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par

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Genetics of *Phytophthora palmivora*
Some preliminary data

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Material and Techniques

The strains used for this study were monozoospore clones of isolates K, 570 L and Fo respectively derived from Sweet Orange, Rough Lemon Egg plant and Rosella (Hibiscus sabdariffa).

Offsprings of crossings beetween these strains were genetically studied with the purpose of definiting general genetical characteristics of this material. First results led us to perfect a method now used for the study of pathogenic characteristics transmission through sexual reproduction.

a) Genetical markers

For each crossing, we have studied the segregation of two features.

The first one was the mating type, strains K and 570 being AI, L and Fo A2.

In K x L crossings, the parental strains differed by their ability to attack and kill young plants of Rosella. L strain, inoculated as a zoospore suspension on the collar of 8 days old plants, kills all of them in less than a week. On the contrary, K strain only causes a collar and stem weak necrosis and plants outlive this attack in most cases.

In 570 x Fo crossings, parental strains differed by their thallus morphology. On PDA medium, Fo develops an exclusively intramatricial mycelium and an irregularly outlined colony, while 570 gives a circular thallus with dense aerial mycelium.

Some mutant strains were also used. They were obtained through U.V. irradiation of L zoospores suspensions. Graphic I shows the survival curve of these zoospores, according to the irradiation dose. A dose of 500 ergs/mm2 was used, later on, for the research of mutants. Among about 4.103 survivors, we have observed some clones with altered characteristics, generally morphological ones. Most of them revealed themselves unreliable and, after a few weeks of culture, showed a reversion to the wild phenotype, thus showing that U.V. treatment had likely produced mere cytoplasmic modifications. In our research of biochemical mutants among these survivors, we used the technique of Total Isolation and we have been able to isolate and maintain two arginine auxotrophic strains, numbered LI and L2.

b) Crossing and oospores germinating methods

Crossings were realised on Pea broth Agar in 10 cm Petri dishes. Cultures were maintained in the dark during 15 days, at a temperature of 26°C, then exposed to fluorescent light for one month. After this treatment oospores were

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separated from the mycelium by mincing and filtration. They were spred one by one on the germinating medium by pipetting. Plates were daily observed during five days and offsprings taken off and cultured on slants of Pea Broth Agar, with or without arginine added. Germination rates thus obtained, according to the tested cross, varied from 10 to about 20 %.

Results

The results of crossings are detailed in tables I to 4. Tables I and 2 respectively give the repartition of numbers for each characteristic and the repartition for each combination of two features.

a) Distribution of mating type Repartition of mating types among offsprings leads to point out that this characteristic is not monogenic. Indeed, in the case of an haploid cycle, the FI expected ratio for a monogenic characteristic is I/I; in the case of a diploid cycle, F.I should be homogenous or with a segregation ratio of 2/I. Our results do not fall in with any of both possibilities.

Offsprings of crossings 570 x Fo (table 2), 570 x L (table 3), 570 x LI and 570 x L2 (table 4) show an important unbalancing of mating type ratios, always at the advantage of AI type.

Among offsprings of crossings K xL (table I), K x LI and K x L2 (table 4), the ratio AI/A2 is respectively 34/60, 46/22, 70/48. We can observe an inversion of ratios beetween K x L and K w LI, K x L2 crosses.

The simplest hypothesis to account for these inverted ratios is that the use of mutant strains introduces a selection factor in crossings, which interferes with germination of oospores bearing it.

Either in the case of haploid cycle, or in the case of diploidy observed ratios for mating types cannot be interpreted easily. However selfing possibilities, as demonstrated by Brasier, seem to point out that, in a crossing, the part of each parent is to suppress the autosterility of its partner. Each confrontation can then lead to the formation of hybrid oospores and, at once, of autofecondated oospores. If the autofecondation rate of the two parents is not the same, the advantaged strain will produce more oopores by this mean, with consequently the appearance, among offsprings, of an excedentary parental class. This hypothesis would allow to explain the disturbed ratios of crossings 570 x Fo, 570 x L, 570 x LI and 570 x L2 where the AI/A2 ratio is about 10/I.

The same hypothesis could also account for the inverted ratios beetween offsprings of K x L and those of K x LI and K x L2. The A2 type, in a minority among progeny of the last crossings, is that of the mutant parent. Now, in addition to their arginine auxotrophy, LI and L2 strains manifest a lowered physiological activity, as expressed especially in a growth rate lower than of the original

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L stain. It is therefore possible that the autofertility rate of these strains can also be lowered by the mutation, which could explain the weak percentage of A2 strains among offsprings of these two crosses.

b) Distribution of phenotypes and problems of heterocaryosis

Phenotypes of table 2 were determined directly on thallus derived from germinating oospore. However, we have tested the reliability of these phenotypes on series of monozoospores clones established from a large number of these offsprings and all germlings with AI or A2 mating type prove to be homocaryotic.

On the contrary, the same analysis among AIA2 offsprings has showed that some of them were heterocaryotic strains. A monozoospore clonage, acting by dissociation of the heterocaryon, is, in fact, sufficient to suppress the apparent homothallism, each new clone being either AI or A2. This fact is important because it leads to think that these AIA2 strains come from oospores with, at least, two fusion nuclei.

We must note, however, that heterocaryosis was proved among some AIA2 strains only. The other offsprings with the double mating type do not produce sporocystes or zoospores and they could not be cloned. Thus, we cannot reject the possibility that these strains be actually homothallic ones and not only heterocaryotic. There would be, then, recombination for the mating characteristic.

c) Behaviour of the second marker in KxL and 570 x Fo crossings

Among offspring of K x L crosses, 12 strains show a pathogenic activity towards Rosella which is different from that of parents. These strains manifest varying degree of virulence, expressed by death rates intermediary beetween those of parental strains. In the case of 570 x Fo cross (table 2) we also notice the presence of an offspring class, morphologically different from the parents. In fact this class gathers several different phenotypes distinct by growth rate, thallus morphology, aerial mycelium/immersed mycelium ratio or thallus sectorisation.

These facts lead to point out that these two characteristics, i.e. pathogenic ability and thallus morphology, are each one under the dependancy of several genes of which recombination give rise to new types.

The three markers thus far examined (mating type, pathogenicity and phenotypes) are polygenic features. Thus their study does not bring any definite information on the nature of the cycle of Phytophthora palmivora. It remains to be seen the segregation of the trophic type in crossings interesting the mutant strains LI and L2.

d) Segregation of Arginine trophic type We must, first of all, notice that the existence of these mutants, in the case of a diploid mycelium, set a problem. Indeed, as food deficiencies are generally recessive

characteristics, the mutant strain must be homozygotic Arg $^-$/Arg$^-$. It is difficult to admit that the two alleles of a same couple might have muted together, but we may suppose that the wild isolate L is already heterozygotic Arg $^+$/Arg$^-$ and that the mutation has hit the Arg$^+$ allele of this couple. In this case it is normal that F.I is homogenous and prototrophic (table 4) (with the hypothesis that parents are both homozygotic and the allele Arg$^+$ dominant). However we must confirm this hypothesis by an analysis of F.2 generations and a back cross. In the case of the back cross expected ratio Arg$^+$/Arg$^-$ is I/I and 3/I for F.2 strains.

Now, it appears in table 4 that all the progeny of these different crossings is homogenous and prototrophic. Thus our hypothesis is not corroborated and the lack of any Arg$^-$ progeny shows that at germination time, there is a selection factor interfering. It seems more than likely that oospores with Arg$^-$ nucleus do not germinate.

This preliminary study leads us to the following conclusions. First of all, it appears that our results do not allow to define the exact nature of the caryological cycle of Phytophthora palmivora. Secondly, we notice that our wild isolates have very different genomes. This is proved by the appearance, among progeny of some crossings, of an important percentage of offsprings showing a spreading of new characteristics. This heterogeneity expresses multiple recombinations. More over, the relative smallness of germination rates and the presence of numerous morphologically abnormal oospores and of empty oogonia show that crossings often lead to lethal genic combinations. We conclude therefore that our bilogical material must be isogenised before any new research. This task is now in progress.