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Cell-Autonomous Recognition of the Rust Pathogen Determines *Rpl*-Specified Resistance in Maize

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The *Rpl* gene of maize determines resistance to the leaf rust pathogen *Puccinia sorghi*. X-ray treatment of heterozygous (*Rpl Oy/rpl oy*) maize embryos generated seedlings with yellow sectors lacking *Rpl*. Yellow sectored seedlings inoculated with rust spores gave rust pustule formation in yellow (*Rpl*-lacking) sectors and hypersensitive resistance in green tissues, thereby demonstrating that the *Rpl* gene product is cell-autonomous in its action. In cases where the hypersensitive reaction was initiated in green (*Rpl*) tissue next to a yellow sector, the hypersensitive response appeared to be propagated poorly, if at all, through *Rpl*-lacking cells.

NUMEROUS GENES PROVIDING resistance to plant pathogenic microorganisms have been identified in a wide variety of plant species. Many of these disease resistance genes are dominant and specify plant resistance to a particular race or races of a specific pathogen (*1*). Often, the resistance phenotype is associated with the hypersensitive response (HR), a local necrosis of plant tissue initiated by and surrounding the site of pathogen contact with the plant host (*2*). This localized cell death presumably isolates the pathogen from host nutrients and is associated with the release of various toxic compounds from the dying plant cells (*2*). Initiation of the hypersensitive response by the infected plant is generally specified by an interaction between a dominant host resistance gene and a dominant recognition factor (or "avirulence locus") in the plant pathogen (*1, 3*). The molecular nature of the events that initiate and propagate disease resistance and the hypersensitive response are not known.

The experiments described herein were designed to investigate the cell autonomy of the HR-associated disease resistance specified by the *Rpl* locus of maize. The results demonstrate that the initiation of the hypersensitive response and resistance to the leaf rust pathogen *Puccinia sorghi* require a cell-autonomous, nondiffusible factor specified by *Rpl*.

Specific alleles of the *Rpl* locus of maize determine dominant, HR-associated resistance to specific races of the fungal pathogen *P. sorghi*. *Rpl* maps 25 centimorgans from the centromere on the short arm of chromosome 10 (*4*). The allele of the oil yellow (*oy*) locus used in this study specifies a recessive yellow plant color trait (*5*) and maps approximately midway between the centromere and *Rpl* on the short arm of chromosome 10. Chromosome breakage between *Oy* and the centromere in developing embryos with the genotype *Rpl Oy/rpl oy* should uncover the recessive *oy* phenotype

Table 1. Sector analysis in 2215 21-day-old seedlings from x-irradiated *Rpl Oy/rpl oy* embryos. The median size of yellow sectors on the first to third leaves is given as a fraction of leaf size. DAP, days after pollination.

Irradiated ear	Time of irradiation (DAP)	Median yellow sector size	Number of seedlings with			
			Yellow sectors per total screened	Pustules in yellow sectors	HR in yellow sectors	Pustules in green tissue
1	5	1/16	2/151	0	0	0
2	6	1/32	2/66	1	0	0
3	7	1/16	11/360	3	0	0
4	7	1/16	13/337	2	1	0
5	9	1/32	8/88	0	0	0
6	9	1/32	16/253	1	0	0
7	10	1/32	21/459	1	0	0
8	12	1/16	11/199	0	0	1*
9	12	1/32	24/302	1	0	0

*Fully susceptible seedling.

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and, if the yellow plant color trait is cell autonomous (*6*), lead to green progeny plants with yellow sectors. Since terminal deletions are much more common than internal deletions, any yellow sectors observed should also lack the dominant *Rp1* resistance gene.

Developing maize embryos were irradiated with 800 rem of hard x-rays 5, 6, 7, 9, 10, or 12 days after pollination to generate chromosome breakage (*7*). Of 2215 21-day-old seedlings screened, 108 had yellow sectors visible to the unaided eye. All of the sectors detected, some as small as four cells in width, had distinct boundaries between the yellow and green tissues. This indicates that the oil yellow–associated plant color trait is cell-autonomous. The oil yellow sectors observed varied in size from approximately 1/64 to 1/8 of the leaf, with the larger sectors found predominantly in the progeny of ears irradiated 5 to 7 days after pollination (Table 1). These data are in agreement with the known timing and arrangement of maize leaf development in embryogeny (*7*).

All of the progeny of x-irradiated ears were inoculated with *P. sorghi* spores 11 days after planting. Ten days later, these seedlings were scored for rust sensitivity. Most of the fully green seedlings inoculated gave numerous hypersensitive response lesions and no rust pustules. One green seedling gave several rust pustules and no HR lesions on each of the three leaves inoculated (Table 1). Whether this fully susceptible seedling was a contaminant or a mutation at the *Rp1* locus (*8*) remains to be determined.

Maize seedlings with oil yellow sectors had HR lesions in their green tissues. Of these, nine seedlings also had one or more rust pustules. The rust pustules observed were found exclusively in yellow sectors (Fig. 1, A to E). Pustules were formed even in oil yellow sectors that were six cells wide or in yellow tissue at the very edge of a green-yellow boundary (Fig. 1, D and E). As a result of low inoculation efficiency, many seedlings exhibited neither pustule formation nor HR necrosis within their yellow sectors. Only one seedling had any HR lesions centered within a yellow sector. Two HR lesions were found on this seedling, which had a single yellow sector, approximately one-quarter leaf in size, that terminated about 80% of the way down the length of the third leaf. Since a developmentally determined sector of this size on the third leaf should always extend to the end of the leaf, this exceptional yellow sector was not derived from chromosome breakage leading to loss of *Oy* and *Rp1*. This aberrant yellow sector was probably due to some environmental damage to the seedling (for example, insect attack).

We also selected ten seedlings with yellow sectors in their emerging fourth, fifth, or sixth leaves for inoculation at a later stage of maturity. These ten plants were inoculated with *P. sorghi* spores 53 days after planting. Eight of these plants developed one or more rust pustules in their yellow sectors, whereas no rust pustules were observed in green tissues on any plant (Fig. 1H). Because of the age of these plants, the hypersensitive lesions observed were much smaller and more difficult to identify (Fig. 1H).

Oil yellow plants are small and slow-growing and have narrow leaves. Similarly, a large oil yellow sector leads to a leaf that is narrower on the sectored side of the midrib than on the fully green side (Fig. 1H). Since the oil yellow phenotype seems to be associated with low vigor, the inability to resist *P. sorghi* infection in yellow sectors could be due to a generalized enfeeblement of physiological processes (including resistance) in oil yellow tissues. To test this possibility, we crossed heterozygous *Rp1 Oy/rp1 oy* plants to an *rp1 oy/rp1 oy* tester. As expected, approximately 50% of the progeny of this cross were oil yellow. Of these oil yellow seedlings, 90% were susceptible to *P. sorghi* infection, while the remainder of the oil yellow seedlings gave a fully resistant HR response (Fig. 1I). This result agrees with the 12% recombination frequency expected between *Rp1* and *Oy1* (*5, 6*) and demonstrates that rust resistance and HR necrosis are manifested in oil yellow tissues that carry an *Rp1* resistance allele.

In cases where a rust spore landed in green (*Rp1*) tissue near a yellow sector, a hypersensitive resistance response was initiated. Once initiated, hypersensitive cell necrosis appeared to proceed a short distance into large yellow sectors (Fig. 1F) or

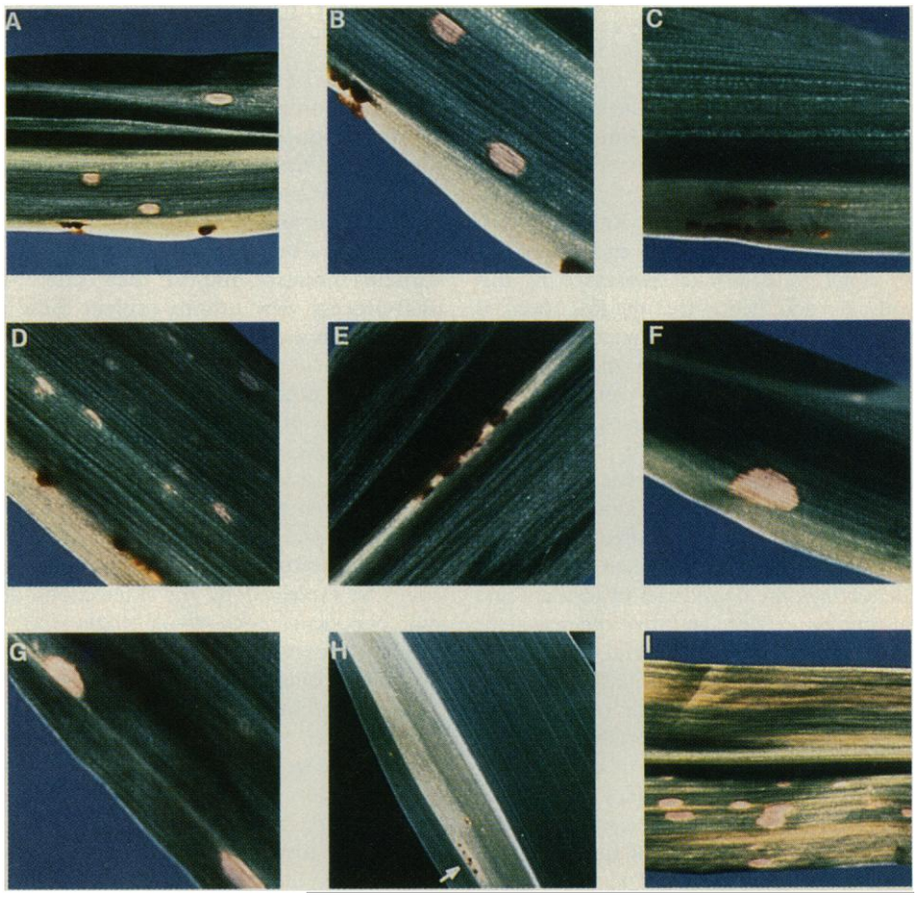

Fig. 1. Resistance and susceptibility to the rust fungus *P. sorghi* on somatically sectored leaves. Developing maize embryos on fertilized ears of the genotype $Rp1^K$ *Oy/rp1 oy* were exposed to 800 rem of hard x-rays. Exposure was 400 rem (measured in air) with 1.42 mm of Cu half-value layer from each of two opposing ports of a General Electric Maxitron 300. The $Rp1^K$ allele of the *Rp1* locus (*4*) and rust culture no. 1 (*8*), used in this study, specify a fully incompatible (that is, resistance) reaction. Brown oval regions are necrotic lesions associated with the hypersensitive resistance response. Rust red spots are pustules of actively growing *P. sorghi*. (**A** to **E**) Pustule formation in yellow sectors. (**F** and **G**) Hypersensitive response initiated in green tissue extending near or into a yellow sector. (**H**) Fifth leaf from a 53-day-old plant irradiated 7 days after pollination. The arrow indicates rust pustules on a yellow sector that occupied 1/4 leaf. (**I**) Hypersensitive necrotic lesions (resistance) on an oil yellow leaf with the genotype $Rp1^K$ *oy/rp1 oy*. Magnifications: (A and I), ×2; (B to G), ×4; (H), ×1/3. (A) and (B) are the same inoculated leaf.

through small yellow sectors (Fig. 1G). Since a single layer of green (*Rp1*) cells may be associated with some very narrow sectors or the boundaries of larger sectors (*7*), we cannot definitively determine whether the necrotic regions in these yellow sectors derive from oil yellow (*rp1*) cells. The hypersensitive necrosis initiated in green tissue at a green-yellow boundary progressed less far into oil yellow sectors than into green tissues (Fig. 1, F and G), leading to an asymmetric HR lesion. This suggests input of the *Rp* locus into propagation as well as initiation of the necrotic response. We do not believe that this result is due to reduced vigor in the yellow sectors, since the size and shape of hypersensitive lesions in *Rp1 oy/rp1 oy* and *Rp1 Oy/rp1 oy* tissues were similar (compare Fig. 1A with Fig. 1I). In this regard, however, the oil yellow sectors in the irradiated material were lighter and more homogeneously yellow than homozygous recessive oil yellow tissues. This indicates that either the hemizygous (*−/oy*) phenotype is more severe than the homozygous recessive oil yellow state or that there is a significant contribution by the hemizygosity of other loci on the short arm of chromosome 10 to the lowered vigor and yellowing of these sectors.

Our data indicate that the *Rp1* locus must be present and active in cells encountered by *P. sorghi* to determine resistance to this pathogen and, hence, that the *Rp1*-specified resistance events are not initiated by a diffusible factor. In experiments that juxtapose resistant and susceptible tissues, various groups have observed hypersensitive necrosis at the graft junction (*9*). Among the difficulties in interpreting these results are the resistance-necrosis reactions induced by cutting and gluing the grafted tissues, the lack of sharpness in (and, often, difficulty in identifying) the boundaries of resistant and susceptible tissues, and the other genetic differences between host and graft tissue sources.

The well-marked, isogenic nature of the sectored plants used in this study proves that a factor on the short arm of chromosome 10, presumably *Rp1*, specifies cell-autonomous initiation of HR-associated resistance upon exposure to *P. sorghi* spores. Determining whether or not *Rp1*-initiated hypersensitive necrosis may be propagated through cells lacking *Rp1* will require detailed microscopic analysis of fungal growth and cell necrosis at green-yellow boundaries.

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Behavioral Dissociation of Dishabituation, Sensitization, and Inhibition in *Aplysia*

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Three forms of nonassociative learning (habituation, dishabituation, and sensitization) have commonly been explained by a dual-process view in which a single decrementing process produces habituation and a single facilitatory process produces both dishabituation and sensitization. A key prediction of this view is that dishabituation and sensitization should always occur together. However, we show that dishabituation and sensitization, as well as an additional process, inhibition, can be behaviorally dissociated in *Aplysia* by (i) their differential time of onset, (ii) their differential sensitivity to stimulus intensity, and (iii) their differential emergence during development. A simple dual-process view cannot explain these results; rather, a multiprocess view appears necessary to account for nonassociative learning in *Aplysia*.

THE RELATION AMONG DIFFERENT forms of learning has been the subject of considerable debate for several decades (*1*). For example, until recently (*2*, *3*) it has been thought that three different forms of nonassociative learning (habituation, dishabituation, and sensitization) could be explained by the interactions of two opponent processes—a single decrementing process that gives rise to habituation and a single facilitatory process that gives rise to both dishabituation (the facilitation of decremented responses) and sensitization (the facilitation of nondecremented responses) (*4*). If this view is correct, then dishabituation and sensitization should always occur together. However, we report here that dishabituation and sensitization, as well as an additional process, inhibition, can be behaviorally dissociated in the siphon withdrawal reflex of the marine mollusk *Aplysia*. This reflex has been used successfully to analyze both nonassociative and associative learning on behavioral and cellular levels (*5*). Our results suggest that a simple dual-process view involving a single decrementing and a single facilitatory process requires revision and that a multiprocess view, perhaps involving inhibitory as well as facilitatory interactions, is necessary to account for the mechanisms underlying nonassociative learning.

To examine dishabituation, we first produced habituation of the siphon withdrawal reflex by administering 20 water-jet stimuli to the siphon at a 30-s interstimulus interval (ISI). We then administered a single stimulus to the tail; this stimulus ranged in intensity from a mild tactile stimulus to multiple electrical shocks (*6*). Finally, we tested the reflex amplitude with water-jet stimuli to assess the magnitude of dishabituation (*7*).

To analyze the time of onset of dishabituation, we compared two conditions. In one condition (Dishab.) animals received tail stimulation after habituation of the siphon withdrawal reflex, while in the other (Recovery) there was no tail stimulus after habituation (*8*). Groups were tested 90 s, 10 min, and 20 min later. The results for Weak

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