Irreversibility of Primary Host Cell Recognition of Pathogen as Demonstrated by Yellowing Response of Barley Leaves to Races of *Erysiphe graminis*

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**Introduction**

Primary event in plant-parasite interaction is most likely the recognition by host cell of microbe as either an associable or a foreign entity. When a microbe was recognized as a foreign entity and was eventually rejected by plant cells, the microbe is nonpathogenic. On the contrary, when plant cells failed to recognize a particular microbe as foreign entity, the organism may establish infection on the plant.

In the previous papers, we coined the term “accessibility” to connote the whole process of this primary recognition and subsequent conditioning toward acceptance (accessibility) or rejection (inaccessibility) and inferred that cells with induced accessibility might be unable to recognize an incompatible race or nonpathogen as foreign entity, while those with induced inaccessibility recognize a primarily compatible race as an entity with which they cannot associate.

In this communication we will describe yellowing response of barley leaves which had been double-inoculated with races of different compatibilities, as a supportive evidence for the above inference.

**Material and Method**

*Plant*: Two cultivars of barley (*Hordeum vulgare* L.), Heil’s Hanna and Yane Hadaka, and one cultivar of common wheat (*Triticum aestivum* L.) Thell. ssp. *vulgare* (Vill.)M. K., Nôrin 4, were used. Seeds of these cultivars were soaked in tap water overnight and germinated on filter paper in a petri dish. Germinated seeds of a uniform size were sown in vermiculite in a plastic container and incubated in a phytotron controlled at 20°C under natural light condition for 10–12 days.

*Fungus*: Races of *Erysiphe graminis* D.C. f. sp. *hordei* Marchal, Hh 4 (compatible with Heil’s Hanna) and Race 1 (compatible with Yane Hadaka and incompatible with Heil’s Hanna), and race 2 of *E. graminis* D.C. f. sp. *tritici* Marchal were cultured on leaves of respective compatible cultivars. For partial synchronization of inoculum conidia, leaves of 10–12 day-old seedlings were inoculated with fresh conidia, incubated at 20°C in a growth chamber illuminated with artificial lamps (Toshiba Plant-lux, 2000 lux) for 8 days, and old conidial chains were blown away, and fresh conidia emerged during subsequent 12 hr were used as inoculum.

*Inoculation*: Leaves of 10 day-old seedlings were inoculated at the middle part of the lower surface with partially synchronized compatible or incompatible conidia by employing a soft hair brush. The first inoculum was removed by rubbing with a wet cotton

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ball 36 hr after inoculation and the same site was inoculated with the second compatible or incompatible race. The seedlings were then incubated at 20 °C in an artificial lamp-illuminated phytotron (12 hr light, 12 hr dark) for 4 days.

Estimation of responses: Yellowing response of and colony development on the inoculated leaves were estimated 4 days after challenge inoculation. The yellowing host reactions were graded into four classes as follows: '0' represents no yellowing; '1' scarcely perceptible yellowing; '2' apparent yellowing; '3' extensive yellowing accompanied by partial chlorosis. Colony development was also graded into four degrees: '0' represents no visible colony; '1' few scattered thin colonies; '2' abundant colonies, yet thin comparing with those of '3'; '3' thick colonies cover whole inoculated area.

Result

Colony development of the fungus and yellowing or chlorotic response of leaves which had been double-inoculated with races of different compatibilities were summarized in Table 1.

Table 1. Yellowing response of and colony formation on barley leaves as determined by the primary recognition of pathogens.

<table>
<thead>
<tr>
<th>Infection Program</th>
<th>Heil's Hanna&lt;sup&gt;1&lt;/sup&gt;-Hh 4 (S)&lt;sup&gt;2&lt;/sup&gt; : Race 1(R)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Yanehadaka&lt;sup&gt;1&lt;/sup&gt;-Race 1(S)&lt;sup&gt;2&lt;/sup&gt; : t2(R)&lt;sup&gt;3&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>1st</td>
<td>2nd</td>
<td>1st</td>
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<tr>
<td>None - S</td>
<td>0 - 1</td>
<td>0</td>
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<tr>
<td>None - R</td>
<td>0 - 1</td>
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<td>S - R</td>
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<td>S - S</td>
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<td>R - None</td>
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<td>R - S</td>
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<td>R - R</td>
<td>2</td>
<td>0</td>
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</table>

1) Host
2) Compatible race
3) Incompatible race
4) Challenger race was applied 36 hr after primary race, and responses were estimated 4 days after challenge inoculation.

It is apparent that in both cultivar-race combinations no appreciable colony was formed on leaves which had been induced to become inaccessible by an incompatible race. On the contrary, leaves which had been primarily inoculated with a compatible race more or less supported the growth of the challenger fungus irrespective of its compatibility to the leaves, even though the degree of colony development depended on their intrinsic affinity. It is worthy of mentioning here that colony development on leaves both induced and challenged by a compatible race was much more extensive comparing with those subjected to single inoculation with a compatible race. Leaves inoculated with compatible race as inducer cannot be compared here because the inoculum had been removed. Inoculation test indicated that colonies on leaves primarily inoculated with a compatible race and challenged with an incompatible race had apparently contained the incompatible conidia. This is consistent with the idea that accessibility had been induced
by the compatible race.

The presence or absence of yellowing response seems to be determined by genetically destined intrinsic affinity between the host and parasite on one hand, and inoculum density on the other. Leaves subjected to a single inoculation did not show any yellowing or slight if ever detected. Heavily inoculated leaves, however, tended to exhibit a slight yellowing even in the single inoculation. Double inoculation, on the contrary, resulted in yellowing especially when an incompatible race was the inducer. When compatible race was inoculated as inducer, only those leaves which had been challenged with an incompatible race responded with yellowing. Leaves that had been induced by an incompatible race responded with an extensive yellowing accompanied by chlorosis on challenge inoculation with a compatible race. The same leaves exhibited a milder yellowing upon challenge inoculation with an incompatible race.

Discussion

Primary inoculation with an incompatible race induced in barley leaves a sensitivity to respond with yellowing against either a compatible or an incompatible race upon challenge inoculation. Preliminary infection by a compatible race, on the other hand, rendered leaves to respond with no or scarcely visible yellowing upon challenge inoculation with compatible or incompatible race. Single inoculation with either race caused no or scarcely perceptible yellowing. Although the extensive yellowing and no visible colony formation on leaves which had been induced by an incompatible race suggest that yellowing or chlorotic response might be an expression of resistance to powdery mildew and in fact Benada\textsuperscript{13} reported a chlorotic spots associated with resistance in barley leaves, the yellowing reaction dealt in this work is essentially an end result of cellular recognition and conditioning and could not be directly related to resistance expression. Nevertheless the results support the idea that the primary recognition and subsequent cellular conditioning toward accessible or inaccessible state become irreversible within short period of time after inoculation and determine a unidirectional cellular response to the fungus that comes later\textsuperscript{3}).

The accessibility-induced leaves showed a slight yellowing but supported the growth of incompatible race. Thus the compatible inducer race should have suppressed the ability of host cell to reject incompatible race, or at least in this experiment, to respond with yellowing against an incompatible race, on challenge inoculation. The results therefore seem to support the concept that induction of accessibility is an active process\textsuperscript{4, 8, 10}.

If accessibility induction was a passive process and compatible race had just damaged invaded cells to the extent that the cells cannot reject the incompatible challenger, the leaves which had been inoculated with incompatible race should also support the growth of challenger, because ultrastructure work revealed much extensive damage in cells with induced inaccessibility than in cells with induced accessibility (Ouchi et al., unpublished data). As elucidated by double or triple inoculation\textsuperscript{3}, accessibility-induced cells are capable of holding an obligate parasitic relation with incompatible challenger, hence should be living at least during the early phase of interaction with the challenger. The effect of inhibitors of protein synthesis also supports the concept of active process for accessibility induction\textsuperscript{13}. Furthermore the fact that even the leaves with induced inaccessibility exhibited a slight yellowing, but hypersensitively responded with yellowing on challenge inoculation, suggests that the primary inducer had just conditioned the cells to inaccessible state, but not to cellular degeneration. Question naturally follows would be why the inaccessibility-induced leaves responded more extensively to primarily compatible race.
than to incompatible race. Localization of induced accessibility and inaccessibility seems
answer the question\(^1\). The cellular conditioning toward inaccessibility was localized so
that incompatible challenger is likely rejected at the penetrated site by the intrinsic ability
of non-induced cells, while compatible challenger race is able to establish infection at
the same non-induced site and grows until the effect of challenger is recognized by the
inaccessibility-induced cells. Ultrastructure study supports this interpretation (Ouchi et al.,
unpublished data). Localization of induced resistance\(^1,3\) and susceptibility\(^1\) has been
reported in some other obligate parasitic diseases. Abnormal sensitivity to compatible race
was also induced in cells located closely to the necrosis caused by an incompatible race in
bean anthracnose\(^1\).

The mechanism of yellowing is another interesting points worthy of unravelling. Both
the phytoalexin production in barley leaves in response to powdery mildew infection\(^9\),
and the injurious effect of a phytoalexin on membrane system\(^12\) suggest that phytoalexin
would possibly participate in this whole process of degeneration.

Acknowledgment

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tion Grant 936004.

Summary

Although barley leaves inoculated singly with races of Erysiphe graminis hordei
exhibited no or slight yellowing, they responded with marked yellowing on double
inoculation, especially when an incompatible race was used as inducer (conditioner) of
reactions. Leaves conditioned by an incompatible race responded much extensively to a
compatible challenger than to an incompatible challenger race. No visible colonies were
formed on leaves hypersensitively responding with yellowing. More colonies were formed
on leaves which had been both induced and challenged by a compatible race than on
those singly inoculated with the compatible race. These results were discussed in relation
to the primary recognition and subsequent cellular conditioning in the host-parasite
interaction.

Literature

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うどんこ病菌接種オオムギ葉の黄化にみられる
宿主細胞の病原菌初期認識の不可逆性

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うどんこ病菌の親和性または非親和性レースを単独接種したオオムギ葉では、若干の黄化が認められることがあるが、通常著しい変化はない。しかしながら、二重接種で、非親和性レースを誘導菌とした場合には、第二次接種後顕著な黄化がみられ、特に第二次接種菌が親和性レースの場合に著しい。黄化した葉にはコロニー形成が認められないが、親和性レースで誘導し親和性レースを後接種した葉においては、親和性レースの単独接種葉よりもコロニー形成が顕著であった。これらの結果を宿主細胞による病原菌の初期認識ならびに細胞内調節との関連において論議した。