Genetical Approach to the Biochemical Nature of Plant Disease Resistance

By SHIGEHISA KIYOSAWA

1st Laboratory of Genetics, Department of Physiology and Genetics, National Institute of Agricultural Sciences

Genetical studies have effectively contributed towards the solution of biochemical nature of biological characters since they were applied for metabolism in *Neurospora crassa*. Now we infer the biochemical mechanisms of disease resistance from many genetical studies on the host-pathogen relationships in various plant diseases.

True resistance*

Most of the genetical studies on true resistance have dealt with specific resistance that a variety with resistance gene(s) is resistant only to some (not all) fungus strains of a pathogen.

Flor[3] studied the inheritance of host-pathogen relationship in the flax and the flax rust system, and demonstrated the gene-for-gene relationship.

Such a relationship was shown in other crop diseases: powdery mildew of barley[18] and wheat,[19] bunt of wheat,[20] stem rust of wheat,[17] blast of rice,[5),11),14] late blight of potato,[4] etc.

The author[7),11] gave the following genetical common characteristics of their gene-for-gene relationship:

1) There are many genes for specific resistance.
2) The function of genes is highly specific.
3) There are many multiple alleles for resistance in host.
4) Resistance genes tend to concentrate on a few chromosomes.
5) Higher resistance (or higher avirulence) is epistatic to lower resistance (lower avirulence).
6) Mostly, resistance (or avirulence) is dominant over susceptibility (or virulence**)

Each genetical characteristic shows at least the following biochemical mechanisms for specific resistance:

1) There are substances to cause as many resistant reactions as resistance genes.
2) The function of the substance is highly specific.
3) A slight difference of base sequence among multiple alleles for resistance leads to the production of substances differing in specificity.
4) Biochemical meaning is not known.
5) Epistatic gene produces actively enzyme or substance, if it is dominant over its allele. Accordingly, resistance and avirulence alleles produce enzymes or substances to cause resistance reaction,

* Disease resistance of plant id divided into two groups, true and field resistances.[12] The former can be detected under greenhouse conditions, and the latter under field conditions not under greenhouse ones. These two are also defined as y_0 and r in equation $y=y_0e^{rt}$, respectively, where y is the number of susceptible type lesions at the early stage of infection, y_0 is the number at the initial stage ($t=0$), and t is time in days.

** In this paper, virulence and aggressiveness refer to specific and nonspecific pathogenicity, respectively.

while susceptibility and virulence alleles do not produce any active substances.

6) Dominant allele is generally an active one although not always. Therefore, resistance and avirulence alleles do produce active substances.

The fifth is above all important in discussing the mechanism of resistance.

Pi-k and *Pi-a* blast resistance genes of rice are taken for example of two resistance genes which control different levels of resistance. The *Pi-k* controls the immune reaction (R^h) and *Pi-a* the resistant reaction only to cause brown spots (R) to blast fungus strain Ina 168. When two varieties with these were crossed, *Pi-k Pi-a* and + + genotypes were obtained.[9]

The *Pi-k Pi-a* and + + genotypes show immune and susceptible reactions to Ina 168 respectively—*Pi-k* gene controlling higher resistance was epistatic to *Pi-a* gene controlling lower one.

Mutants (Ina 168-a^+) attacking plants with *Pi-a* gene were rarely obtained spontaneously from Ina 168. More frequently, mutants (Ina 168-k^+) overcoming the resistance controlled by *Pi-k* gene were isolated from Ina 168.[6] These mutants removed both avirulence genes, *Av-a* and *Av-k*, of Ina 168.

Mutants (Ina 168-a^+-k^+) that came to attack plant with both resistance genes were obtained from Ina 168-a^+. These original and mutant strains show the reactions as shown in Table 1.

Table 1. Host-pathogen relationship in the rice-rice blast system

Genotype of host	Fungus strain and its genotype			
	Ina 168 *Av-k Av-a*	Ina 168-k^+ + *Av-a*	Ina 168-a^+ *Av-k* +	Ina 168-a^+-k^+ + +
Pi-k Pi-a	R^h	R	R^h	S
Pi-k +	R^h	S	R^h	S
+ *Pi-a*	R	R	S	S
+ +	S	S	S	S

By such a method, gene-for-gene relationship can be demonstrated if there is no sexual stage as in blast fungus. Genes *Av-k* and *Av-a* show R^h and *R* reactions on *Pi-k Pi-a* plant, respectively. A combination of two avirulence genes shows R^h reaction on *Pi-k Pi-a* plant, which means higher avirulence is epistatic to lower avirulence.

When a susceptible pair of specific host and pathogen genes (*Pi-k*: *Av-k*$^+$) is combined with other resistance pair (*Pi-a*: *Av-a*), a resistant reaction is shown. This indicates that resistant gene pair (*Pi-a*: *Av-a*) produces substance responsible for resistant reaction but not susceptible gene pairs (*Pi-k*: *Av-k*$^+$, *Pi-k*$^+$: *Av-k* and *Pi-k*$^+$: *Av-k*$^+$) for susceptible reaction.

The author[7] investigated whether or not the various hypotheses published already could explain these characteristics of specific resistance and concluded that every hypothesis cannot interpret them, particularly dominance and epistasis of resistance and avirulence over susceptibility and virulence, respectively.

He proposed some hypotheses that might explain the characteristics of specific host pathogen relationship. Dominant and particularly epistatic genes are in general an active gene and produces a gene product. Therefore, resistance and avirulence genes are active and they produce any gene products.

Resistant and avirulent reaction is expected to result from an interaction between these gene products. Various possible positions in pathway from the gene to the product were presumed as shown in Fig. 1. However, contrary, a product of resistance gene can possibly interact in pathogen with a product of avirulence gene.

Afterward, Hadwinger and Schwochau[5] proposed the hypothesis that the inducer produced by avirulence gene in pathogen stimulated phytoalexin production via de-repression of phytoalexin gene by correspondingly inhibiting synthesis of the respective repressor. This hypothesis is similar to the ① hypothesis in Figure 1 proposed by Kiyosawa.[7]

Hadwinger and Schwochau,[5] furthermore, presumed that in the case of the pisatin-producing system in *Pisum sativum* there existed in the pea genome a pisatin operon with an operator site and a polycistronic

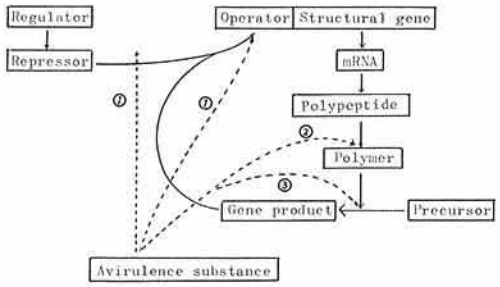

Fig. 1. Possible interactions between host and pathogen

① An avirulence gene in pathogen acts as a regulator or inducer of resistance gene in host and the regulator gene or inducer activates a gene which produces an enzyme inducing a resistant reaction.

② A substance produced by an avirulence gene (polypeptide or its polymer) and polypeptide produced by a resistance gene in host are specifically bound with each other and their products catalyze a resistant reaction.

③ A polypeptide or polymer produced by an avirulence gene binds with a polymer produced by a resistance gene in host and its product forms an enzyme which catalyzes a resistance reaction.

④ A polymer produced by ② or ③ directly gives a decisive effect on living ability of host cells and causes a hypersensitive reaction.

structural gene, where all the enzymes of the pisatin pathway were encoded.

They believed that these hypothetical induction mechanisms were not limited to the phytoalexin-type host response, but applied quite generally to every type of resistance response—hypersensitive reaction.

English and Albersheim[2] provided a new hypothesis based on two important facts. Firstly, the amount of α-galactosidase activity detected in the culture medium of fungus, *Colletotrichum lindemuthianum*, grown on isolated hypocotyl cell walls of *Phaseolus vulgaris*, is related to the aggressiveness of the isolated, and secondly, the various fungus strains secrete more α-galactosidase when they are grown in hypocotyl walls isolated from susceptible plants than when they are grown in walls isolated from resistant plants.

According to the hypothesis, what appears to be critical in this host-pathogen interaction is not whether fungus has a genetic capacity to secrete α-galactosidase but whether the environment of the fungus permits production and secretion of relatively large amount of the enzyme.

Furthermore, they lead to the hypothesis that constituents (effectors) within the cell walls of host control the synthesis of α-galactosidase by repression or induction, or by both, and that fungus strains respond to different effectors, respond differently to the same effectors, or are capable of differentially extracting an effector from the cell walls of different varieties.

Expanding the English-Albersheim's hypothesis, Albersheim, Jones and English[1] built up the following hypothesis: Different resistance genes have the function of encoding an enzyme which adds glucose side chains to a polysaccharide in the cell wall through different linkages (for example, A and B genes through α-glucosidic and β-glucosidic linkages, respectively).

Fungus strains produce glucosidase which specifically corresponds to the product of each resistance gene. If avirulence is dominant over virulence, avirulence gene a, which corresponds to resistance gene A adding α-glucoside to the wall, inhibits the production of β-glucosidase in the pathogen. The gene b inhibits the production of α-glucosidase.

If avirulence is recessive, virulence gene a produces α-glucosidase to specifically interact with the cell wall containing α-glucoside produced by resistance gene A and to remove the α-glucosyl residues from the wall galactan of the host. The gene b removes the β-glucosyl residues by the B gene.

The removed glucose represses the synthesis of α-galactosidase in pathogen which determines the degree of pathogenicity.

It is well known as a hypothesis to explain the specificity of host-pathogen relationship that virulence gene in *Cochliobolus carbonum* and *C. victoriae* produces the host-specific toxin.[21),22)]

In this case, resistance of the hosts to specific toxin is controlled by a single dominant gene in the corn and by a single recessive gene in oat.[16),23)] Dominance of pathogenicity is not known because the fungus is pathogenic in haploid phase.

As mentioned above, it was concluded from the viewpoint of genetical studies that the toxin hypothesis was not the case of the mechanism of the specific resistance as shown by Flor.[3)] The difference between the corn and oat-*Cochliobolus* system and the flax-flax rust system is considered from the viewpoint of genetical studies as follows:

First, it is assumed that the toxins produced by virulence genes, a and b, are selectively detoxified by the product of resistance genes, A and B, respectively.[22)] The a-A or b-B combination induces a resistant reaction. The other combinations, a-A$^+$, a$^+$-A and a$^+$-A$^+$, or b-B$^+$, b$^+$-B and b$^+$-B$^+$, lead to susceptible, resistant and resistant reactions, respectively.

Considering two genes in each host and pathogen, the reactions caused by all the possible combinations are expected as shown in Table 2. In this table, reactions shown in

Table 2. Host-pathogen relationship in the toxin-detoxification system

Host variety	Fungus strain			
	ab	a+	+b	++
A B	R	R	R	R
A+	S	R	S	R
+B	S	S	R	R
++	S	S	S	R

Toxins produced by virulence genes, a and b, are selectively detoxified by enzymes produced by resistance genes, A and B, respectively.

[ ] have been confirmed experimentally.[21)].

Secondly, it is assumed that susceptibility genes A and B produce a receptor which binds specificially with the toxin produced by virulence genes a and b to induce susceptible reaction, respectively.[22)] All the possible combinations of these two gene pairs are expected to show reaction pattern as shown in Table 3.

Table 3. Host-pathogen relationship in the toxin-receptor system

Host variety	Fungus strain			
	ab	a+	+b	++
A B	S	S	S	R
A+	S	S	R	R
+B	S	R	S	R
++	R	R	R	R

Toxins produced by virulence genes, a and b, correspondingly interact with receptors formed by susceptibility genes, A and B, to induce susceptible reactions, respectively.

Accordingly, we could not conclusively differentiate these two systems by classical genetical studies, although dominance or recessiveness of resistance may suggest what is the case. It is noted that these reaction patterns are clearly different from that of the Flor's gene-for-gene relationship. Therefore, there are at least two categories in specific resistance.

The characteristic difference between two categories is a reaction resulting from a combination of resistant and susceptible gene-pair (for instance, A-a and B$^+$-b) individually inducing *R* and *S* reactions, respectively. This combination leads to resistant reaction in the flax-flax rust system (Table 1), and to susceptible reaction in the cereal-*Cochliobolus* system (Tables 2 and 3).

Furthermore, resistant genotype is obtained from the combination of susceptible genotypes (A+ and +B) generally in the cereal-*Cochliobolus* system, but not in the flax-flax rust system. (Compare the last in Table 1 with the first column in Tables 2 and 3).

Specific resistance genes often constitute the sets of multiple alleles. In blast resistance of rice, three sets are known.[14)] Rice varieties with these alleles show the reactions as shown in Table 4. Of mutants of the pathogen so far obtained from Ken 54-20 and Ina 168 in relation to *Pi-k* locus in the host, some attacking *Pi-k* genes do not attack *Pi-k*^{h}, but all the

Table 4 Reactions of genes on *Pi-k*, *Pi-ta*, *Pi-z* loci to various fungus strains

Resistance gene	Fugus strain																
	P-2b	Ken 53-33	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Iha 168	Ken 54-20-k^+	Ken 54-20-kh^+	Ina 168k^+	Ina 168-kh^+	Ken Ph-03	Ken Ph-03-ks^+	Ina 72-ta^+	Ken 53-33-zt^+	Ina 168-zt^+	TH68-184
Pi-k^s	S	S	S	S	S	S	S	S	S	S	S	R^h	S				
Pi-k	MR	S	S	R^h	R^h	R^h	R^h	S	S	S	S	R^h	R^h				
Pi-k^p	S	S	S	R	MR	R	R	S	S	S	S	R	R				
Pi-k^h	M	S	S	R	MR	R	R	R	S	R	S	R	R				
Pi-ta	S	S	M	MR	M	MR	S							S	S	S	MR
Pi-ta^2	S	M	R	R	R	R	MR							M	M	MR	MR
Pi-z	M	M	M	MR	M	MR	M								M	M	S
Pi-z^t	R^h	R^h	R^h	R^h	R^h	R^h	R^h								S	S	R^h
	R^h	R	MR	M	MS	S											

More resistant ←——→ *more susceptible*

mutants attacking *Pi-k*^{h} alleles overcome *Pi-k* and *Pi-k*^{p} alleles.

Of three multiple allele loci, *Pi-k* and *Pi-ta* loci are different from loci for specific resistance in other crops in the point that reverse reactions to two fungus strains are not found.

In other crops, reverse reactions are generally recognized as in the *Pi-z* locus in rice. This difference is due to any one of the three following:

(1) Reverse reactions should be found but are not yet found in *Pi-k* and *Pi-ta* loci because the number of fungus strains tested is too small.

(2) Multiple alleles which do not show reverse reactions should be found, but are not yet discovered in other crops.

(3) Rice blast is between obligate parasite with specific (with reverse reaction) pathogenicity (=virulence) and saprophyte without it, because of its facultative nature.

As mentioned above, a resistance gene specifically corresponds to an avirulence gene. This property may be said to show the semi-fine structure of resistance gene.[14]

The *Pi-k*^{s} allele is most limitedly effective for fungus strains tested. The active site of *Pi-k*^{s} allele is named as E, and fungus strains which show avirulent reaction (or resistant reaction) on the variety with *Pi-k*^{s} (referring to *Pi-k*^{s} variety) are considered to have an active site e which is specifically functional to site E of the host.

Varieties with *Pi-k* allele also show R^h reaction to other strains than Ken Ph-03 which is avirulent only to *Pi-k*^{s} variety. This indicates that *Pi-k* has an active site C, other than E. A fungus strain, P-2b, expresses MR reaction on *Pi-k* variety differing from R^h reaction of strains Ken 54-20 and Ina 168.

Accordingly, P-2b has a different site from that of Ken 54-20 and Ina 168. This is explained by the absence (P-2b) or the presence (Ken 54-20 and Ina 168) of the site c, and the site b is given as avirulence of P-2b on *Pi-k* variety. Ken 54-20-k^+ and Ina 168-k^+ attack *Pi-k* variety, but not *Pi-k*^{h} variety.

Therefore, *Pi-k*^{h} allele has a site A, which is not contained in *Pi-k* allele. And the site a in pathogen corresponding to the site A in host is not contained in Ken 54-20-kh^+ and Ina 168-kh^+ but in Ken 54-20-k^+ and Ina 168-k^+. This causes the difference of reactions between *Pi-k* and *Pi-k*^{h} alleles to Ken 54-20 and Ina 168 (R^h and R).

The site D does not control any resistance

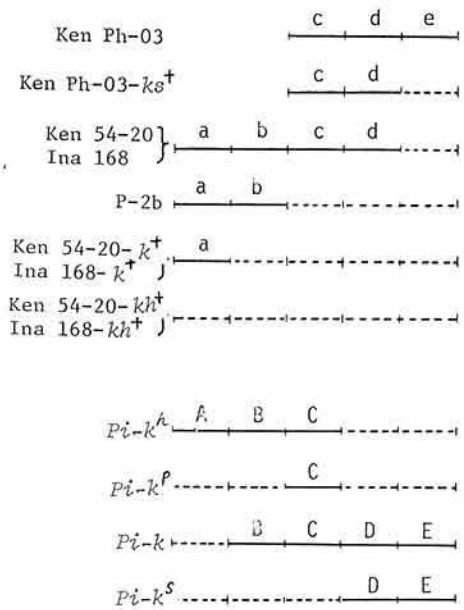

Fig. 2. Semi-fine structure of the locus *Pi-k* and its corresponding avirulence genes

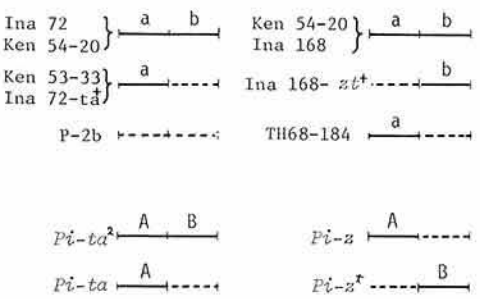

Fig. 3. Semi-fine structure of the loci *Pi-ta* and *Pi-z* and their corresponding avirulence genes

by itself. It intensifies the function of site C and E, and changes R reaction by them into R^h reaction. The semi-fine structures of various avirulence genes of fungus strains and the resistance alleles at the *Pi-k* locus are presumed as in Fig. 2.

The semi-fine structures of *Pi-ta* and *Pi-z* loci are given in Fig. 3. Each site presumably existed in the host interacts only with specific one in the pathogen. Therefore, each site should be large enough to interpret the specific interaction.

The genetic analysis of pathogenicity of blast fungus is not possible at present because it does not have a perfect stage required for crossing. Active sites in the pathogen do not always need to be arranged in order as shown in Figs. 2 and 3. Practically, these sites might be scattered on different chromosmes.

In Fig. 2, the active site of avirulence gene is arranged in order of mutation frequency from left (low) to right (high). If the active sites of avirulence gene are clustered in one locus and their mutation is of frameshift type, the order shown in Fig. 2 can well explain a different mutation frequency between active sites.

Field resistance

There are a few studies on the inheritance of field resistance. The field resistance is, however, known to be controlled by multigenes or polygenes. On the field resistance of rice to blast, the author[13),15)] conducted a few experiments. He studied the field resistance based on the consideration that the field resistance is a weak resistance and can be tested by inoculation with a weak aggressive strain even in a greenhouse.

The weak resistance of varieties, Norin 22,[15)] Homare Nishiki and Ginga,[13)] which are highly resistant in field, are controlled by one major and two or more minor genes in a greenhouse.

The field resistance is originally a complex with functions of a host to inhibit germination, penetration, growth and sporulation of fungus.[10)] This complexity may be associated with the multigenic or polygenic nature of the field resistance.

As mentioned above, the author[7)] emphasized that toxin, phytoalexin, cell wall degrading enzyme, nutrient, and preformed inhibitor in the host could not be hypothesized to cause the specific resistance represented by the flax-flax rust system.

The production of toxin and cell wall degrading enzyme is probably associated with the aggressiveness of the fungus. And the

production of phytoalexin and preformed inhibitor, and the lack of some nutrients are concerned with the nonspecific resistance of the host.

As resistance is opposite to pathogenicity, some information on mechanism of resistance can be made clear by studying pathogenicity. The author has not published some mutants of a low aggressiveness obtained from fungus strains collected in the field.

The reactions of one of these mutants (Hoku 1-Lp) on some varieties which do not have the true resistance were compared with those of a weakly aggressive fungus strain (Ken 54-04) collected in the field, but correlation between them (Fig. 4) was not found.

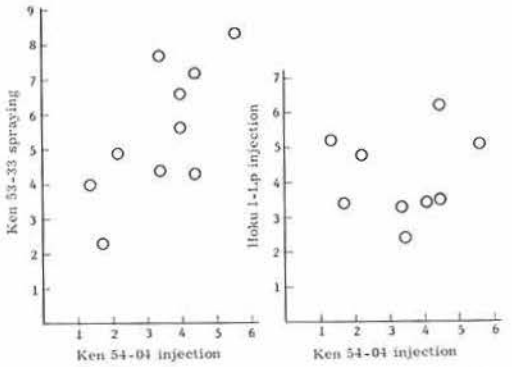

Fig.4. Comparison of resistance by the number of susceptible lesions of some varieties without true resistance to three fungus strains

The reaction of the fungus strain Ken 54-04 closely correlated with that of another fungus strain collected in the field, Ken 53-33. This suggests that Hoku 1-Lp has a different mechanism of low aggressiveness of Ken 54-04, and furthermore there are some difference mechanisms of field resistance.

The genetical studies on the field resistance have not been so advanced yet, and its gene analysis is generally very difficult. However, by the above-mentioned way and biochemical studies, its mechanism could be analyzed.

References

1) Albersheim, P., Jones, T. M. & English, P. D.: Biochemistry of the cell wall in relation to infective processes. *Ann. Rev. Phytopath.*, **7**, 171–194 (1969).
2) English, P. D. & Albersheim, P.: Host-pathogen interactions: I. A correlation between α-galactosidase production and virulence. *Plant Physiol.*, **44**, 217–224 (1969).
3) Flor, H. H.: The complementary genetic systems in flax and flax rust. *Adv. Genetics*, **8**, 29–54 (1956).
4) Frandsen, N. O.: IV. Grundlagen und Methoden der Züchtung. A. Resistenzeigenschaften und ihre Vererbung. 1. Resistenzzüchtung. *Handbuch der Pflanzenzüchtung*, **3**, 71–97 (1958).
5) Hadwinger, L. A. & Schwochau, M. E.: Host resistance responses—an induction hypothesis. *Phytopath.*, **59** 223–227 (1969).
6) Katsuya, K. & Kiyosawa, S.: Studies on mixture inoculation of *Pyricularia oryzae* on rice. 2. Inter-strain difference of mixture inoculation effect. *Ann. Phytopathol. Soc. Japan*, **35**, 299–307 (1969).
7) Kiyosawa, S.: Pathologico-genetic aspect of specific disease resistance in plant. *Shokubutsu Boeki (Plant Prot.)*, **19**, 353–360 (1965). [In Japanese.]
8) Kiyosawa, S.: Genetic studies on host-pathogen relationship in the rice blast disease. Proc. Symp. "Rice Diseases and Their Control by Growing Resistant Varieties and Other Measures". Tokyo, 137–153 (1967).
9) Kiyosawa, S.: Inheritance of blast-resistance in Chinese rice varieties and their derivatives. *Japan. J. Breed.*, **18**, 193–205 (1968).
10) Kiyosawa, S.: The present condition and problems of the epidemic studies on crop diseases. *Shokubutsu Boeki (Plant Prot.)*, **23**, 10–15 (1969). [In Japanese.]
11) Kiyosawa, S.: Genetic properties of specific disease resistance. *Shokubutsu Boeki (Plant Prot.)*, **23**, 465–471 (1969). [In Japanese.]
12) Kiyosawa, S.: The concept of true resistance and field resistance. *Nogyo Gijutsu (Agr. Tech.)*, **25**, 21–25 (1970). [In Japanese.]
13) Kiyosawa, S.: Inheritance of blast resistance of the rice varieties Homare Nishiki and Ginga. 1. Resistance of Homare Nishiki

and Ginga to the fungus strain Ken 54-04. *Bull. Natl. Inst. Agr. Sci. Japan*, **D21**, 73–105 (1970).

14) Kiyosawa, S.: A presumption of semi-fine structure of resistance and avirulence genes on rice blast disease. *Nogyo Gijutsu* (*Agr. Tech.*), (1970). [In Japanese.]

15) Kiyosawa, S., Matsumoto, S. & Lee S. C.: Inheritance of resistance of rice variety Norin 22 to two blast fungus strains. *Japan. J. Breed.* ,**17**, 1–6 (1967).

16) Konzak, C. F.: Genetics in plant breeding. *Brookhaven Symp. Biol.* **9**, 157-176 (1956).

17) Loegering, W. Q. & Powers, H. R. Jr.: Inheritance of pathogenicity in a cross of physiologic races 111 and 36 of *Puccinia graminis* f. sp. *tritici*. *Phytopath.*, **52**, 547–554 (1962).

18) Moseman, J. G.: Host-parasite interaction of the gene for resistance in *Hordeum vulgare* and for pathogenicity in *Erysiphe graminis* f. sp. *hordei*. *Phytopath.*, **49**, 469–472 (1959).

19) Powers, H. R., Jr. & Sando, W. J.: Genetic control of the host-parasite relationship in wheat powdery mildew. *Phytopath.*, **50**, 454–457 (1960).

20) Schaller, C. W., Holton, C. S. & Kendrick, E. L.: The inheritance of the second factor for resistance to bunt, *Tilletia caries* and *T. foetida*, in the wheat variety Martin. *Agron. J.*, **52**, 280–282 (1960).

21) Scheffer, R. P., Nelson, R. R. & Ullstrup, A. J.: Inheritance of toxin production and pathogenicity in *Cochliobolus carbonum* and *Cochliobolus victoriae*. *Phytopath.*, **57**, 1288–1291 (1967).

22) Scheffer, R. P. & Pringle, R. B.: Pathogen-produced determinants of disease and their effects on host plants. The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction (ed. by Mirocha, C. J. and Uritani, I.), 217–236 St. Paul, Minnesota (1967).

23) Ullstrup, A. J. & Brumson, A. M.: Linkage relationship of a gene in corn determining susceptibility to a Helminthosporium leaf spot. *Jour. Am. Soc. Agron.*, **39**, 606–609 (1947).