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Starch Content in Leaves of Spring Barley in Relation to Adult Plant Resistance to Powdery Mildew (*Erysiphe graminis* f. sp. *hordei*)

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흰가루病에 對한 봄 보리의 成體植物 抵抗性과 葉內 澱粉含量의 關係

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ABSTRACT

Starch contents in healthy and powdery mildew-infected spring barley leaves at first and fifth leaf stages were measured at different intervals after inoculation. Different patterns of starch accumulation in the susceptible cultivar Peruvian and the adult plant resistant cultivar Asse were found in infected first and fifth leaves. At the early phase of infection, the amount of starch in infected first leaves was slighlty changed in both cultivars. During colony development and sporulation, the decrease in starch content was more marked in the susceptible Peruvian, whereas the amounts of starch in the adult plant resistant Asse increased during colony development and then decreased sharply before and after sporulation. In heavily infected first leaves, the susceptible cultivar Peruvian showed a drastic decrease in starch content. In the adult plant resistant cultivar Asse, the higher amount of starch retained in these infected leaves, regardless of infection intensity. The regulation of starch accumulation in mildewed barley plants at different leaf stages was discussed in relation to adult plant resistance of spring barley.

INTRODUCTION

Powdery mildew, which is caused by *Erysiphe graminis* f. sp. *hordei*, is one of the most important limiting factors in barley production in barley-growing countries. In recent years interest has increased in the possibility of improving the stability of disease resistance in barley cultivars. Increased emphasis has been placed on cultivars which possess a stable, durable type of resistance, in spite of shifts in races of powdery mildew fungus, or which may reduce the selection pressure for new races. Slow-mildewing and/or adult plant resistance which is distinctly expressed on the plants at late develop mental stages may be a desirable type of resistance. It has been reported in several cases for *Erysiphe graminis* and its cereal hosts[3,4,6,7,8,9,14,16,18].

Adult plant resistance of spring barley to powdery mildew was characterized in detail on the basis of

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mildew development and plant metabolism at various leaf stages[5].

Starch is one of the major products of photoassimilation and a major form of stored energy in plants. Starch accumulation is most active in plant leaves when demands for essential cell reactions are met and the cell is in a condition of energy excess. Turnover of starch and its reutilization has been studied in a number of fungus-plant interactions. Starch breakdown occurs in the presence of pathogen enzymes that catalyze its hydrolysis to glucose which then can be utilized as a source of carbon. Starch accumulates in plants infected by biotrophic fungi. Usually, the starch content decreases around infection sites soon after infection, increases before and during sporulation and then decreases sharply after sporulation[11]. The importance to the fungus of starch accumulation is not known. It could be used by the fungus for late sporulation. Little information is availale on a role of starch accumulation to the expression of resistance to powdery mildew at late developmental stages of barley plants.

The purpose of this report was to study changes in starch accumulation in infected first and fifth leaves of barley cultivars susceptible and adult plant resistant to powdery mildew.

MATERIALS AND METHODS

Barley and Inoculum

The spring barley cultivars used were (i) Peruvian, susceptible at all of the plant growth stages and (ii) Asse, susceptible at the early leaf stage, but resistant at the late leaf stage. Environmental conditions for cultivation of plants and mildew fungus are described earlier[5].

Plants at 1st and 5th leaf stages were inoculated by uniformly spraying a conidial suspension of *Erysiphe graminis* f. sp. *hordei*(C_{17} Amsel) in FC43 (Fluorinert Electronic Liquid, Commercial Chemicals Division/3M, St. Paul, Minnesota, USA). One m*l* of conidial suspension(ca. 7.10^4~21.10^4 conidia/m*l* FC 43) was sprayed on the 1st leaves of both cultivars. As a result, the leaf areas infected were ca, 40% for Peruvian and ca. 20% for Asse. At the 5th leaf stages, the infected leaves with different number of colonies were selected for starch analysis. The infection types for Peruvian were 4 and 2~3, and for Asse, 4 and 2 at the 1st and 5th leaf stages, respectively. Uninoculated plants were used as control.

Starch Determination

One gram fresh weight of first or fifth leaf blades from both healthy and infected plants at 1st and 5th leaf stages was harvested at 11AM, cut into 1-cm segments, and boiled in 80% (v/v) ethanol (three changes) to remove chlorophyll and soluble sugars. The extracted leaf sections were placed in 100m*l*-Erlenmeyer flasks. The starch remaining in the leaves was then hydrolyzed with 15m*l* of 52% perchloric acid for 20h on a shaker(40rpm, amplitude= 5cm) at room temperature[11]. Two-milliliter samples were taken and neutralized with an equal volume of 9N NaOH. Glucose concentrations were assayed by the arsenomolybdate method[13].

RESULTS AND DISCUSSION

Figure 1 shows the changes in starch content of first leaves in the susceptible cultivar Peruvian and the adult plant resistant cultivar Asse during infection with *E. graminis* f. sp. *hordei*. Until 2 days after inoculation, the amount of starch in infected leaves was slightly changed as compared to those of healthy leaves. As mycelial development became visible, the regulation of starch accumulation in infected leaves were different in both cultivars, Peruvian and Asse. During colony development and sporulation, the decrease in starch content was more marked in the susceptible Peruvian, whereas the amounts of starch in the adult plant resistant Asse increased sharply on the 4th day after inoculation and then decreased sharply until 9 days after inoculation.

The evidence presented indicates that starch accu mulates in barley leaves infected with *E. graminis* f. sp. *hordei*. The results show that starch accumulations in infected seedling leaves were differently regulated in both susceptible and adult plant resistant cultivars. After sporulation, starch decreased in both cultivars. In particular, the susceptible cultivar

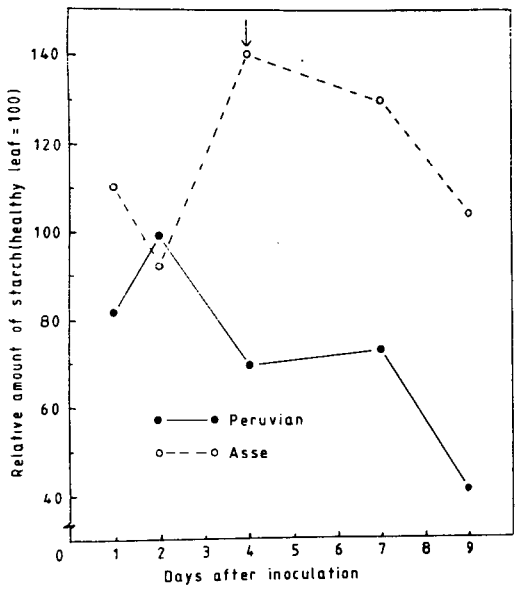

Fig. 1. Relative amount of starch in powdery mildewinfected first leaves of spring barley cultivars(Peruvian=susceptible, Asse=adult plant resistant) at intervals after inoculation. Arrows indicate the beginning of colony formation. On the 9th day after inoculation, the leaf areas infected were ca. 40% in Peruvian and ca. 20% in Asse.

Peruvian showed a drastic decrease in starch accumulation. The actual timing of starch accumulation in tissue during the infection process seems to vary with the host genotypes. In fungal diseases starch has been found to accumulate during vegetative growth of the fungus and then decrease during sporulation[1,10,11,12]. Accumulation of metabolites in infected tissues has been shown to be an active process in biotrophic fungi such as rust and powdery mildew[2,15,17]. It probably accounts for the carbohydrates accumulated as starch or soluble sugars and for other metabolites required for fungal development. The fact that there is a marked decline of starch content in the infected susceptible Peruvian may suggest a drastic exhaustion of starch for rapid fungal development by accelerated carbohydrate catabolism in susceptible cultivars. In contrast, the infected barley cultivars having adult plant resistance retained most of the accumulated starch in infected leaf tissue and the starch probably is slowly utilized through conversion to glucose, as an energy source, for fungal development and sporulation.

Different patterns of starch accumulation in the susceptible Peruvian and adult plant resistant Asse were found in infected first and fifth leaves at different infection intensities(Fig. 2). At low infection intensity on first leaves, the cultivar Peruvian showed a slight increase in starch content, but at higher infection intensity, starch decreased sharply In the cultivar Asse, starch contents in infected first leaves were 1.3 times greater than that of healthy controls, regardless of infection intensity. In the cases of infected fifth leaves, the cultivar Peruvian showed a slight increase similar to the cultivar Asse at low infection intensity, whereas at higher infection intensity its starch contents decreased sharply. In the cultivar Asse, starch contents in infected fifth leaves increased slightly at higher infection intensity

These results indicate that the starch accumulated in heavily infected tissue of first or fifth leaves may be greatly degraded into hexoses to utilize energy for fungal growth in susceptible cultivars relativ

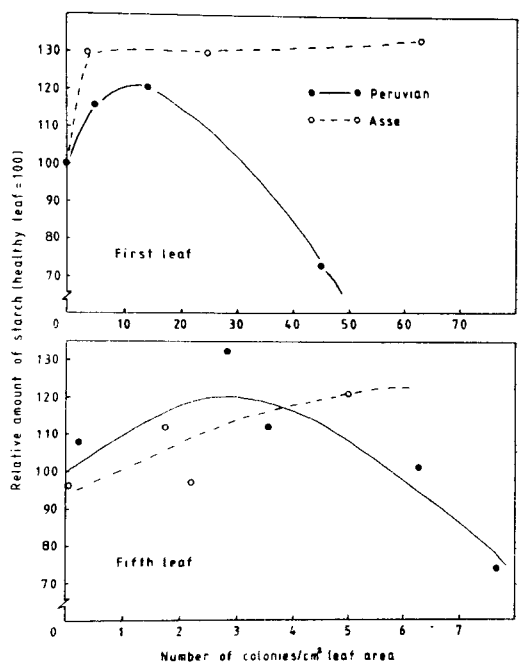

Fig. 2. Relative amounts of starch in powder mildew-infected first and fifth leaves c spring barley cultivars(Peruvian=susceptibl Asse=adult plant resistant) at differer infection intensities on the 7day after inoculation.

to adult plant resistant cultivars. Hwang[5] reported that the increase of glucose and fructose at higher infection intensities was more pronounced in Peruvian, whereas a higher amount of sucrose accumulated in Asse. The conspicuous increase of hexoses in the infected susceptible Peruvian may be supported by an increased degradation of starch. In contrast, the continuous retention of starch accumulated in infected leaves of the adult plant resistant Asse could be a reflection of less impairment of carbohydrate metabolism and less utilization of nutrients by the fungus itself. As a result, the accumulation of higher amount of starch in infected plants of adult resistant cultivars may contribute to the ultimate higher yields of barley.

摘 要

봄보리 1.5葉期에 흰가루 病菌을 接種하여 病進展에 따른 葉內澱粉含量變化를 測定하였다. 1.5葉期에 接種하였을 때 感受性 品種 Peruvian과 成體植物抵抗性 品種 Asse에서 相異한 澱粉蓄積樣相을 보였다. 感染初期에서는 두 品種 모두 澱粉含量에 다소 變化가 있었다. 病斑形成과 胞子形成 동안 感受性 品種 Peruvian에서 澱粉含量減少가 뚜렷하였으나 Asse에서는 病斑形成 동안 增加하였고 胞子形成前後에 급격히 減少하였다. 感染이 甚한 1.5葉에서 Peruvian은 급격한 澱粉減少를 보였으나 Asse는 感染强度에 관계 없이 感染잎에 높은 澱粉含量을 유지하였다. 봄 보리의 成體植物抵抗性과 연관시켜 相異한 發育時期에서 흰가루병 感染된 잎의 澱粉蓄積役割을 論하였다.

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