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ROLE OF N, P & K CONTENTS IN RESISTANCE AGAINST ASCOCHYTA BLIGHT OF LENTIL (*LENS CULINARIS* MEDIK)

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Abstract

Nitrogen and phosphorus contents of un-inoculated plants of lentil lines included in susceptible group were higher than those of the resistant one whereas, potassium, contents were more in the un-inoculated lines of resistant as compared with the susceptible group. Upon inoculation with *Ascochyta lentis,* the nitrogen and potassium contents, increased invariably in both the resistant and susceptible group of lentil lines. On the other hand, phosphorus contents increased in inoculated lentil lines of the susceptible group but decreased in those of the resistant group. In general, it was found that the plants lower in nitrogen and phosphorus were resistant to lentil blight while those with higher nitrogen and phosphorus were susceptible to lentil blight disease caused by *Ascochyta lentis.*

Introduction

Lentil is an important pulse crop 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 also 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). Consequently, 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 (FAO 2004). Lentil is the second most important pulse crop of Pakistan after chickpea. In 2004-05, lentil was grown in Pakistan over an area of 43.4 thousand hectares with an average yield of 599 Kg ha^{-1} only (Anon., 2005)

Lentil contains 28.6 percent protein, 3.1 percent ash, 4.6 percent crude fiber, 44.3 percent starch, 36.1 percent amylose, 63.1 percent total carbohydrates providing 420 Cal. gross energy100 g^{-1} (Bhatty & Wu, 1974). Moreover, lentils are lower in anti-nutritional factors such as haemaglutinins, oligosaccharides and flavones as compared with most of the other legumes. Although they contain tannins in the seed coat, but 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, in most of the cases is removed before this type of lentil is used in food preparation. The *macrosperma* (large-seeded lentils) 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 as compared with most of other pulses, make lentil very suitable for human consumption (Williams *et al*., 1974).

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The low yield of lentil in Pakistan may be attributed to continuous cultivation of cultivars with low yield potential and excessive vegetative growth, narrow adaptability, poor yield stability, and susceptibility to stress conditions (Rajput & Sarwar, 1989). 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 the four reaction groups. 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 N, P and K contents of different lentil lines showing resistance and susceptibility to *Ascochyta lentis* the cause of lentil blight.

Materials and Methods

A virulent isolate of *Ascochyta lentis* was recovered from diseased pods. The fungus was purified by spore streak method (Ricker & Ricker, 1936). The pure culture was maintained on 6 percent Lentil Seed Meal Agar (LSMA) in the test tubes (30 ml cap.) and stored in a refrigerator for further studies. *A. lentis* was mass cultured on boiled and subsequently sterilized chickpea seeds.

Seven advanced lentil lines each of the two reaction groups (resistant and susceptible) were sown in the field. The test 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 were inoculated with *A. lentis* spore suspension ($2x10^3$ spore ml^{-1}). Plant samples comprising of shoots of inoculated and un-inoculated plants of resistant and susceptible lines, were collected from the field on 30 days after inoculation. These were washed in 0.2 percent detergent solution in order to remove any dirt followed by washing in 0.8

percent 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 then 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 N, P and K following (Bhargava & Raghupathi 1995). Nitrogen and phosphorus content was recorded as percentage of dry weight whereas content of rest of the elements were recorded as ppm (parts per million). The data collected were analyzed following (Steel *et. al*., 1996), (Gomez & Gomez 1984), (Petersen 1989a,b) and using computer programme MstatC. To elucidate the data, graphs, bar diagrams etc. were prepared using computer programme HG4.

Results

The samples of un-inoculated and inoculated plants from both the reaction groups were collected, oven dried, ground to fine powder and subjected to standard analytical methods for the determination of Nitrogen, Phosphorus and Potassium contents. The results are described as below:

Nitrogen (percent dry weight): The data on nitrogen (percent) of un-inoculated and inoculated plants of both the susceptible and resistant entries are given in Table 1a. The analysis of variance for nitrogen content is given in Table 1b. Although percent nitrogen was slightly higher in the lentil lines of susceptible group, yet both the reaction groups did not differ significantly from one another statistically. Inoculation with the pathogen significantly increased percent nitrogen in susceptible from 2.992 to 3.148, and in resistant group from 2.851 to 3.007. The overall range of percent nitrogen in un-inoculated and inoculated plants was 2.193 to 3.390 (average 2.921) and 2.40 to 3.513 (average 3.078), respectively. The range of percent nitrogen in un-inoculated and inoculated plants of susceptible group was 2.757 to 3.157 (average 2.992) and 3.063 to 3.243 (average 3.148), respectively while in case of resistant group this range was 2.193 to 3.39 (average 2.851) and 2.40 to 3.51 (average 3.007), respectively.

Phosphorus (percent dry weight): The data on phosphorus content (percent) of the un-inoculated and inoculated plants of susceptible and resistant lentil entries is given in Table 1a. According to analysis of variance, percent phosphorus content did not differ significantly in both the reaction groups (Table 1b). The phosphorus content of un-inoculated plants of susceptible lentil lines was higher than those of resistant ones while the reverse situation was observed in case of inoculated plants. It means that inoculation with the pathogen did not significantly change the phosphorus content of lentil lines/cultivar of both the reaction groups. The mean percent phosphorus in un-inoculated and inoculated resistant plants was 0.4471 (0.3367 to 0.5167) and 0.47 (0.38 to 0.54), respectively, while in case of un-inoculated and inoculated susceptible plants, the mean percent phosphorus was 0.471 (0.113 to 0.7567) and 0.2895 (0.2033 to 0.4133). Although variable response to inoculation was observed within the lentil lines, yet it was more pronounced within the plant of susceptible group where as it decreased considerably as a result of inoculation.

Table 1a. Nitrogen (percent dry weight), phosphorus (percent dry weight) and potassium (ppm dry weight) content for the reaction groups and lines/cultivars of lentil included in the reaction group.

Lines/ Cultivar	Nitrogen (%)		Phosphorus (%)		Potassium (ppm)	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Susceptible group						
91549	3.137 abc*	3.133 abc	0.5400 ^{NS}	0.4133	414.3 g	954.7 a
93523	3.157 abc	3.243 a	0.5200	0.2367	380.7 h	889.3 b
93536	2.757 f	3.063 cd	0.4933	0.3033	167.0 i	472.0 f
93564	3.160 abc	3.100 bcd	0.3767	0.2400	130.7 j	377.3 h
93566	2.970 de	3.140 abc	0.4967	0.3667	612.7 e	822.0 c
95560	2.877 ef	3.223 ab	0.7567	0.2633	44.0 j	698.0 d
96504	2.877 ef	3.133 abc	0.1133	0.2033	481.7 f	687.7 d
LSD	**0.1410**		**0.4884**		**20.60**	
Mean	2.992 b	3.148 a	0.4710 ^{NS}	0.2895	333.0 b	700.1 a
Resistant group						
94503	2.950 ^{NS}	3.097	0.4600 cde	0.5033 cde	754.3 j	997.7 f
94506	3.390	3.513	0.5167 ab	0.5400 a	743.7 j	963.3 g
95509	3.090	3.170	0.4833 bcd	0.5400 a	888.7 hi	1053.0 d
95513	2.737	3.047	0.4333 de	0.4533 cde	893.0 h	1024.0 e
95515	2.807	2.933	0.4467 de	0.3800 fg	866.0 i	1051.0 d
95527	2.193	2.400	0.3367 g	0.4567 cde	993.7 f	1204.0 b
Masoor-93	2.790	2.890	0.4533 cde	0.4167 ef	1150.0 c	1379.0 a
LSD	**0.1685**		**0.05329**		**24.37**	
Mean	2.851 b	3.007 a	0.4471 ^{NS}	0.4700	898.5 b	1095.9 a

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

Table 1b. Analysis of variance for nitrogen (percent dry weight), phosphorus (percent dry weight) and potassium (ppm dry weight) content for the reaction groups and lines/cultivar of lentil.

Source of variance	Degree of freedom	Mean squares		
		N	P	K
Replications	2	0.024^{NS}	0.054^{NS}	226.86^{NS}
Reaction group	1	0.417^{NS}	0.129^{NS}	4851368.68**
Error (a)	2	0.039	0.025	17.71
Tr. within Susc. group	1	0.256*	0.346^{NS}	1415335.71**
Error (b)	2	0.006	0.085	10.29
Tr. within Resist. group	1	0.256*	0.005^{NS}	409072.02**
Error (c)	2	0.003	0.001	558.95
Lines Within Susc. group	6	0.055**	0.084^{NS}	204082.44**
Tr. x Lines within Susc. group	6	0.036**	0.047^{NS}	38546.66**
Error (d)	24	0.007	0.084	149.48
Lines/Cult. within resist. group	6	0.735**	0.015**	120770.43**
Tr. x Lines/Cult. within resist. group	6	0.009^{NS}	0.006**	2338.86**
Error (e)	24	0.010	0.001	209.10
Total	**83**			

Table 2a. Correlation between mineral contents of un-inoculated (U) and inoculated (I) plants of lentil lines/cultivar susceptible to Ascochyta Blight.

		N	P	K
U	N	_		
I				
U	P	0.006	_	
I		-0.238		
U	K	0.135	0.348	_
I		0.587	0.461	

Table 2b. Correlation between mineral contents of un-inoculated (U) and inoculated (I) plants of lentil lines/cultivar resistant to Ascochyta Blight.

		N	P	K
U	N	_		
I				
U	P	0.985**	_	
I		0.569		
U	K	-0.569	-0.464	_
I		-0.614	-0.503	

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

Potassium (ppm of dry weight): The data on potassium content is given in Table 1a. On the basis of analysis of variance of these data (Table 1b), highly significant difference was observed in both the reaction groups (mean square 4851368.68). In case of un-inoculated plants of resistant lentil lines/cultivars, potassium was almost 300 percent of that of susceptible lentil lines. Although the potassium content of susceptible lines was considerably lower than that of resistant ones, yet the increase was much more pronounced, compared with the resistant lentil lines, in response to inoculation with the pathogen. Mean potassium content of un-inoculated and inoculated plants of susceptible cultivars was 333.0 (130.7 to 481.7) and 700.1 (377.3 to 954.7), respectively, while in case of resistant group, the mean potassium content in un-inoculated and inoculated plants was 898.5 (743.7 to 1150.0) and 1095.9 (963.3 to 1379.0), respectively.

Correlation: The correlation coefficient (r) values were calculated for mineral content of un-inoculated and inoculated plants of susceptible and resistant lines/cultivar (Table 2 a&b). In case of un-inoculated susceptible plants, all the possible correlations were non-significant, while in case of un-inoculated resistant plants, correlation was highly significant between nitrogen and phosphorus content (0.985).When the mineral content of inoculated plants was considered, all the possible correlations were non- significant.

Discussion

Although statistically insignificant, the susceptible lentil lines contained more nitrogen in both un-inoculated and inoculated plant than the resistant ones. The plants with more nitrogen have a tendency to have an increase in cell number and cell size with

an overall increase in leaf production (Njoku, 1957). A significant correlation between leaflet width and disease reaction has already been reported (Bayaa *et al*., 1994). As the nitrogen is mobile within the plant (Devlin & Witham, 1983), the younger leaves definitely contain more nitrogen than the older ones. Perhaps, that is the reason that the topmost leaves are more susceptible to the disease. Although the mineral content were assayed for the whole shoot, yet it seems that if only topmost foliage would have been assayed, this would have given a clearer concept of the picture. Nitrogen if in excess, favours the development and growth of a lot of plant pathogens as it is an integral part of their nutrition, thus reducing the resistance against the pathogens. This situation seems to be true in this case as well. A higher amount of nitrogen in the inoculated plants of susceptible group may be attributed to the spread of the pathogen in a nitrogen richer foliage and accumulation of fungal mycelia, the latter being richer in nitrogen. There was a general increase in the nitrogen content of both the groups upon inoculation. This increase was, hence, relatively higher in the susceptible group as compared with the resistant one. These results are controversial with the previous reports. Reddy & Khare (1984) reported that nitrogen content, although higher in lentil varieties susceptible to rust than in the resistant ones, yet decreased in both the groups. Randhawa (1994) reported increase in nitrogen content in chickpea cultivars resistant to Ascochyta blight while there was a decrease in the susceptible cultivars.

Phosphorus is utilized by the plants for the formation of nucleic acids, phospholipids, the coenzymes NAD and NADP, ATP and other high-energy compounds (Devlin & Witham, 1983).

The phosphorus content although higher in un-inoculated plants of susceptible lentil lines than the resistant ones, yet it decreased, as a result of inoculation with *Ascochyta lentis* while it increased in the resistant lentil lines. Depletion of phosphorus in susceptible lentil lines may be due to increased but disrupted respiration in inoculated plants. But the increase in phosphorus content in resistant lentil lines indicates intact and consistent uptake and translocation of this mineral. On the other hand, Reddy & Khare (1984) reported decrease in phosphorus in lentil cultivars both resistant and susceptible to *Uromyces fabae* but Randhawa (1994) reported increase in phosphorus in case of chickpea cultivars of both susceptible and resistant groups against infection by *Ascochyta rabiei*.

Potash is important for plants for the processes such as respiration, photosynthesis and chlorophyll development and is also a part of some important enzymes and coenzymes. Un-inoculated plants of resistant lentil lines contained almost 250 percent of the potash as that of susceptible ones, which even increased in the inoculated plants. In case of susceptible lines, the increase in potash as a result of inoculation with the pathogen was more pronounced than in case of resistant lentil lines. These results comply with those of Randhawa (1994) and Reddy & Khare (1984). The pronounced increase in the potash content of susceptible lines may be attributed to rapid and extensive growth, multiplication and sporulation of *Ascochyta lentis* in the susceptible lentil lines as compared with the resistant ones.

In general, it was observed that the plants lower in nitrogen and phosphorus were resistant to the disease while those with higher nitrogen and phosphorus were susceptible.

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