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Changes in Amino Acid Content in Infected Leaves of Spring Barley Plants Resistant to Powdery Mildew at Adult-Plant Stage

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흰가루病에 成體植物抵抗性을 지닌 봄보리의 감염잎에서 아미노산含量의 變化

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ABSTRACT

Ethanol-soluble amino acids in healthy and powdery mildew-infected leaves of the susceptible cultivar Peruvian and the adult-plant-resistant cultivar Asse of spring barley were quantitatively analysed. At 1 day after inoculation, the levels of amino acids in the infected first leaves of the two cultivars were similar to those of comparable healthy controls. During sporulation, increases in amino acids were more pronounced in Peruvian than those in Asse. The changes in amino acid content in the infected first and fifth leaves were closely related to the number of colonies per leaf. The susceptible cultivar Peruvian showed higher amounts of amino acids in infected first and fifth leaves at all infection intensitives than did Asse.

Key words: Erysiphe graminis, barley, adult-plant resistance, amino acids.

要 約

흰가루病에 대하여 感受性인 봄보리品種 Peruvian과 成體植物 抵抗性品種 Asse의 健全잎과 感染잎에 含有되어 있는 에탄올可溶性 아미노산을 分析하였다. 接種 1日 後에는 이들 두 品種의 第1葉內의 아미노산 含量은 健全잎과 비슷한 水準이었다. 胞子形成 동안 Asse보다 Peruvian에서 아미노산의 增加가 뚜렷하였다. 感染된 第1葉, 第5葉에서 아미노산 含量은 葉當 病斑數가 많을수록 增加하였고, 感受性 品種 Peruvian의 感染잎이 Asse보다 아미노산 含量이 더 높았다.

INTRODUCTION

Amino acids are not only substrates for plant pathogens, but may also affect certain metabolic processes capable of producing preformed substances associated with resistance (13). Substantial changes in amino acid content of plants infected by fungi reflect the alteration in various metabolic processes favorable or unfavorable for fungal development. Although there are some reports on changes of amino acid metabolism in tissues infected with biotrophic fungi, the results inconsistent(2,3,10,11,12,14,15).

In previous investigations, qualitative and quantita-

tive differences in resistance to powdery mildew between spring barley cultivars during plant development could be easily detected under controlled conditions on the basis of race specificity, infection type or rate(4) and infection process(5). Amino acid metabolism altered during plant development, possibly with some relation to the appearance of adult-plant-resistance in the barley cultivars tested(8). The inhibitory effect of barley leaf extracts at the fifth-leaf stage was more pronounced than that at the first-leaf stage(6). We also suggested that gene action may be changed during development in barley cultivars differing in resistance to powdery mildew(9).

In contrast to race-specific, mostly hypersensitive type of resistance, there is very little information about the physiological or biochemical mechanisms governing the processes which retard the development and limit the growth of powdery mildew in compatible host-pathogen relationships. The different quantitative levels of resistance in barley cultivars may depend on and affect the metabolic processes within the infected plants. Our previous results also demonstrated that carbohydrate metabolism was less altered in powdery mildew-infected plants of adult-plant-resistant barley cultivars than in susceptible cultivars(7). This paper reports the results of experiments that examined amino acid contents in powdery mildew-infected plants of susceptible and adult-plant-resistant barley cultivars.

MATERIALS AND METHODS

Plant and inoculum. The spring barley (*Hordeum vulgare* L.) cultivars used were Peruvian which is susceptible to powdery mildew at all plant-growth stages, and Asse which is susceptible at the early leaf stages but resistant at the late leaf stages. To avoid any contamination by powdery mildew, the plants were grown in cases (130x90x130 cm) made of glass and muslin in a greenhouse. The conditions for cultivation of plants were as follows: (i) illumination by 8,000 lux (400 W HQL lamps) for 16 h of light/day from 6 a.m. to 10 p.m., (ii) temperature maintained at 18±1°C during the light period and 15±1°C during darkness and (iii) relative humidity 60±10%/85±10% (day/night). Plants for experiments were grown in plastic pots (13x13x13 cm) containing sterilized compost soil mixed with the fertilizer Hakaphos (7 g per 20 kg compost). During cultivation of the plants no more fertilizer was applied.

Plants at the first- and fifth-leaf stages were inoculated by uniformly spraying a conidial suspension of *Erysiphe graminis* f.sp. *hordei* Marchal (race C_{17} Amsel) in FC 43 (Fluorinert Electronic Liquid, Commercial Chemicals Division/3M, St. Paul, Minnesota) on the abaxial surface of the leaves. The same inoculum density was sprayed on the seedling leaves of both cultivars. As a result, the leaf areas infected were about 40% for Peruvian and about 20% for Asse. The plants showing different colony numbers after inoculation (Fig. 2) were selected for determining the effect of infection intensities on the amino acid level of barley plants at the first- and fifth-leaf stages.

Analysis of amino acids. One gram fresh weight of leaf tissue at the required stages of development was harvested at 11 a.m., cut into 1-cm segments and boiled in 20 ml of 80% (v/v) ethanol (three changes).

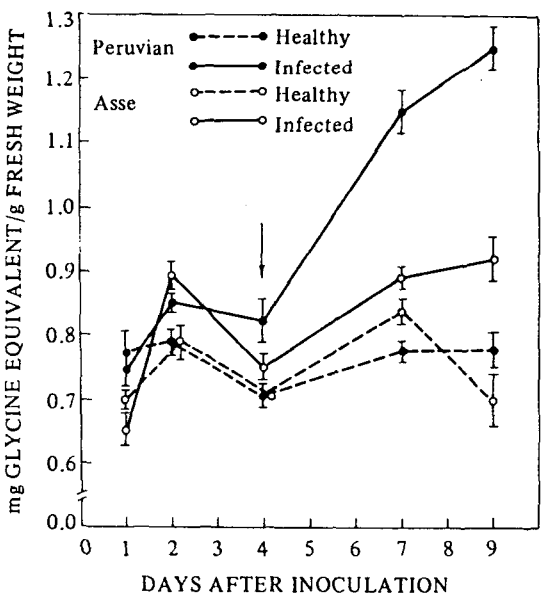

Fig. 1. Contents of ethanol-soluble amino acids in healthy and powdery mildew-infected first leaves of spring barley cultivars (Peruvian= susceptible, Asse=adult-plant-resistant) at intervals after inoculation at the first-leaf stage. Each value represents a mean ± one standard deviation of three replicate samples. Arrows indicate the beginning of colony formation. The leaf areas infected were about 40% in Peruvian and 20% in Asse.

Prior to sampling, the conidia and mycelium produced were carefully removed by brushing the infected leaves. The pigments were removed from the ethanol extracts by shaking in 50 ml of 80% ethanol with 30 ml petroleum benzine. Total amino acids were determined by the method of Yemm and Cocking (16) using glycine as a standard.

RESULTS AND DISCUSSION

The experiments were done using barley plants at first-and fifth-leaf stages, because mature plants at later growth stages could not be uniformly infected by artificial inoculation of *E. graminis*. Our previous studies demonstrated that when using these inoculation methods, adult-plant resistance of spring barley to powdery mildew can be detected and estimated at the third or fifth-leaf stage (4,5).

Figure 1 presents the amounts of total soluble amino acids in healthy and infected first leaves of the susceptible cultivar Peruvian and the adult-plant-resistant cultivar Asse at intervals after inoculation of *E. graminis*. In healthy leaves, the amount of total soluble amino acids was similar in Peruvian and Asse throughout the experiment. At 1 day after inoculation, the levels of amino acids in the infected leaves of the two cultivars were similar to those of comparable healthy controls. As the fungal structure became established on the leaf surface, the soluble amino acids began to increase. During sporulation at 7 and 9 days after inoculation, increases in amino acids were more pronounced in Peruvian than those in Asse.

Amino acids are of central importance in the metabolism of plants, principally because they are required as building blocks for protein biosynthesis. The increase in amino acids in infected leaves is likely to be related to the stimulation of protein synthesis or the rapid decomposition of proteins. The occurrence of continuous protein turn-over in plants involves a continuous requirement for new amino acid biosynthesis, since the amino acids from the degraded proteins are further broken down and not returned to the synthetic pool(1).

At the seventh day after inoculation, the amounts of total soluble amino acids in the infected first and fifth leaves were related to the number of colonies per leaf (Fig. 2). The susceptible cultivar Peruvian showed higher amounts of amino acids in infected first-and fifth-leaves at all infection intensities than did Asse.

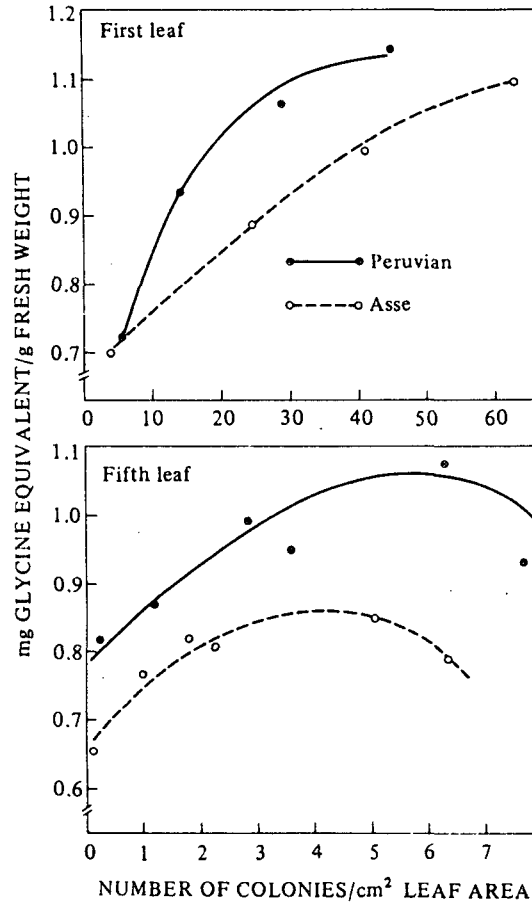

Fig. 2. Contents of ethanol-soluble amino acids in healthy and powdery mildew-infected first and fifth leaves of spring cultivars (Peruvian= susceptible, Asse=adult-plant-resistant) on the 7th day after inoculation at the first-and fifth-leaf stages, respectively. Each value represents a mean of three determinations.

These findings suggest that in susceptible cultivars the metabolic activity in infected plants is markedly promoted in the direction favorable for powdery mildew development by the increased activity of enzymes when compared with adult-plant-resistant cultivars. The less impairment of amino acid metabolism of adult-plant-resistant cultivars by powdery mildew may contribute to the higher yields of barley.

REFERENCES

1. BIDWELL, R. G. S., BARR, R. A. & STEWARD, F. C. (1964). Protein synthesis and turn-over in cultured plant tissue: sources of carbon for synthesis and the fate of the protein breakdown products. *Nature* 203:367-373.
2. FRIC, F. (1964). Veränderungen in Kohlenhydrat und Stickstoffmetabolismus in Gerstenblättern im Verlauf der Infektion mit dem obligaten Parasiten *Erysiphe graminis* f. sp. *hordei* Marchal. *Biologia* 8:597-610.
3. HÜMME, B. (1979). Untersuchungen des Amino-N-und Zucker-Effluxes aus Blattgewebe von *Phaseolus vulgaris* nach Infektion mit *Uromyces phaseoli* sowie die Charakterisierung und Wirkung eines "Elicitors" aus Keimschlauchwänden der Uredosporen des Bohnenrostes. *Dissertation Göttingen.*
4. HWANG, B. K. & HEITEFUSS, R. (1982). Characterization of adult plant resistance of spring barley to powdery mildew (*Erysiphe graminis* f. sp. *hordei*). I. Race specificity and expression of resistance. *Phytopathol. Z.* 104:168-178.
5. HWANG, B. K. & HEITEFUSS, R. (1982). Characterization of adult plant resistance of spring barley to powdery mildew (*Erysiphe graminis* f. sp. *hordei*). II. Infection process at different leaf stages. *Phytopathol. Z.* 104:179-190.
6. HWANG, B. K. & HEITEFUSS, R. (1982). Antifungal compounds in leaves of spring barley in association with adult plant resistance to powdery mildew (*Erysiphe graminis* f. sp. *hordei*). *Phytopathol. Z.* 104:272-278.
7. HWANG, B. K. & HEITEFUSS, R. (1986). Sugar composition and acid invertase activity in spring barley plants in relation to adult-plant resistance to powdery mildew. *Phytopathology* (in press).
8. HWANG, B. K., IBENTHAL, W. D. & HEITEFUSS, R. (1983). Age, rate of growth, carbohydrate and amino acid contents of spring barley plants in relation to their resistance to powdery mildew (*Erysiphe graminis* f. sp. *hordei*). *Physiol. Plant Pathol.* 22:1-14.
9. HWANG, B. K., WOLF, G. & HEITEFUSS, R. (1982). Soluble proteins and multiple forms of esterases in leaf tissues at first and flag leaf stages of spring barley plants in relation to their resistance to powdery mildew (*Erysiphe graminis* f.sp. *hordei*). *Physiol. Plant Pathol.* 21:367-372.
10. RAGGI, V. (1974). Synthesis of free ^{14}C-amino acids in rusted bean (infected and noninfected leaves). *Phytopathol. Z.* 81:262-272.
11. RAGGI, V. (1974). Free and protein amino acids in the pustules and surrounding tissues of rusted bean. *Phytopathol. Z.* 81:289-300.
12. SADLER, R. & SCOTT, K. J. (1974). Nitrogen assimilation and metabolism in barley leaves infected with the powdery mildew fungus. *Physiol. Plant Pathol.* 4:235-247.
13. SCHÖNBECK, F. & SCHLÖSSER, E. (1976). Preformed substances as potential protectants. In *Physiological Plant Pathology,* Ed. by R. Heitefuss & P. H. Williams, pp. 653-678. Springer-Verlag, Berlin.
14. SEMPIO, C., TORRE, G. D., FERRANTI, F., BARBERINI, B. & DRAOLI, R. (1975). Defence mechanism in beans resistant to rust. *Phytopathol. Z.* 83:244-266.
15. SUBRAHMANYAM, P., GOPAL, G. R., MALAKONDAIAH, N. & REDDY, M. N. (1976). Physiological changes in rust infected groundnut leaves. *Phytopathol. Z.* 87:107-113.
16. YEMM, E. W. & COCKING, E. C. (1955). The determination of amino acids with ninhydrin. *The Analyst* 80:209-213.