Inhibition of Hypersensitive Necrosis by Ascorbic Acid Supply in Powdery-mildewed Barley Leaves

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オオムギうどんこ病における過敏感え死のアスコルビン酸処理による阻害

In the hypersensitive necrosis of powdery-mildewed barley leaves, the fluorescent appearance in mesophyll cells was induced by the fungal infection and followed by mesophyll browning¹⁾. Both fluorescent appearance and browning in mesophyll cells were effectively inhibited by the glucose supply, and the fungal development was promoted in parallel with increasing inhibition rate of these reactions²⁾. These results suggested that the fluorescent or browning materials produced in mesophyll cells restricted the development of fungi after the establishment of penetration.

Thus, the purpose of the present study was to elucidate which reaction of fluorescing or browning in barley mesophyll cells had a critical role in the restricted development of an incompatible race of the powdery mildew fungus. Therefore, the effect of ascorbic acid on the mesophyll browning and fungal development in barley leaves was examined at various stages of infection since a number of studies have demonstrated that the browning reaction in plant tissues was caused by the oxidation of phenolic compounds³⁾ and the oxidation was suppressed by the administration of reducing agents such as ascorbic acids⁴⁾.

The resistant cultivar of barley (*Hordeum vulgare* L.), Turkey 290, was grown in a modified Hoagland solution⁵⁾ and inoculated with conidia of *Erysiphe graminis hordei*, race I, under the same conditions as described previously⁶⁾. The water-culture solution was replaced with ascorbic acid solution (2.0, 5.0 and 7.5 mg/ml water) adjusted to pH 7.0 with 0.1 M phosphate buffer at the various days before or after inoculation, and the renewal of the solution was conducted every day until observation. Fluorescent microscopic observation of fluorescent appearance in mesophyll cells or light microscopic observation of mesophyll browning and fungal development was carried out according to the procedure reported previously¹⁾.

The effect of ascorbic acid supply on the fluorescing or browning of mesophyll cells was first examined in barley seedlings supplied with 7.5 mg/ml ascorbic acid one day after inoculation (Fig. 1), since it was known that the appearance of fluorescence and browning in mesophyll cells were found 2 and 3 days after inoculation,

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Fig. 1. Effect of ascorbic acid supply on the fluorescent appearance (—○—) and browning (—●—) of mesophyll cells of barley primary leaves of resistant cultivar, Turkey 290, inoculated with *E. graminis hordei*, race I. A : Control (non-supply), B : Ascorbic acid (7.5mg/ml) was supplied one day after inoculation.

respectively¹⁾. In this treatment, the fluorescent appearance was not inhibited, but the browning of fluorescent mesophyll cells was effectively deduced. The effect of the supply on mesophyll browning was further examined by changing the concentration of ascorbic acid solution and the time of supply (Fig. 2). The most inhibitory effect on mesophyll browning was observed in the treatments with 5.0 and 7.5 mg/ml ascorbic acid one day before inoculation, and their inhibition rates were more than 70 and 80 %, respectively, while the effective inhibition was not found in the treatment with 2.5 mg/ml ascorbic acid at the same periods. Although the similar inhibition pattern was recognized when treated 2 days after inoculation, the rates were not so high as that in above treatemnts. The inhibitory effect was not almost observed in any treatment 3 days after inoculation. On the other hand, the fluorescent appearance was not inhibited by any treatment.

The effect of ascorbic acid supply on the fungal development was examined (Table 1). The area of fungal pustule was measured 6 days after inoculation as the criterion of the fungal development. The fungal development was not promoted even when the browning reaction in mesophyll cells was effectively inhibited by those treatments. On the other hand, none of the treatments in this experiment affected the rate of formation of primary haustoria, and the supply did not prevent the growth of the fungi in the compatible host-parasite combinations.

In the resistant barley leaves, Turkey 290, inoculated with *E. graminis hordei*, race I, 15-20 % of inoculated conidia could invade into epidermal cells, form the mature haustoria and elongate the secondary hyphae. The fungi elongating secondary hyphae were accompanied by the hypersensitive necrosis in mesophyll cells and the

Fig. 2. Effect of ascorbic acid supply on the mesophyll browning of barley primary leaves of resistant cultivar, Turkey 290, inoculated with *E. graminis hordei*, race I. Ascorbic acid was supplied one day before inoculation (A), and 2 and 3 days after inoculation (B and C, respectively).

—○— : non-supply
—■— : 2.5 mg/ml ascorbic acid
—▲— : 5.0 mg/ml ascorbic acid
—▼— : 7.5 mg/ml ascorbic acid

Table 1. Effect of ascorbic acid supply on the fungal development of *E. graminis hordei*, race I, on barley primary leaves of resistant cultivar, Turkey 290

Time of supply	Area (mm ²)* of fungal pustule in ascorbic acid supply			
	Concentration (mg/ml)			
	0	2.5	5.0	7.5
Non-supply (control)	0.66	—	—	—
Before inoculation				
1 day	—	0.073	0.065	0.077
After inoculation				
1 day	—	—	—	0.069
2 days	—	0.060	0.065	0.064
3 days	—	0.060	0.052	0.055

* The area was measured 6 days after inoculation.

fungal development was finally restricted. The fluorescing of mesophyll cells in the hypersensitive necrosis was successfully inhibited and the fungal development was also promoted by the glucose supply²⁾. However, the promotion of the fungal development was not almost detected when only the mesophyll browning was effectively inhibited by the ascorbic acid supply. These results suggest that the fluorescent compounds had a restrictive function to the fungal development.

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