Unstable Aneuploid Progenies of *Phytophthora ramorum*[1]

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Abstract

Phytophthora ramorum has three populations belonging to the same species: the two North American populations (NA1 and NA2) and the European population (EU1). All three populations are genetically distinct lineages, as revealed by different molecular marker systems. *P. ramorum* is also heterothallic, with two opposite mating types, A1 and A2. All NA1 and NA2 isolates are of the A2 mating type. All EU1 isolates have been identified as A1, except for three A2 isolates collected in Belgium in 2002 and 2003. Since 2004, no isolates of the A2 mating type have been reported in Europe, which limits the risk of sexual recombination in Europe. In contrast, several EU1 isolates have been reported in nurseries in North America, some even at sites where NA1 isolates were also found. Although sexual reproduction in nature has not been observed yet, the presence of isolates of both mating types at a single site might lead to genetic recombination, which could lead to an increase in pathogen fitness and host range. The formation of sexual resting oospores might also increase the long-term survival of *P. ramorum*. The ability of plant pathogen populations to respond to novel challenges from their environment, such as host resistance and pesticide exposure, depends on their mechanisms for generating genetic variation.

Germinating oospores have been obtained *in vitro* from crosses between EU1-A1 and NA1-A2 isolates as well as between EU1-A1 and EU1-A2 isolates (Boutet and others, unpublished), but at a low frequency. To determine the nature of sexual recombination in *P. ramorum*, germinating oospores of a cross between an NA1-A2 isolate and an EU1-A1 isolate were genotyped with microsatellite markers that were heterozygous in the parental isolates. All crosses contained alleles of both parents, confirming exchange of genetic material, but meiotic irregularities occurred frequently. Non-Mendelian inheritance events were also observed. This included inheritance of more than two alleles at a single locus or loss of alleles from one of the parents at a separate locus. The aneuploid progeny was also mitotically unstable: zoospore and hyphal tip derivatives of the progenies showed genotypic rearrangements as well as phenotypic variation. These data indicate that progenies from crossings between *P. ramorum* isolates are often aneuploid and genetically unstable.

Introduction

Phytophthora species are known to exhibit phenotypic variation during asexual growth. This is due to genome instability caused by transposable elements, gene conversion, mitotic recombination, and/or dispensable chromosomes (Chamnanpunt and others 2001, Francis and St. Clair 1997, Goodwin 1997, Judelson 2002). Additional variation can be created if the sexual cycle in *P. ramorum* is functional;

exchange of genetic material from both parents can result in an increased fitness and host range. Non-Mendelian inheritance is not uncommon in *Phytophthora* species (Förster and Coffey 1990, Carter and others 1999, Dobrowolski and others 2003, Judelson 1996, Judelson and others 2002, Van der Lee and others 2004). Sexual progeny of *P. cinnamomi* and *P. sojae* were also mitotically unstable. Sectoring in colonies has been seen in progeny of *P. cinnamomi* (Zheng and Ko 1996, Dobrowolski and others 2003). This morphological variation could be associated with the loss of alleles in the asexually derived subcultures. Chamnanpunt and others (2001) reported that in hybrid strains of *P. sojae,* unique genetic types could be rapidly generated by mitotic gene conversion. Mitotic gene conversion resulted in the reassortment of loci that show close linkage during meiotic recombination at remarkably high frequencies, as high as 3.10^2 conversions per locus per nucleus per generation. The objective of this study was to determine whether this type of genetic instability is also observed in progeny of *P. ramorum*.

Materials and Methods

In total, 41 oospore progenies from pairings between complementary *P. ramorum* isolates were analyzed with microsatellite markers, among which 28 progenies originated from EU1 x EU1 pairings, and 13 originated from EU1 x NA1 pairings. Separate subcultures were made from five different growth sectors within the colony of an EU1 x NA1 progeny. Separate single zoospore cultures were also created from different progeny. DNA was extracted from each subculture. The microsatellite marker profiles of the parents, the original progeny, and all subcultures were determined as described in Vercauteren and others (unpublished). Eight loci (KIPrMS18, KIPrMS64, KIPrMS82a-b, ILVOPrMS133, and ILVOPrMS145a-c) were explored for allelic rearrangements. When no difference between the progeny and the parents was detected for these loci, extra microsatellite markers were used for further genotyping.

Results and Conclusions

Microsatellite analysis revealed that each of the 13 EU1 x NA1 progenies contained alleles of both parents. In 20 EU1 x EU1 progenies, loss of heterozygosity was observed for at least one of the eight microsatellite loci analyzed. These progenies show the loss of at least one allele from the loci that are heterozygous in the parents as a result of becoming homozygous. For the eight other EU1 x EU1 progenies, up to seven extra microsatellite markers were used, after which rearrangements were observed in all of them. Therefore, microsatellite fingerprinting profiles showed genetic rearrangements in all progenies. Based on this data there is evidence that all progenies were recovered from germinating oospores and that the sexual cycle in *P. ramorum* is functional, with an exchange of genetic material from both parents. Selfing was not detected in any of the EU1 x NA1 progenies. Non-Mendelian inheritance of alleles was common. In all EU1 x NA1 progenies at least one locus contained alleles from only one parent or had more than two alleles.

Mycelial colonies from germinating oospores often contained sectors with a reduced growth rate. Microsatellite marker analysis confirmed genetic rearrangements in these sectors. The separate single zoospore cultures derived from one progeny also had differences in allele number at some loci. However, genotypic changes may have

occurred prior to zoosporogenesis, during growth of the subculture. Genotypic differences in these single-zoospore subcultures were correlated with phenotypic differences. In contrast to the genetic rearrangements observed in the mycelium and zoospore cultures derived from the progeny, no such rearrangements were observed in the parental strains, even after years of storage and subculturing.

The likelihood of sexual recombination of *P. ramorum* in nature is very low, but the risk remains. Mitotic gene rearrangements after sexual recombination may result in a wide genetic diversity. This could then lead to the development of strains that are adapted to new environmental challenges or different hosts.

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