	60.67 a	52.00 b	797.7 e	837.7 d
Masoor-93	75.67 f	72.33 f	30.33 e	43.00 c	700.7 f	935.0 b
LSD	8.724		4.110		25.12	
Mean	**91.71 b**	**98.19 a**	**43.19 b**	**46.95 a**	**715.3 b**	**883.1 a**

*Means sharing similar letters in the same column do not differ from each other at p=0.05

Correlation: The correlation coefficient (r) values, calculated for mineral content of un-inoculated and inoculated plants of susceptible and resistant lines/cultivars are given in Table 3a, b. In case of un-inoculated susceptible plants, significant correlation was observed between iron and zinc (0.823) only. Similarly in case of un-inoculated resistant plants, correlation between zinc and sodium was also significant (-0.816).

When the mineral content of inoculated plants was considered, there was significant correlation between calcium and sodium (0.867) in case of susceptible lines. On the other hand, in case of resistant entries, significant correlation was observed in case of zinc with magnesium (-0.846).

Discussion

Successful colonization of plant by pathogens requires efficient utilization of nutrient resources available in the host tissue (Ghorbani *et al.*, 2008). Nitrogen and potassium play a major role in resistance to pathogens as N frequently reduces the level of resistance while, K improves it by affecting plant morphology, hardening the tissues that restrict the penetration of the pathogen, moreover, potassium nutrition increases the content of phenols which can also play a beneficial role in plant resistance (Pervez *et al.*, 2007). The micronutrients are also involved in various physiological and biochemical process that can affect the response of plants to pathogens (Marschner, 1995). Impaired nutrients both macro and micro affect disease resistance directly, as the plants which are nutrient-deficient show poor defense towards the disease (Dordas, 2008). In the present studies, sodium content of susceptible lentil lines was slightly less than that of the resistant ones. Upon inoculation with the pathogen, the sodium content increased in both the groups, being more prominent in case of susceptible lentil lines. Due to lack of literature on this aspect, the role of sodium in resistance/susceptibility to *Ascochyta lentis* could not be justified.

Table 3a. Correlation between mineral contents of un-inoculated (U) and inoculated (I) plants of lentil lines/cultivars susceptible to *Ascochyta* Blight.

		Na	Ca	Mg	Cu	Zn	Fe
U	Na	_					
I							
U	Ca	0.210	_				
I		0.867*					
U	Mg	-0.067	0.699	_			
I		-0.329	-0.548				
U	Cu	-0.300	-0.195	0.247	_		
I		0.010	-0.145	0.519			
U	Zn	-0.399	-0.515	-0.700	0.316	_	
I		-0.525	-0.463	-0.463	-0.534		
U	Fe	-0.308	-0.083	-0.359	0.462	0.823*	_
I		0.159	0.144	-0.516	0.304	0.260	

Table 3b. Correlation between mineral contents of un-inoculated (U) and inoculated (I) plants of lentil lines/cultivars resistant to *Ascochyta* Blight.

		Na	Ca	Mg	Cu	Zn	Fe
U	Na	_					
I							
U	Ca	0.679	_				
I		0.430					
U	Mg	0.202	-0.215	_			
I		0.015	-0.344				
U	Cu	-0.084	-0.496	0.625	_		
I		-0.251	-0.678	0.148			
U	Zn	-0.816*	-0.395	-0.163	0.047	_	
I		-0.280	0.108	-0.846*	0.257		
U	Fe	0.351	-0.357	0.607	0.351	-0.291	_
I		0.578	0.221	-0.377	0.287	0.244	

Correlation Coefficient (r) = 0.754 (5%), 0.874 (1%)

Calcium is mainly important as a constituent of plant cell wall in the form of clacium pectate. If partly removed from the middle lamella, the cell plasticity is increased (Devlin & Witham, 1983). It is then evident that plant deficient in calcium would have more plastic cells, which may render them more susceptible to different biotic as well as abiotic stresses. Increased cell plasticity may ease the entry of the pathogen into the host cells similarly the tissues with less amount of Ca are more prone the attack of the pathogen as compared to the normal level (Dordas, 2008). The calcium content of lentil lines susceptible to blight was significantly lower than the resistant ones, which supports the above hypothesis. Same response in the lentil cultivars has already been reported against rust (Reddy & Khare, 1984).

The (Ca+Mg)/K ratio play an important role in the management of nematode diseases in various crops (Bains *et al*., 1984). Magnesium content was higher in case of susceptible lentil lines as compared to the resistant ones. Upon inoculation, the magnesium decreased in case of susceptible and increased in case of resistant lentil lines. On the contrary, Reddy & Khare (1984) reported increase in magnesium content of plants of both the susceptible and resistant lentil lines upon inoculation with *Uromyces fabae*. Magnesium plays very important role in synthesis of chlorophyll and consequently in photosynthesis and carbohydrate metabolism (Devlin & Witham, 1983). Depletion of magnesium in the susceptible lentil lines upon inoculation suggests reduced photosynthesis and carbohydrate metabolism in them due to reduced photosynthetic area. The photosynthetic area is reduced mainly due to necrosis of the leaf tissues as a result of large-scale infection of this tissue. In addition to the above functions, magnesium is also a part of middle lamella as magnesium pectate along with calcium pectate. As has already been discussed, the loss or depletion of these compounds renders plasticity to the plant cells; this fact favours the previous conception that more plastic cells would be more vulnerable to infection by the pathogen. On the other hand, increased magnesium in the resistant lentil lines suggests increased and sound photosynthesis and carbohydrate metabolism in the resistant group. But Reddy & Khare (1984) reported increased magnesium in both susceptible and resistant lentil cultivars, as a result of inoculation with *Uromyces fabae*.

Zinc content of susceptible lentil lines was higher than that of the resistant ones prior to inoculation with the pathogen and it even increased in both the groups, increase being more pronounced in case of susceptible group. These results are similar to those recorded by Randhawa (1994) in case of chickpea-*Ascochyta rabiei* interaction but opposite to those obtained by Reddy & Khare (1984) in lentil *Uromyces fabae* interaction. Zinc has also been reported to completely inhibit the mycelial growth of *Aspergillus carneus* and *A. ellipticus* at 500 mg L^{-1} (Moslem & Parvez, 1992). Zinc is considered to be involved in the biosynthesis of the plant auxin indol-3-acetic acid through its involvement in the synthesis of tryptophane (Tsui, 1948). It may therefore be concluded that the susceptible lentil lines would exhibit more growth due to enhanced indol-3-acetic acid (IAA) synthesis and hence may render them more vulnerable to infection by the pathogen.

As far as copper content is concerned, it was more in the susceptible lines as compared with the resistant ones. Upon inoculation, there was a slight increase in the copper content of resistant and quite marked increase in case of susceptible lentil lines. This was exactly reverse to the one reported by Reddy & Khare (1984). While Randhawa (1994) reported decreased copper in chickpea cultivars resistant to *Ascochyta rabiei* and increased copper content in susceptible cultivars. Copper is an essential component of phenolases (Nason & McElroy, 1963) which are responsible for the biosynthesis of different phenolic compounds. Perhaps, this suggests the increased production of phenolic compounds in the susceptible lentil lines prior to inoculation compared with the resistant ones.

Iron content of the susceptible group was lower than that of the resistant group prior to inoculation with the pathogen but after inoculation iron content increased in both the groups and iron accumulated more in the inoculated plants of susceptible group as compared that of the resistant group. This is quite confusing and opposite to that reported by Reddy & Khare (1984) and Randhawa (1994). The only justification may be the involvement of iron as a component of various flavoproteins (metalloflavoproteins) active in biological oxidations (Devlin & Witham, 1983), which may increase as a result of inoculation with the pathogen. Iron is also found in iron-porphyrin proteins, which include cytochromes, peroxidases and catalases. It may be assumed that the above-mentioned proteins may be responsible for increased catabolism in the inoculated plants.

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