Proposal for Standardized Nomenclature and Identification of Races of *Plasmopara halstedii* (Sunflower Downy Mildew)

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Abstract

A proposal, formulated by pathologists from six countries, is made for a standardized set of nine, publicly available lines to be used as differential lines to identify races of sunflower downy mildew (*Plasmopara halstedii*). In addition, a nomenclature system using a triplet code which is concise and easy to use is presented. It should allow pathologists to name unambiguously a new race and at the same time convey information on its virulence pattern.

Introduction

Until 1980, the situation with races of *Plasmopara halstedii*, the obligate pathogen causing downy mildew of sunflower, was very simple, because only two races were known. Since that time, however, there have been a multitude of races identified, first in North America (Gulya et al., 1991b; Rashid, 1993), and subsequently in several European countries following extensive surveys (Gulya et al., 1991 b ; Spring et al., 1994 ; Viranyi and Gulya, 1995 ; Roeckel-Drevet et al., 1997), and ultimately in all countries where sunflower downy mildew has been recorded. The use of a variable number of lines to characterize races, and the absence of a universally accepted nomenclature system has led inevitably to a situation where information on races was not readily understandable by sunflower researchers in other countries. Sackston et al. (1990) summarized sources of resistance known in 1990 and what was known of the genetics, and suggested a system of race nomenclature in which the virulence pattern (a list of the total number of resistance genes attacked) was used for race identification. Gulya et al. (1991a) expanded upon this by proposing a set of 15 lines, and continued the practice of identifying races according to a list of the genes they were virulent upon. When resistance genes in a given line had not been verified, a letter designating the anticipated gene was used. Thus, what was once referred to as race 5, a very virulent race, was designated as race 1, 2, 3, 4, 5, a, b, c, d, e. This denoted that race 5 was virulent on *Pl*1, *Pl*2, *Pl*3, *Pl*4, and *Pl*5, and genes a, b, c, d, and e, whose inheritance had not been demonstrated conclusively in printed research.

While this nomenclature system presented exact information on virulence, it obviously led to cumbersome race labels which would be difficult to remember and awkward to use in print or speech. In addition, segregation studies have shown that some *Pl* genes are, in fact, clusters of genes, each giving resistance to one or a few races. (Vear et al, 1997). It is therefore not possible, at present, to use gene names to name races. Gulya (1995) suggested adopting a triplet coding system as proposed by Limpert and Muller (1994) for plant pathogens in general. The set of differential lines was decreased from 15 to nine lines, each having a different profile of reactions to presently known races. The nine lines are grouped into three sets of three lines each. The nine differentials are all publicly available inbred lines (or composites) and have been distributed to researchers around the world. The adoption of the triplet coding system, however, has been slow, and many publications still employed either a numerical race name or referred to new races as variants of existent races (Rashid, 1993). With the proliferation of new races, the need for international standardization became more crucial.

The following proposal is the summation of a discussion held at the conclusion of the ISA Downy Mildew Symposium, and is believed to be an accurate synopsis of the final consensus of opinion reached between participants.

Standardized set of differentials

Several requirements for ideal differential lines were proposed. The genotypes should be (1) publicly available, (2) fixed inbred lines, rather than hybrids or composites, (3) offer consistent downy mildew reactions, (4) and preferably have had some classical or molecular research into the genetics of downy mildew resistance. In some cases, lines with supposedly identical downy mildew reactions are known (e.g. Rha-265, Rha-266, and AD-66 all resistant only to race 1 = European race) and have been used interchangeably by researchers. From the standpoint of uniformity, however, the group agreed that a single line, with no substitutions, should be used. Additionally, because many of the proposed lines were originally released up to 25 years ago, with the possibility of divergent selections existing, it was agreed that a single seed source be used for distribution. Requests for seed should therefore be made directly to the breeders (J. Miller, USDA; D. Skoric, IFVC; F. Vear, INRA).

The nine differentials will be grouped into three sets of three lines. The first set will consist of three USDA lines. HA-304, proposed as the universal susceptible, is a single-headed confection line which has been shown to have consistent susceptibility and profuse sporulation with all races, also making it an excellent choice for producing single-spore isolates. The other two lines are also USDA releases, namely Rha-265 and Rha-274. Rha-265 has resistance to race 1, Rha-274 is known to confer resistance to races 1 and 2 (Red River race).

The second and third set of differentials are different from those previously proposed. The second set will consist of PMI3, PM-17 and 803-1. PMI3, an INRA selection of the USDA composite DM-2, was felt to offer more uniformity in downy mildew reaction because it is a fixed line as contrasted with DM-2. PM-17, a line selected from PI 406022 by Rama Urs of Dahlgren, is proposed as a substitute for IS-2000, the latter a proprietary line of Interstate (now Advanta). When Pioneer Hi-Bred Int. acquired Dahlgren research and its seed inventory, Pioneer officials agreed to let this line be distributed publically as a differential line. The line 803-1 was selected by the breeding staff of the Institute of Field & Vegetable Crops, Novi Sad, Yugoslavia. Ljubich (1989) demonstrated that 803-1 possess resistance to downy mildew races 1, 2, 3, and 4, but was susceptible to the then newly discovered race 5.

The third set of differentials consists of HAR-4, QHP1 and HA-335. HAR-4 is a USDA synthetic bred from an Argentine genotype developed by INTA (Saenz Pena 74-1-2). All five HAR synthetic were originally selected for resistance to four North American races of rust (*Puccinia helianthi*), with HAR-4 and HAR-5 noted as having resistance to all downy mildew races known at the time of their release. QHP1 was selected from a cross including HAR-5 made by INRA. Lastly, the line HA-335 is proposed as the line which at the moment is resistant to all known mildew races.

We have decided to give the nine lines new designations to simplify the system. The differentials will be referred to as D-1 through D-9. Since the USDA and INRA system of naming germplasm is very different, and there are already two lines with very similar designations (PMI3 and PM-17), we felt that it would be advisable to give the differentials a uniform and simple label. The new designations, original names, and pedigrees of the differentials are found in Table 1.

Table 1 : Sunflower lines proposed as differentials for downy mildew race identification

Designation	Original Name	Pedigree	Source of Resistance
D-1	HA-304 (USDA)	Commander	None
D-2	Rha-265 (USDA)	Peredovik/ 953-102	953-102 (Canada)
D-3	Rha-274 (USDA)	HA-119/ HA-62	953-88 (Canada)
D-4	PMI3 (INRA)	selection of DM-2	Novinka (Russia)
D-5	PMI-17 (USDA/Dahlgren)	PI 406022	? (Iran)
D-6	803-1 (USDA)	*H. tuberosus* cross	*H. tuberosus* (Yugo)
D-7	HAR-4 (USDA)	Saenz-Pena 74-1-2 selection	? (Argentina)
D-8	QHP1 (INRA)	HAR-5 x PRS7 selection	Guayacan INTA (Argentina)
D-9	HA-335 (USDA)	HA-89 x	wild *H. annuus*

Supplemental differentials

In an attempt to make the identification of mildew races as convenient as possible, the initial set of differentials will be limited to nine. Publicly available lines, presumably with other resistance genes, are known, and could be employed, but it was felt these additional lines could be relegated to supplemental status. Such lines include HIR34 and Rha-325 (susceptible to race 6, whereas Rha-274 is resistant). The remainder of the HA-335 series should also be considered as supplementary differentials, and would be especially useful when an isolate is found that is virulent on HA-335. As HA-337, HA-338 and HA-339 derive their resistance from *Helianthus praecox*, and Rha-340 derives its resistance from *H. argophyllus*, there is strong probability that the lines possess different genes, although research supporting this inference is lacking. The inbred line YVQ (INRA), whose resistance is derived from HA-335, is at present the opposite of Rha-274, since it is resistant to all races except 1 and D. The line QPR2 (INRA), with resistance derived from Progress via the USDA synthetic DM3, may also be necessary, since it appears to distinguish races A and 4. Work in progress by INRA and USDA pathologists will determine whether QPR2 could replace PM-17 in the standard set of differentials, or possibly if both are needed.

Addition of new differentials in the future:

To insure continuity in race naming, it is suggested that the present nine differentials be used from now forward. Once it becomes apparent that new lines are necessary to characterize new mildew virulence patterns, we must add a complete new set of three lines in order to continue using the triplet code. Our thoughts are that the differential lines can be reviewed and discussed every four years at the general meeting of the International Sunflower Association (e.g. in Toulouse, France in 2000).

Triplet coding system

An essential component of this proposal is the triplet coding system, which numerically assigns a value if a differential is susceptible, and thus conveys information on the virulence of an isolate. If the first line of a set of three is susceptible, it imparts a value of 1 ; if the second line is susceptible it imparts a value of 2 ; and if the third line is susceptible it imparts a value of 4. The virulence code is additive within each set, so that if the first and second line are susceptible, they impart a value of 1 + 2, or 3. To continue the example, if the first and third lines are susceptible, they would impart a virulence code of 1 + 4, or 5, while if all three lines were susceptible the virulence code would total a 7. This is graphically illustrated in Table 2, where the triplet code for historical races 1 and 2 is given, along with a fictitious race. In this system the higher the triplet code is, the more virulent the race. The final virulence code or race name of an isolate will be a 3-digit code, one digit from each of the three sets of lines.

Table 2 : Triplet coding system for defining sunflower downy mildew races adapted from Limpert and Muller (1994)

Differential Line	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10	Triplet Code
Value if **S.**	1	2	4	1	2	4	1	2	4		
Race 1	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**		
		1+0+0=**1**			0+0+0=**0**			0+0+0=**0**			**100**
Race 2	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**		
		1+2+0=**3**			0+0+0=**0**			0+0+0=**0**			**300**
Race ?xyz?	**S**	**S**	**S**	**S**	**R**	**S**	**R**	**S**	**R**		
		1+2+4=7			1+0+4=**5**			0+2+0=2			**752**

S = susceptible; **R** = resistant; Race xyz = hypothetical race

Testing methods and interpretation of results

In order for independent laboratories to characterize mildew isolates for their race reaction and achieve the same results, there must be some standardization in methods and interpretation, but this does not imply that everyone must follow the same, exact procedure. There are currently many variations in the whole seedling immersion technique, with differences in inoculum concentration, inoculation period, growing medium, etc. This topic has been reviewed by Gulya et al. (1991), Mouzeyar et al. (1993, 1994) and Gulya (1996).

First, it is recognized that there are more than two discrete phenotypes following downy mildew infection by seedling immersion. Seedling reaction may fall into at least four categories, as demonstrated by Vear (1978), Wethje et al. (1979), Ljubich (1989), Sackston et al. (1990) and Mouzeyar et al., (1993, 1994). These are :

(#1) The fungus invades a very few cells, resulting in a hypersensitive reaction, with no microscopic evidence of colonization or sporulation at all.

(#2) The fungus colonizes the radicle and hypocotyl, but there is no sporulation on either the cotyledons or true leaves.

(#3) Sporulation on cotyledons only, where the fungus colonizes the radicle, hypocotyl and cotyledons, but stops at the cotyledonary node. This can be due either to the presence of a resistance gene (Cotyledon-Limited-Infection: CLI) or to unfavorable test conditions, resulting in sporulation on the cotyledons but nothing on the true leaves of susceptible genotypes.

(#4) Fully-systemic infection, in which the fungus colonizes the entire plant, with stunting, and sporulation on cotyledons and true leaves.

It is generally accepted that categories #1 and #2 are a valid expression of resistance, and the category #4 is susceptible, but interpretation of category #3 (sporulation on cotyledons only) varies according to author. For this reason, it was decided to conduct a further study to evaluate all suggested lines with a wide range of mildew races, and then to publish their findings in a refereed journal. This future publication will build upon this present proposal, but will present a defined inoculation methodology, and will also present information on exact symptomotology of all differentials with a wide range of mildew races.

One last point which needs to be stressed is the uniformity of infection on all differentials. Assuming that all lines are homozygous for mildew genes, and that the seedlots are free from contamination, then one would expect to see either all seedlings resistant (although phenotypes may vary between classes #2 and #3 above) or all seedlings susceptible. If infection is variable in all lines, the conclusion should be that inoculation conditions were less than optimal, and the tests should be repeated. If infection is variable on some, but not all lines, this would suggest that the inoculum is a mixture of more than one race. In this case, the only way to correctly identify the race is to create single sporangial isolates

Conclusion

A set of nine lines, developed by the USDA, IFVC and INRA, are suggested to be used as differentials to identify races of *Plasmopara halstedii.* The use of a triplet coding system, coupled with worldwide use of the nine lines, will allow unambiguous naming of races. As a start, the authors undertake to characterize their collections of downy mildew races using this system and to test, the supplementary differentials to determine their interest. The proposed system should be reviewed periodically and the addition of new lines considered.

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