

The Formation of Elongating Secondary Hyphae of *Erysiphe graminis* and the Segregation of *Ml* Genes in Barley

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ABSTRACT

Barley plants grown from seed derived from heterozygous parent plants with genotypes *Mla mla*, *Mlg mlg*, *Mlp mlp*, or *Mlk mlk* were inoculated with culture CR3 of *Erysiphe graminis* f. sp. *hordei*. Barley plants from homozygous-recessive and homozygous-dominant parents were also inoculated and used as controls. Inoculated plants were held under environmental conditions known to favor parasite development. The percentages of conidia that produced elongating secondary hyphae (ESH) on individual plants were recorded 26 to 28 hr after inoculation. The genotype of each progeny plant was determined by the segregation of disease reaction of its progenies. The range of the per cent ESH was greater on homozygous progeny derived from plants heterozygous for an *Ml* gene than on homozygous progeny derived

from homozygous plants. The per cent ESH ranged from 45 to 85 on homozygous-recessive progenies derived from heterozygous parent plants. The per cent ESH ranged from 70 to 85 on homozygous-recessive progenies derived from homozygous parent plants. The per cent ESH on plants homozygous-dominant for an *Ml* gene was also dependent on the genotype of the parent plant. For example, the per cent ESH formed on *Mla Mla* plants ranged from 0 to 15 when the parent plants were homozygous *Mla Mla*, and 0 to 40 when the parent plants were *Mla mla*. The results suggested a carry-over effect from heterozygous parent plants to homozygous progenies.

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A defined system for examining the early infection process of *Erysiphe graminis* f. sp. *tritici* on wheat and *E. graminis* f. sp. *hordei* on barley has been reported (2). The development of the fungus was shown to consist of stages that can be distinguished by changes in morphology and differential sensitivity to various environmental conditions. With the appropriate environmental conditions, the synchrony of morphogenesis can be increased and the percentage of successful infections can be maximized.

Elongating secondary hyphae (ESH) have been used as an indication of the establishment of compatible, functional host-parasite relationships, since the formation of elongating secondary hyphae is completely dependent on the formation of haustoria in the host epidermal cells (2).

The objectives of this study were to examine the action of genes for incompatibility in the parasite and host on the sequential development of the parasite, and to determine the relationship between the percentage of elongating secondary hyphae to the segregation of genes for incompatibility in the host.

MATERIALS AND METHODS.—*Culturing of powdery mildew.*—*Erysiphe graminis* DC. Merat f. sp. *hordei* em. Marchal culture CR3 was maintained on susceptible barley (*Hordeum vulgare* L. 'Manchuria'). Barley plants were grown in 4-inch pots and were

inoculated when they were 6 to 7 days old. We inoculated sets of barley plants daily by dusting conidia produced 7 days after inoculation onto the leaves. Inoculated plants were maintained in a controlled environment chamber provided with adequate air circulation (5). Conidia for experimental uses were abundant by 6 days after inoculation.

Lines of barley used and designation of genotypes.—Manchuria barley was used as the standard to which other lines were compared. Four other lines, each possessing a different gene affecting mildew development, were back-crossed to the cultivar Manchuria. The four near-isogenic lines and their derivations were as follows: *Mla* (Algerian C.I. 1179 \times ${}^4_{14}$ Manchuria C.I. 2330), *Mlg* (Goldfoil C.I. 928 \times ${}^4_{14}$ Manchuria C.I. 2330), *Mlp* (Psaknon C.I. 6305 \times ${}^4_{14}$ Manchuria C.I. 2330), *Mlk* (Kwan C.I. 1016 \times ${}^4_{14}$ Manchuria C.I. 2330). The symbol " \times ${}^4_{14}$ " refers to the original cross with Manchuria, three generations of backcrossing to Manchuria, and then selfing the heterozygous progeny in each of several generations. After 12 to 14 generations of selfing, homozygous-dominant and homozygous-recessive lines were selected. These lines were nearly isogenic to each other and are referred to as paired lines. Heterozygous plants were also selected. The barley cultivar Manchuria contains no known major genes affecting

TABLE 1. Gene designations for barley lines

| Actual genotype | Abbreviated designation |
|--|-------------------------|
| <i>mLa mLa mlg mlg mlp mlp mlk mlk</i> | <i>Ml</i> or Manchuria |
| <i>Mla Mla mlg mlg mlp mlp mlk mlk</i> | <i>Mla</i> |
| <i>mLa mLa Mlg Mlg mlp mlp mlk mlk</i> | <i>Mlg</i> |
| <i>mLa mLa mlg mlg Mlp Mlp mlk mlk</i> | <i>Mlp</i> |
| <i>mLa mLa mlg mlg mlp mlp Mlk Mlk</i> | <i>Mlk</i> |

development of *E. graminis*. By definition, therefore, it contains the recessive alleles at the four loci that are known to affect mildew development. The system of abbreviation used to indicate the host genotype for each host line is shown in Table 1.

The genotype of culture CR3 of *E. graminis* f. sp. *hordei* used is *Pa Pg Pp Pk* according to the gene designations of Loegering (1).

Methods of controlled inoculation.—Single 5- to 6-day-old plants grown in 2-inch pots were inoculated by the rolling method (4) in all experiments where the development of the powdery mildew fungus during primary infection was studied. A uniform distribution of single conidia ranging from 80 to 120/cm² of leaf area was obtained by this method. Only single, well-separated parasite units were counted at each observation to eliminate the possibility of inhibition due to crowding.

Test of the segregation of *Ml* genes in barley.—Seeds derived from heterozygous parent barley plants with genotypes *Mla mla*, *Mlg mlg*, *Mlp mlp*, or *Mlk mlk* were planted individually in 2-inch pots. Barley seeds from homozygous-recessive or homozygous-dominant parents were planted as a control. The percentage of conidia that produced ESH on a portion of the primary leaf on individual plants was recorded 28 hr after inoculation. Plants were then grown to maturity in the greenhouse. The genotype of each plant was determined by the segregation of infection types among progeny 7 days after inoculation. The infection types were recorded as: 0 = no observable mildew development; 1 = chlorotic flecking; 2 = necrotic reaction; 3 = significant reduction in mildew development; and 4 = abundant mildew development.

Environmental conditions for experiments.—All experiments were carried out in Sherer-Gillett Model CEL 512-37 and Model CEL 25-7 growth chambers. The conditions necessary for synchronous growth of each developmental stage of primary infection were as follows: (i) For the 1st hr, inoculated plants were kept in darkness, at 18 ± 1C, and at ca. 100% relative humidity; (ii) for the 2nd through 6th hr, inoculated plants were kept in 260 ft-c of light (200 ft-c from white VHO fluorescent tubes and 60 ft-c from incandescent bulbs), 22 ± 1C, and relative humidity of 65 ± 5%; (iii) for the 7th through 20th hr, the conditions were the same as (ii), but in darkness; (iv) for the 21st through 36th hr, the conditions were the same as (ii).

Examination of fungal development.—The per-

centage of the parasite population in each stage of development was observed by microscopic observations (with a B & L microscope equipped with apochromatic objectives and at a magnification of X 150) on 1-cm leaf sections (excluding the 1-cm tip section) at various times after inoculation. The leaf was discarded after the percentage of parasitic units in each stage of development was recorded. Observations at subsequent hours were made on other inoculated plants.

RESULTS.—*Effect of *Ml* genes on the formation of elongating secondary hyphae of *E. graminis* f. sp. *hordei*.*—The effects of different *Ml* genes in the four near-isogenic barley lines were tested by inoculation with culture CR3 of *E. graminis* f. sp. *hordei*, a strain that possesses the complimentary genes *Pa*, *Pg*, *Pk*, and *Pp* for incompatibility with the four genes *Mla*, *Mlg*, *Mlk*, and *Mlp*, respectively. The formation of ESH was used as a criterion for the establishment of compatible relations between host and parasite. The effects of each gene pair, e.g., *Pa/Mla* (parasite/host) genotype, on the formation of ESH of the barley powdery mildew fungus were recorded from 20 to 26 hr after inoculation (Fig. 1). Approximately 8, 16, 32, and 37% ESH were observed 26 hr after inoculation with the genotypes (parasite/host) *Pa/Mla*, *Pg/Mlg*, *Pk/Mlk*, and *Pp/Mlp*, respectively, specifying incompatibility. The possibility that genes other than the *Ml* genes contributed to the results was considered unlikely, because essentially the same results were obtained on paired lines with no known dominant *Ml* genes, as with Manchuria. The kinetics of formation of ESH on each near-isogenic homozygous-recessive line was shown to closely parallel the results on Manchuria (Fig. 2).

*Effect of *Ml* genes on the infection type produced by the powdery mildew fungi on barley.*—We inoculated near-isogenic lines of barley with or without *Ml* genes by dusting the conidia of the fungus on 5- to 6-day-old seedlings. The inoculated plants were incubated under environmental conditions similar to those used for the maintenance of stock cultures. The infection types were recorded 5 to 6 days after inoculation. Table 2 summarizes the effect of the different genes on final infection types, as well as the primary infection process. Incompatibility was expressed as a lower infection type observed 6 days after inoculation.

*Formation of elongating secondary hyphae as a criterion for the identification of segregating *Ml* genes.*—It should be possible to identify the segregation of genes in the host by their effect on the formation of ESH, since the percentage of the parasite units applied which eventually form ESH is different for each of the *Ml* genes. The purpose of this experiment was to determine if the three types of progenies, the homozygous dominant *Ml*, the homozygous recessive *ml*, and the heterozygote obtained from the selfing of heterozygous plants could be identified from the percentage of ESH formed.

Seeds derived from the selfing of four near-isogenic heterozygous barley parents were planted

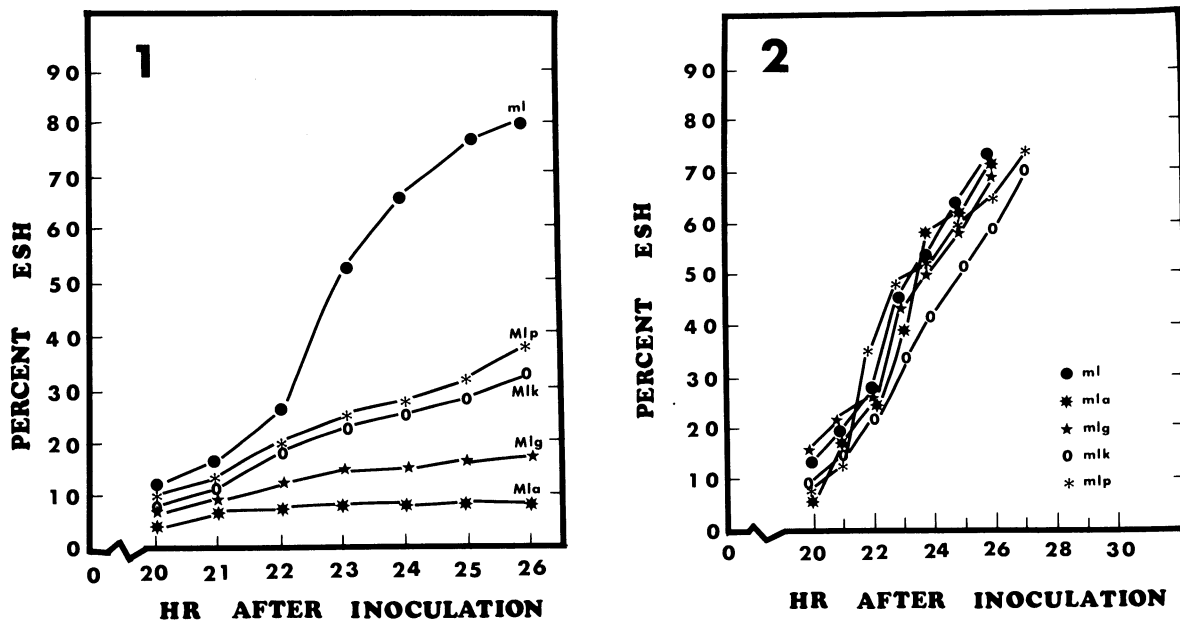


Fig. 1-2. 1) Formation of elongating secondary hyphae (ESH) by *Erysiphe graminis* f. sp. *hordei* (CR3) on near-isogenic barley lines that possess different *Ml* genes. 2) Formation of elongating secondary hyphae (ESH) by *Erysiphe graminis* f. sp. *hordei* (CR3) on five near-isogenic barley lines all with recessive *ml* genes. The line designated *m1a*, for example, was the homozygous recessive derived from the cross Algerian × ⁴₁₄ Manchuria. (The symbol "× ⁴₁₄" refers to the original cross with Manchuria, three generations of backcrossing to Manchuria, and then selfing the heterozygous progeny in each of 14 generations).

TABLE 2. Action of *Ml* genes on development of *Erysiphe graminis* f. sp. *hordei* CR3 on barley

| | Hours after inoculation | | | |
|-----------------------------|-------------------------|----------------------|-------------------------------|-------------------------------|
| | 0-4 | 5-8 | 22-28 | 120 |
| Near-isogenic barley lines: | % Germination | % Mature appressoria | % Elongating secondary hyphae | % Infection ^a type |
| Manchuria | >95 ^b | >90 ^b | >75 ^b | 4 |
| <i>M1a</i> | >95 | >90 | >10 | 0 |
| <i>M1g</i> | >95 | >90 | 10-20 | 0, 1 |
| <i>M1k</i> | >95 | >90 | 30-40 | 2 |
| <i>M1p</i> | >95 | >90 | 35-45 | 1, 2 |

^aInfection type: 0 = no observable mildew development. 1 = chlorotic flecking. 2 = necrotic reaction. 3 = significant reduction in mildew development. 4 = abundant mildew development.

^bPercentage of total parasitic population observed at the end of each period.

individually in 2-inch pots. Approximately 50 seedlings of each line, 5 to 6 days old, were inoculated with culture CR3. A 1-cm section of each seedling was removed, and the percentage of applied parasite units which formed ESH on that section 26 to 28 hr after inoculation was recorded. Infection types on the remaining portion of the inoculated leaves were observed 6 days after inoculation. The plants were grown to maturity and the genotype of each seedling was determined by the segregation of infection types among its progenies. The number of seedlings with a given percentage range of ESH was recorded.

The percentages of ESH produced on homozygous-recessive plants derived from homozygous-recessive parents of all four near-isogenic lines were always in the range of 75 to 90%. Per cent of ESH produced by control homozygous-dominant plants derived from homozygous parents of *M1a M1a*, *M1g M1g*, *M1k M1k*, and *M1p M1p* were observed to be 0 to 10%, 10 to 20%, 10 to 40%, and 25 to 45%, respectively. However, the ranges in the percentage of ESH produced on homozygous plants of genotypes *M1a M1a*, *M1g M1g*, *M1k M1k*, and *M1p M1p* which were derived from heterozygous parents were 0 to 40%, 10

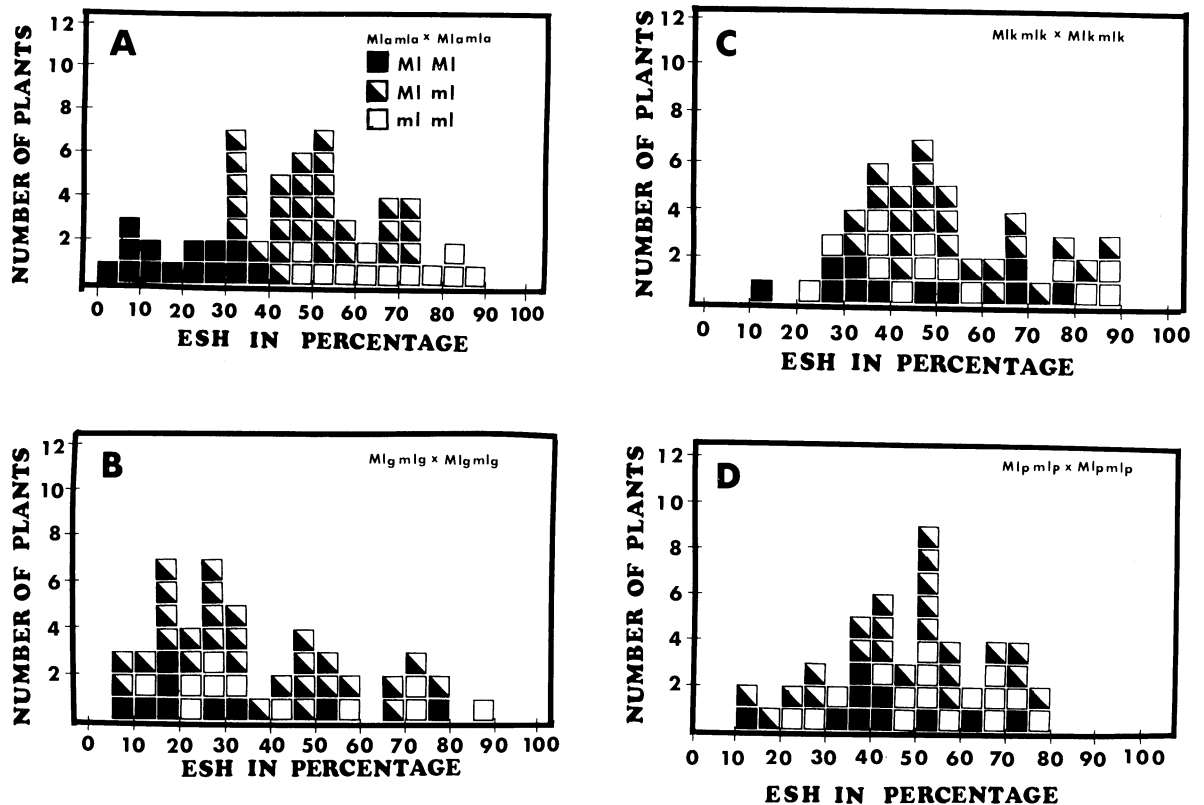


Fig. 3. The number of plants homozygous *MI MI*, heterozygous *MI ml*, and homozygous *ml ml* on which a particular range of percentage ESH was produced. All plants were derived from the selfing of heterozygous *MI ml* plants. A) *Mla*. B) *Mlg*. C) *Mik*. D) *Mlp*.

to 80%, 10 to 80%, and 10 to 75%, respectively (Fig. 3-A,B,C,D). A greater range in the percentages of ESH was observed on homozygous-recessive progenies derived from heterozygous parents than from homozygous parents.

The percentage of ESH could be used to distinguish homozygous progeny *Mla Mla* from *m la m la* derived from selfing *Mla m la* plants. The percentage of ESH cannot be used to distinguish the two types of homozygous progeny derived from selfing plants of the genotypes *Mlg Mlg*, *Mik Mik*, or *Mlp Mlp*. With all four genes, the range in the percentages of ESH produced by the parasite on either homozygous-dominant or homozygous-recessive plants derived from heterozygous parents is significantly larger than the ranges observed on homozygous plants derived from homozygous parents.

DISCUSSION.—The reaction of *Ml* genes in the host with their corresponding *P* genes in the parasite was not detected before the penetration process by the pathogen. The effects of genes for incompatibility were the reduction in the percentage of ESH formed in the primary infection process, and a lower infection type at 6 days after inoculation.

The use of the percentage of ESH formed for identifying genes for incompatibility gave surprising

results when applied to the identification of segregating *Ml* genes in barley. The identification of three different genotypes, the homozygous recessive (*ml ml*), the homozygous dominant (*MI MI*), and the heterozygote (*MI ml*) was attempted by the inoculation of progeny obtained from the selfing of heterozygous plants (*MI ml*). In all four near-isogenic barley lines tested, the range of the percentages of ESH on the homozygous-dominant (*MI MI*) and the homozygous-recessive (*ml ml*) plants derived from selfing of heterozygous parents (*MI ml*) was considerably larger than on homozygous-dominant (*MI MI*) plants derived from homozygous-dominant parents (*MI MI*) and homozygous-recessive (*ml ml*) plants derived from homozygous-recessive (*ml ml*) parents. The functioning of both dominant and recessive genes in the parent is suggested. The carry-over effect of the parent on the homozygous progeny cannot be from the genotype of the endosperm, unless double fertilization occurred, because the endosperm would be homozygous. Both *Mlg* and *Mlp* were semidominant as evaluated by infection type 6 days after inoculation (3). It was also observed that an intermediate infection type, type 2 (see Table 2 for infection type designation) was found on some plants derived from the selfing of *Mla m la*. When the progenies of plants

with infection type 2 were inoculated, two types of segregation were observed. Progeny from some plants gave all infection type 0. Progeny from other plants had progeny with infection types 0, 2, or 4, but not in the ratio of 1:2:1 expected with partial dominance. The results strongly suggest a carry-over effect from heterozygous parents to the progeny and a function of both *Mla* and *m1a* genes which affect the expression of the genotype of the progeny.

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