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Biochemical Basis of Resistance in Lentil (*Lens culinaris* Medik.) Against Ascochyta Blight: I Phenols

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Abstract: The amount of total phenols was higher in case of susceptible group prior to inoculation with the pathogen, compared with the resistant group. Upon inoculation total phenols increased significantly in both the groups, the increase being more pronounced in case of resistant group.

Key words: *Lens culinaris*, *Ascochyta lentis*, Phenols

Introduction

Lentil (*Lens culinaris* Medik.) contains 28.6% protein, 3.1% ash, 4.6% crude fiber, 44.3% starch, 36.1% amylase, 63.1% total carbohydrates and 420 Cal. 100 g^{-1} gross energy (Bhatty and Wu, 1974). Moreover, lentils are 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, but not in the cotyledons (Vaillancourt *et al*., 1986). 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*., 1993). In 1998-99, lentil was grown over an area of 3.404 million hectares in the world, 35.25% being occupied by India, 5.88% by Bangladesh and 1.91% by Pakistan, the total share in world production by these countries being 29.55, 5.46 and 1.24% respectively (Anonymous, 1997). Lentil is the second most important pulse crop of Pakistan after chickpea. In 1998-99, lentil was grown over an area of 65 thousand hectares with an average yield of 571 Kg ha^{-1} only (Anonymous, 1997). This is extremely low as compared to other countries of the world e.g. 736 Kg ha^{-1} in India, 814 Kg ha^{-1} in Bangladesh, 728 Kg ha^{-1} in Syria and 1370 Kg ha^{-1} in USA (Anonymous, 1997).

The low yield in Pakistan may be attributed to continuous cultivation of cultivars with low yield potential and excessive vegetative growth, narrow adaptability, low stability of yield, and susceptibility to stress conditions (Rajput and Sarwear, 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* (Bond. and Vassilj.) and may cause considerable losses in yield especially under cool and moist conditions. 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.

There are numerous reports on the role of phenolics and Phytoalexins in contributing resistance to the plants, by a number of host-parasite interactions. These substances act in the chemical defence of higher plants mainly in three ways. First, they are present in the healthy plants at concentrations sufficient to inhibit growth and sporulation of a pathogen (generally referred to as pre-formed resistance factors); Secondly, as a response to infection, their concentration is markedly increased imparting resistance against the invading microorganisms. Finally, certain post-infection products (phytoalexins), which are not normally found in healthy plants, are synthesized or increase in amounts after infection.

Thus, it seems imperative to undertake studies on the determination of role of phenolics in resistance against *Ascochyta lentis*.

Materials and Methods

Isolation of *A. lentis* was done from diseased pods. The fungus was purified by spore streak method (Riker and Riker, 1936). The pure culture was grown on 6% LSMA in the test tubes (30 ml cap.) and stored in a refrigerator for further studies. *A. lentis* was mass cultured on chickpea grains (being cheaper than lentil).

Four advanced lines each of the reaction group (resistant and susceptible) were sown in the field during the growing season of 1997-98. 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 (2×10^3 spore ml^{-1}).

Samples from growing tips of green plants of resistant and susceptible (inoculated as well as un-inoculated) lentil lines were collected on 23rd of March, 1997 (30 days after inoculation with *A. lentis*). The samples were immediately weighed with the help of electronic balance (Mettler PE 3600). They were cut into small pieces (about 5 mm) and put in boiling methanol (BDH) until the green colour came out. The methanol was decanted and the plant pieces were ground in a pestle and mortar into fine powder. This powder was mixed in the decanted methanol and it was then filtered (Whatman No. 1) into a small glass bottle (50 mL capacity). The residue was washed with 80% acidified (0.1% HCl) methanol. 5 mL of water was added in the filtrate, and methanol was evaporated at 45°C 100 rpm, using Buchi Rotavapor R-114 with Buchi Waterbath B-480. The aqueous portion was washed with n-hexane until the green colour was removed. The concentration of phenolic compounds in the above water extract, was determined using the Folin-Ciocalteau reagent by adopting the modified method keeping chlorogenic acid as standard and results were calculated as

Table 1: Total phenols (mg g^{-1} fresh weight) for the reaction groups and the lentil lines/cultivar

Lines/Cultivar	Treatment		Mean	Percent Increase
	Uninoculated	Inoculated		
	Susceptible Group			
91549	1.244 e	1.816 c	1.530 d	45.98
93566	1.812 c	2.088 a	1.950 a	15.23
95560	1.589 d	1.791 c	1.690 c	12.71
96504	1.656 d	1.950 b	1.803 b	17.75
LSD	0.09744			0.6890
Mean	1.575 b	1.911 a	1.743	21.33
	Resistant Group			
94506	1.479 cd	1.999 b	1.739 b	35.16
95513	1.444 d	2.244 a	1.844 ab	55.40
95527	1.364 d	2.286 a	1.825 a	67.60
Masoor-93	1.843 c	2.364 a	2.003 a	43.88
LSD	0.1949			0.1378
G. Mean	1.529	2.067	1.798	35.19

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

Table 2: Analysis of Variance for total phenols (mg g^{-1}) fresh weight for the reaction groups and the lentil lines/cultivar

Source of variance	Degree of Freedom	Means Squares
Replications	2	0.010^{NS}
Reaction Group	1	0.144^{NS}
Error (a)	2	0.018
Treatments within Susceptible Group	1	0.678*
Error (b)	2	0.005
Treatments within Resistant Group	1	3.295
Error ©	2	0.015
Lines within Suusceptible Group	3	0.189**
Treatment × Lines within Susceptible Group	3	0.040**
Error (d)	12	0.003
Unes/Cultivar within Resistant Group	3	0.073**
Treatment × Lines/Cultivar within Resistant Group	3	0.043*
Error (s)	12	0.012
Total	47	

NS = Non Significant * = Significant at p = 0.05

mg g^{-1} fresh weight. This was done by using Beckman Model 25 Spectrophotometer at 750 nm.

The data collected were analyzed following Steel and Torrie (1980), Gomez and Gomez (1984) and Petersen (1989a, b) using computer programme MstatC. To elucidate the data, graphs, bar diagrams etc. were prepared using computer programme HG4.

Results

The data regarding the total phenols is given in Table 1. In susceptible group, the inoculation with the pathogen caused 45.98, 15.23, 12.71 and 17.75% increase, in the total phenols of the lines 91549, 93566, 95560 and 96504, respectively, the average being 21.33%. The increase in phenols of all the lines was statistically significant. In case of resistant group, the entries 94506, 95513, 95527 and Masoor-93 expressed 35.16, 55.40, 67.60 and 43.88% increase, in the total phenols, respectively, with an average of 50.07%, as a consequence of inoculation with the pathogen. The analysis of variance for total phenols is given in Table 2. The difference in total phenols of the two reaction groups was non-significant (mean square 0.144), though the total phenol content of resistant entries (being smaller than that of susceptible ones in un-inoculated plants) increased more as compared with susceptible entries (mean square 3.295 and 0.678 for resistant and susceptible groups, respectively). The mean total phenols were 1.575 (1.244-1.812) mg g^{-1} fresh weight in un-inoculated plants of susceptible group, which increased to 1.911 (1.791-2.088) mg g^{-1} after inoculation with the pathogen. While in case of resistant group, the total phenols increased from 1.482 (1.364-1.643) mg g^{-1} in uninoculated plant to 2.224 (1.999-2.364) mg g^{-1} in inoculated plants. It is apparent from the above figures that the increase in total phenols was more pronounced in case of resistant group compared with the susceptible one.

Discussion

Total phenols were more in case of un-inoculated plants of susceptible group as compared with the resistant group, which was quite unusual because majority of the reports in this respect indicate more phenolic compounds in the resistant than in the susceptible lines. But such type of relationship has also been reported (Wakimoto *et al*., 1960; Ramakrishnan, 1989) in rice where cultivars resistant to the blast disease contained less amount of polyphenols prior to inoculation with the pathogen. It clearly indicates that all the phenolic compounds may not impart role in resistance to plant pathogens but some of them may favour disease development on the other hand as well. As a result of inoculation with the pathogen, there was significant increase in the content of total phenols in both the reaction groups, increase being more pronounced in the lentil lines of resistant groups as compared with the susceptible group. Increased phenolic compounds accumulation in the resistant cultivars have been reported in many host-parasite interactions (Reddy and Khare, 1984; Parashar and Sindhan, 1986; Jamil *et al*., 1990; Randhawa, 1994).

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