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ASSESSMENT OF ENDOGENOUS ORGANIC ACID LEVELS IN ASCOCHYTA BLIGHT [*Ascochyta rabiei* (Pass.) Labr.] SUSCEPTIBLE AND RESISTANT CHICKPEAS (*Cicer arietinum* L.)

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ABSTRACT

Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labr., is one of the most important foliar diseases of chickpea (*Cicer arietinum* L.) in many countries. The present study was designed to determine whether there were different concentrations of organic acids between kabuli chickpea genotypes known as resistant and susceptible to ascochyta blight. ILC 263, (susceptible to ascochyta blight), FLIP 95-51C and FLIP 95-60C (resistant to ascochyta blight) were used to determine the levels of endogenous citric, malic, oxalic, quinic, and succinic acids. Citric and oxalic acid concentrations were lower in resistant genotypes than the susceptible genotype. However, malic acid was higher in the resistant genotypes than the susceptible one. Results suggested that high level of malic acid may be used as pre-selection criteria for resistance to ascochyta blight in chickpea breeding material.

Keywords: Ascochyta blight, chickpea; citric acid; malic acid; oxalic acid; quinic acid; sucsinic acid.

INTRODUCTION

All external surfaces of the chickpea (*Cicer arietinum* L.) plants, with the exception of corolla, are covered by glandular and aglandular hairs that secrete acids (Cubero, 1987; van der Maesen, 1972, 1992; Singh, 1997). Secretion consists of almost exclusively of malic acid, with very small amounts of oxalic acid. The possibility of finding some specific response to drought or reflectance of radiation was examined but no significant differences were observed with or without malic acid (Khanna-Chopra and Sinha, 1987). Rembold (1981) reported that there was a clear correlation between malic acid content of leaves and susceptibility to pod borer (*Heliothis* spp.) damage in cultivars grown under rainfed conditions in India. Cultivars exuding malic acid above a threshold level were relatively resistant to *Heliothis*. Later work on spore germination and germtube development of ascochyta blight [*Ascochyta rabiei* (Pass.) Labr.], one of the most important diseases of chickpea (Nene et al., 1996; Akem, 1999), indicated that there was no difference in the exudates between resistant and susceptible cultivars (Nene and Reddy, 1987). Considering the conflicting results of the previous work on the exudates, therefore this study focuses on whether there are significant differences among levels of organic acids (citric, malic, oxalic, quinic, and succinic acids) between kabuli chickpea genotypes known as resistant or susceptible to ascochyta blight.

MATERIALS AND METHODS

Plant material: Kabuli chickpeas have white and pale cream seeds and no pigmentation on the stem (Muehlbauer and Singh, 1987). Three kabuli chickpea lines, ILC 263 (susceptible to ascochyta blight), FLIP 95-51C and FLIP 95-60C (both resistant to Ascochyta blight) supplied from International Center for Agricultural Research in the Dry Areas (ICARDA) were used. Genotypes were sown in the first week of December in 2000 and the second week of December 2001 at the experimental area of Akdeniz University in Antalya, Turkey. Genotypes were grown in a randomized complete block design with two replications under field conditions. The experimental plots consisted of one row of 4 m length with inter and intra row spacing of 45 x 10 cm. The susceptible check, ILC 263, was repeated every two-test rows in order to enhance epidemics. Weed control in the experimental area was done by hand prior to generative stage.

Weather and soil conditions: Antalya has mild and wet winters and hot and dry summers. Monthly and seasonal distribution of precipitation was irregular that is typical for a Mediterranean climate. In the experimental area, generally organic matter and macro plant nutrients were found at the low level with total nitrogen 0.1%. Soil texture of experimental area was loam with a pH of 8.05.

Inoculation of plants: Several breeders or pathologists have focused on ascochyta blight and pointed out that the fungus survives in the diseased chickpea debris and in seeds from infected plants (Kaiser and Hannan, 1988; Maden, 1983; Maden et al., 1975; Navas-Cortez et al., 1998; Trapero-Casas and Kaiser, 1992). Infected debris is an important source of infection in the following seasons since the fungus survives for 2 years in infected tissues. In this study, infected debris was used for inoculation of the plants.

Disease assessment: Screening methods for *Ascochyta rabiei* (Pass.) Labrouse were given in detail (Jiménez-Díaz et al.,1993) elsewhere. Disease rating scale was scored by using 1-9 class scale as described by Toker et al., (1999), where 1 = Immune, 5 = Tolerant and 9 = Very Highly Susceptible (all plants killed by the disease). Scoring was made after pod filling stage.

Organic acid analyses: Genotypes were evaluated for their reaction to ascochyta blight for two years and after the first year's observation in the test location organic acid analyses were done in the second year. Whole leaves with leaflets and rachis and the youngest ones fully emerged in shoot, and having green pods with immature seeds were used in the analyses. Harvest was done in the second week of May in 2002, when plants were at the early pod filling stage. Plant organs were cut with scissors without touching by hand. Citric, malic, oxalic and succinic acids were determined as percent of total organic acids by using HPLC. Firstly, 10 g of samples from homogenised plant shoot was blended with 40 ml of 0.01 M KH_2PO_4 and centrifuged for 30 min. at 6000 rpm. Then the supernatant was filtered through a 0.45 µm membrane and passed through Sep-Pack C18 column. 20 µl of filtrate was injected. A Varian LC Star HPLC System with UV-VIS Detector (214 nm) was employed. HPLC column was Nucleosil 5C18 (250x4.6 mm ID.) and mobile phase was 2% KH_2PO_4 contained 0.1g/l hexane sulphonic acid sodium salt as ion pair reagent with 0.9 ml/min flow rate at the ambient temperature.

Statistical analysis: The data were recorded in percentage (%) of citric, malic, oxalic and succinic acids, determined as percent of total organic acids in fresh weight by using HPLC, and then the data obtained were analysed by using MSTATC statistical software package (Freed et al., 1989). Each genotype for total values of all recorded traits was compared using orthogonal contrast (susceptible vs. resistant genotypes) comparison feature of the software.

RESULTS

Reaction to the pathogen

Analysis of variance revealed that genotype effect was statistically significant ($p< 0.01$) only for succinic acid. As for orthogonal contrast that is between ascochyta susceptible and resistant genotypes, the contrast was statistically significant for citric acid ($p< 0.05$). As can be seen in Figures 1-5, FLIP 95-51C and FLIP 95-60C, resistant to ascochyta blight, were scored with 2, namely resistant over two years under field conditions. As expected, susceptible genotype, ILC 263, was scored more than 8 on the 1-9 class scale over two years. Especially in the second year, ILC 263 was killed by the pathogen, *Ascochyta rabiei*, in the all rows, except several plants in one row and scored with 9.

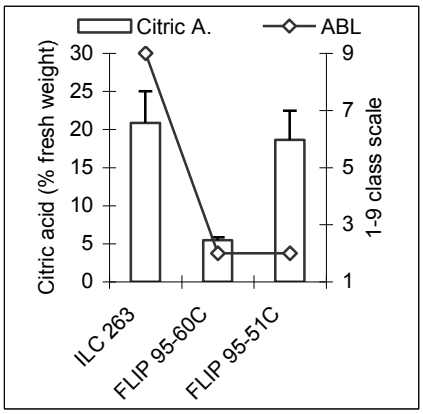

Figure 1. Mean values of citric acid (% fresh weight) in shoots with green pod during pod filling stage of kabuli chickpeas, susceptible and resistant to ascochyta blight when genotypes subjected to *A. rabiei*. ABL is ascochyta blight score on a 1-9 class scale. Values are means ± standard deviations.

Organic acids

Succinic acid was the highest and followed by citric, malic, and quinic acids, respectively. Oxalic acid level was the lowest (Figures 1-5). The pathogen, *A. rabiei*, was not likely effect on endogenous levels both of quinic acid and succinic acid. Organic acids levels were varied in 5.46 to 20.85% for citric acid (Figure 1); 9.25 to 15.69% for malic acid (Figure 2); 4.91 to 8.67% for oxalic acid (Figure 3); 9.23 to 12.4% for quinic acid (Figure 4); and 45.21 to 68.84% for succinic acid (Figure 5). Quinic and succinic acid levels changed from genotype to genotype, independent from the susceptible/resistant class (Figures 4 and 5). In contrast, malic acid was lower in susceptible genotype, ILC 263, (9.25%) than in both of the resistant genotypes, FLIP 95-60C with 11.89% and FLIP 95-51C with 15.69% (Figure 2).

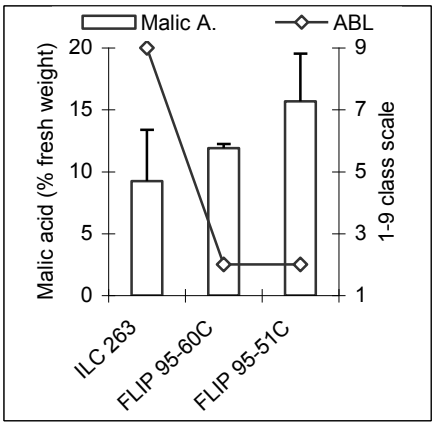

Figure 2. Mean values of malic acid (% fresh weight) in shoots with green pod during pod filling stage of kabuli chickpeas, susceptible and resistant to ascochyta blight when genotypes subjected to *A. rabiei*. ABL is ascochyta blight score on a 1-9 class scale. Values are means ± standard deviations.

Despite the fact that genotypic effect on malic acid concentration was not statically significant at $p< 0.05$, there

was a consistency between the classes for the quantity of malic acid. On the other hand, citric acid level in FLIP 95-60C with 5.46% and in FLIP 95-51C with 18.62%, resistant to ascochyta blight, was lower than susceptible genotype, ILC 263 (Figure 1). Likewise, oxalic acid level was the highest in ILC 263 (Figure 3). In other words, oxalic acid in resistant genotypes, FLIP 95-51C and FLIP 95-60C, was lower than in ILC 263, the susceptible genotype.

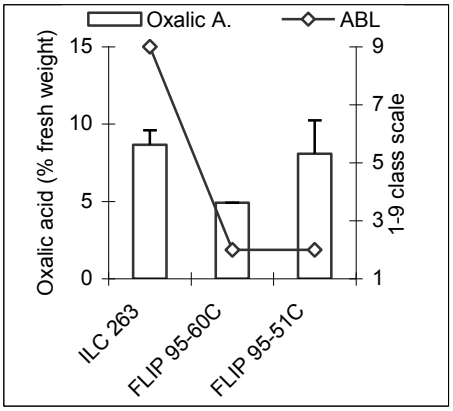

Figure 3. Mean values of oxalic acid (% fresh weight) in shoots with green pod during pod filling stage of kabuli chickpeas, susceptible and resistant to ascochyta blight when genotypes subjected to *A. rabiei*. ABL is ascochyta blight score on a 1-9 class scale. Values are means ± standard deviations.

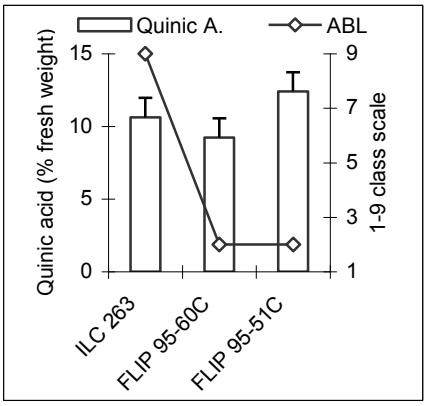

Figure 4. Mean values of quinic acid (% fresh weight) in shoots with green pod during pod filling stage of kabuli chickpeas, susceptible and resistant to ascochyta blight when genotypes subjected to *A. rabiei*. ABL is ascochyta blight score on a 1-9 class scale. Values are means ± standard deviations.

DISCUSSION

Although pathotypes used in the study were unknown, it should have been Pathotype-1 and/or Pathotype-2 due to the fact that resistant genotypes, FLIP 95-51C and FLIP 95-60C were scored with rating 2 on 1-9 class scale. This is especially lower score when compared the scores of resistant genotypes in ICARDA (ICARDA, 1998; 1999). *A. rabiei* isolates were classified into 3 groups (Pathotype-1, Pathotype-2, Pathotype-3). The classification was based on the reactions to a set of differentials (Udupa et al., 1998; Khan et al., 1999; Jamil et al., 2000). Santra et al. (2001) showed that 48 isolates from different countries were placed to 5 group using RAPD markers. It was also reported that Pathotype-3 was the most virulent one (ICARDA, 1998; Jamil et al., 2000). Similarly, studies on races 0 (*Foc*-0) and 5 (*Foc*-5) were done with the chickpea cultivars P-2245 and PV-61 on development of Fusarium wilt (*Fusarium oxysporum* f.sp. *ciceris*) and found that *Foc*-5 proved much more virulent than *Foc*-0 in chickpea (Navas-Cortes et al., 2000). ILC 263, susceptible to ascochyta blight and International check of ICARDA, rated 9 as expected.

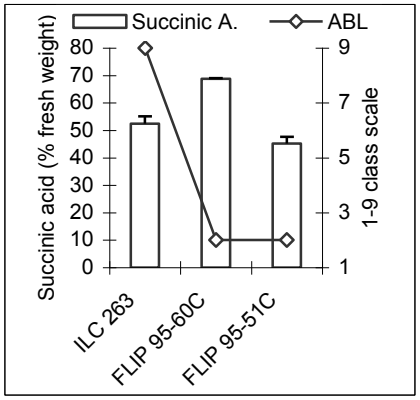

Figure 5. Mean values of succinic acid (% fresh weight) in shoots with green pod during pod filling stage of kabuli chickpeas, susceptible and resistant to ascochyta blight when genotypes subjected to *A. rabiei*. ABL is ascochyta blight score on a 1-9 class scale. Values are means ± standard deviations.

Since whole fresh organs were used to determine of organic acids, succinic acid was the highest among the acids studied. On the other hand, it was shown that malic acid was the highest on gland secretions (Khanna-Chopra and Sinha, 1987; Yoshida et al., 1995). The authors further reported that malic acid content on the surface of leaves and fruit wall of chickpea varied in different stages of growth. Malic acid level of green shoot in chickpea genotypes under ascochyta blight epidemic conditions was lower than succinic acid and it was almost equal to citric acid. As reported previously by Khanna-Chopra and Sinha (1987), oxalic acid showed the lowest level among the organic acids. Singh et al. (1998) studied the role of malic acid in pycnidiospore germination of *A. rabiei* in chickpea. They concluded that pycnidiospore germination of *A. rabiei* was significantly enhanced in low concentrations of malic acid ($p<0.05$ and 0.01). Twelve resistant and susceptible genotypes were evaluated and the endogenous level of malic acid on surface washing of leaves and shoot tissues was higher in susceptible ones than in resistant chickpeas. In contrast, the endogenous level of malic acid, determined in leaves, was higher in resistant than susceptible types. The findings were similar to our results. Bashir et al. (1997) pointed out that ascochyta blight infection, when the plants sprayed with 20 mM oxalic acid, was reduced by 59% in CM 72 (susceptible to ascochyta blight) and 40% in C727 (resistant to ascochyta blight) in comparison to the control. In our study, ILC 263, susceptible to ascochyta blight, had the highest percentage of oxalic acid. Differences in the accumulation of the β-1,3 glucanases in ascochyta blight resistant and susceptible chickpeas when

they exposed to *A. rabiei* were described (Armero and Tena, 2001). The authors concluded that accumulation of the β-1,3 glucanases alone cannot be the reason for resistance. In a work on washed surface of leaves in four chickpea genotypes, it was explained that the accumulation of oxalic acid was considered to be one of the mechanisms of *Helicoverpa armigera* resistance in chickpea. But there was no effect on larval growth of malic acid (Yoshida et al., 1995).

In conclusion, among the organic acids studied, malic acid was higher in the resistant genotypes than the susceptible one. Consequently, the high level of malic acid may be used as pre-selection criterion for resistance to ascochyta blight in chickpea breeding material. A detailed correlation study with more entries of breeding lines remains a further task in order to determine indirect selection criteria via organic acids for resistance to the ascochyta blight.

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LITRATURE CITED

Akem, C., 1999. Ascochyta blight of chickpea: present status and future priorities. Int. J. Pest Manage. 45: 131-137.

Armero J., M. Tena, 2001. Possible role of plasma membrane H^+-ATPase in the elicitation of phytoalexin and related isoflavone root secretion in chickpea (*Cicer arietinum* L.) seedlings. Plant Sci. 161: 791-798.

Bashir, N, M.I. Hasmi, F.F. Jamil, 1997. Induction of systemic acquired resistance by oxalic acid in chickpea (*Cicer arietinum* L.) against *Ascochyta rabiei.* Pakistan J. Phytopath. 9: 18-20.

Cubero, J.I., 1987. Morphology of chickpea. In: Saxena MC, Singh KB, (eds.). The Chickpea. CAB International. Oxon. pp. 35-66.

Freed, R., S.P. Einensmith, D. Guetz, D. Reicosky, V.W. Smail, P. Volberg, 1989. User's Guide to MSTATC, An analysis of agronomic research experiments. Michigan State University.

ICARDA, 1998. Germplasm program: legumes annual report for 1998. ICARDA, Aleppo, Syria.

ICARDA, 1999. Germplasm program annual report for 1999. ICARDA, Aleppo, Syria.

Jamil, F.F., N. Sarvar, M. Sarvar, J.A. Khan, J. Geistlinger, G. Kahl, 2000. Genetic and pathogenic diversity within *Ascochyta rabiei* (Pass.) Lab. populations in Pakistan causing blight of chickpea (*Cicer arietinum* L.), Physiol. Mol. Plant P. 57 243-254.

Jiménez-Díaz, R.M., P. Crino, M.H. Halila, C. Mosconi, A.T. Trapero-Casas, 1993. Screening for resistance to fusarium wilt and ascochyta blight in chickpea. In: Saxena MC, Singh KB, (eds.). Breeding for Stress Tolerance in Cool Season Food Legumes. John Wiley & Sons; Baffins Lane, Chichester, pp. 77-95.

Khan, M.S.A., M.D. Ramsey, R. Corbiére, A. Infantino, A. Porta-Puglia, Z. Bouznad, E.S. Scott, 1999. Ascochyta blight of chickpea in Australia: identification, pathogenicity and mating type. Plant Pathol 48: 230-234.

Khanna-Chopra R., S.K. Sinha, 1987. Chickpea: physiological aspects of growth and yield. In: Saxena MC, Singh KB, (eds.). The Chickpea. CAB International. Oxon, pp. 163-189.

Kaiser, W.J., R.M. Hannan, 1988. Seed transmission of *Ascochyta rabiei* in chickpea and its control by seed-treatment fungicides. Seed Sci. Technol. 16: 625-637.

Maden, S., 1983.Transmission of seed-borne infections of *Ascochyta rabiei* (Pass.) Labr. to seedling and its control. J. Turk. Phytopath. 12: 77-82.

Maden, S., D. Singh, S.B. Mathur, P. Neergaard, 1975. Detection and location of seed-borne inoculum of *Ascochyta rabiei* and its transmission in chickpea (*Cicer arietinum* L.). Seed Sci. Technol. 3: 667-681.

Muehlbauer, F.J., K.B. Singh, 1987. Genetics of chickpea. In: Saxena MC, Singh KB, (eds.). The Chickpea. CAB International. Oxon. pp. 99-125.

Navas-Cortéz, J.M., A.T. Trapero-Casas, R.M. Jiménez-Díaz, 1998. Influence of relative humidity and temperature on development of *Didimella rabiei* on chickpea debris. Plant Pathol. 47: 57-66.

Navas-Cortés, J.A., A.R. Alcalá-Jiménez, B. Hau, R.M. Jiménez-Díaz, 2000. Influence of inoculums density of races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* on development of fusarium wilt in chickpea cultivars. Eur. J. Plant Pathol. 106: 135-146.

Nene, Y.L., M.V. Reddy, 1987. Chickpea diseases and their control. In: Saxena MC, Singh KB, (eds.). The Chickpea. CAB International. Oxon. pp. 233-270.

Nene, Y.L., V.K. Sheila, S.B. Sharma, 1996. A world list of chickpea and pigeonpea pathogens. ICRISAT. Patancheru, Andhra Pradesh, India. p. 27.

Rembold H., 1981. Malic acid in chickpea exudates. A marker for *Heliothis* resistance, Int Chickpea Newsl 4: 18-19.

Santra, D.K., G. Singh, W.J. Kaiser, V.S. Gupta, P.K. Ranjekar, F.J. Muehlbauer, 2001. Molecular analysis of *Ascochyta rabiei* (Pass.) Labr., the pathogen of ascochyta blight in chickpea. Theor. Appl. Genet. 102: 676-682.

Singh, K.B., 1997. Chickpea (Cicer arietinum L.), Field Crop Res. 53: 161-170.

Singh, P.J., P. Mahendra, C. Devakumar, M. Pal, 1998. Role of malic acid in pycnidiospore germination of *Ascochyta rabiei* and chickpea blight resistance. Indian Phytopathol. 51: 254-257.

Trapero-Casas, A., W.J. Kaiser, 1992. Development of *Didimella rabiei* the teleomorph of *Ascochyta rabiei* on chickpea straw. Phytopathology 82: 1261-1266.

Toker, C., M.I. Cagirgan, B. Uzun, 1999. Screening and selection for resistance to ascochyta blight [*Ascochyta rabiei* (Pass.) Labr.] of chickpea (*Cicer arietinum* L.) under field conditions. J. Turk. Phytopath. 28: 101-110.

Udupa, S.M., F. Weigand, M.C. Saxena, G. Kahl, 1998. Genotyping with RAPD and microatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. Theor. Appl. Genet. 97: 299-307.

van der Maesen, L.J.G., 1972. *Cicer* L., A monograph of the genus with special reference to chickpea (*Cicer arietinum* L.), its ecology and cultivation. H.Veenman & Zonen N.V. Wageningen.

van der Maesen, L.J.G., 1992. *Cicer arietinum* L., In: van der Maesen LJG,, Somaatmadja S, (eds.). Plant Resources of South-East Asia. No 1. Pulses. Prosea Foundation, Bogor, Pudoc-DLO, Wageningen. pp. 42-4326.

Yoshida, M., S.E. Cowgill, J.A. Wightman, 1995. Mechanism of resistance to *Helicoverpa armigera* (Lepidoptera: Nuctuidae) in chickpea: role of oxalic acid in leaf exudates as an antibiotic factor. J. Econ. Entomol. 88: 1783-1786.