

SEXUAL COMPATIBILITY IN *Trichometasphaeria turcica* LUTT

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ABSTRACT

In view of the scarcity of data available in the literature, especially in Brazil, concerning the genetics of *Trichometasphaeria turcica*, a comparative study of monoconidial and mono-ascosporic isolates of the fungus was conducted to determine the genetics of the sexual compatibility pattern starting from crosses *in vitro*.

The exact determination of the compatibility group is hampered by the great genetic heterogeneity of the nuclei present in the multinucleate conidia. The results of monoconidial crosses and the physical factors that may be operating for successful crossing are discussed.

INTRODUCTION

Trichometasphaeria turcica is a heterothallic fungus whose perfect stage (sexual form) is not found in nature but can be readily induced in the laboratory by crossing two sexually compatible isolates. Sexual compatibility in *T. turcica* has been scarcely documented in the literature. The first study on this topic was published by Luttrell (1959), who obtained approximately 1:1 segregation for the "plus and minus" conjugant types, suggesting that the sexual reaction is determined by a pair of genes. The same investigator, however, postulated the existence of one or more secondary genes that may act on the intensity of sexual reaction.

Nelson (1959a) also obtained 1:1 segregation among 218 monoconidial isolates from 5 crosses and 210 monoascosporic isolates from 6 different crosses. The

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author believes that the sexual process may be controlled by large numbers of genes. Hamid and Aragaki (1975), in a study of the sexual compatibility of 366 mono-ascosporic isolates, obtained 1:1 segregation for conjugant types, concluding that sexual compatibility in *T. turcica* is only controlled by a pair of genes.

In Brazil, Hermes (1979) was the first investigator to try to obtain *T. turcica* ascospores in Rio Grande do Sul. Among 5 isolates, the author obtained 2 that were sexually compatible, thus confirming the heterothally of the fungus also for Brazilian isolates and opening new perspectives with respect to the perfect stage of the fungus and its viability in the laboratory. The objective of the present investigation was to study the sexual compatibility pattern of the fungus starting from crosses *in vitro*.

MATERIALS AND METHODS

Monoconidial crosses

Monoconidial cultures were crossed amongst themselves to obtain pseudothecia and ascospores isolates and to study sexual compatibility. Eight samples from different locations in the State of Rio Grande do Sul were used and numbered as follows according to their origin: 2, Sto. Antônio da Patrulha; 20, Uruguaiana; 25, Pelotas; 30, Pelotas; 35, Pelotas; 40, Pelotas (sorghum); 45 Guaíba; 50, Pelotas.

Approximately 10 barley grains, previously autoclaved at 120°C and 1.5 atm pressure, were placed on Petri dishes containing Sach agar, and an inoculum of a different monoconidial culture was placed on each side. The same procedure was repeated using agar-water with a fragment of maize leaf, which was also autoclaved and placed on the dish. Two replications of each monoconidial cross were performed on Sach agar (Luttrell, 1958) and two on agar-water (20% agar in 1000 ml water). Modified Sach agar (Nelson, 1959a) was also tested, but with no improved results. The dishes containing the crosses were sealed with 3-cm wide cellulose tape and placed in an incubator at a constant temperature of 25°C for 25 ± 5 days, after which they were examined and ascospores were isolated.

Monoascosporic crosses

Ascospores isolated from the monoconidial crosses were crossed amongst themselves by the same technique as described above (Luttrell, 1958). Sixteen of the 33 ascospores from cross 20 x 30 were utilized and were isolated as follows: from pseudothecium 6, the 7 ascospores from a single ascus; from pseudothecium 7, ascospores nos. 16, 22 and 30; from pseudothecium 1, ascospores nos. 8, 10 and 13; and from pseudothecium 3, ascospores nos. 9, 11 and 14.

After the sexual compatibility group of these 16 ascospores was determined,

4 of them (nos. 4, 3, 20 and 13) were selected and crossed with the ascospores obtained from two other crosses, 35 x 40 and 20 x 35. Ascospores nos. 41, 42 and 43 from cross 35 x 40, and ascospores nos. 38 and 40 from cross 30 x 35 were used. The ascospores originating from these two crosses, in addition to being crossed with the 4 initial ascospores (4, 3, 20 and 13), were also crossed with one another.

Backcrosses

To confirm and exploit the advantages of this method, all of the 16 ascospores used in the crosses were backcrossed to their respective parents using the technique described above.

RESULTS

Monoconidial crosses

The data obtained from the crosses between the 8 samples are shown in Table I. Results were considered to be positive when young or ripe pseudothecia were produced, in small or large numbers, and even when the pseudothecia did not show a well-developed ascigerous mass or ascospores.

Analysis of the contents of the pseudothecia present on the 64 plates showed the absence of ripe ascospores, with only young ascospores being present. There was a wide variation in the number of pseudothecia, a fact that led us to classify the crosses as weak or strong according to the production of pseudothecia, in agreement with Luttrell (1958).

Additional tests showed that a cross may be positive in one or two replications and produce no pseudothecia in others under the same laboratory conditions, indicating the importance of the physical medium for the development of sex structures, regardless of the genetic factors involved.

The determination of the sexual compatibility groups for the monoconidial isolates of *T. turcica* was hampered by the fact that crosses of isolates theoretically having the same compatibility group exhibited developing pseudothecia or stromas.

Time for pseudothecium ripening was longer among the isolates considered weak than among the isolates with abundant production of sex structures. The absence of sexuality in many compatible crosses in contrast to the exuberant production of pseudothecia in others showed that *T. turcica* isolates vary in compatibility level.

Monoascosporic crosses

In contrast to the data reported by Knox-Davies and Dixon (1960), the

Table I - Comparison of 8 *T. turcica* samples crossed in all possible combinations in terms of pseudothecia production.

Compatibility group	Sample	Reaction of crosses between samples							
		A				a			
		2	30	50	35	20	25	40	45
A	2	-	-	-	D	+	+	+	0
	30	-	-	D	-	+	0	0	+
	50	-	D	-	-	+	+	+	+
	35	D	-	-	-	+	0	+	+
a	20	+	+	+	+	-	D	-	-
	25	+	0	+	0	D	-	-	-
	40	+	0	+	+	ü	-	-	D
	45	D	+	+	+	-	-	D	-

-, Absence of pseudothecia in comparisons of samples from the same compatibility group.

+, Production of pseudothecia in comparisons of samples from groups of opposite compatibility.

0, Absence of pseudothecia in comparisons of theoretically compatible samples: groups of opposite compatibility.

D, Production of pseudothecia in comparisons of samples from the same comparibility group.

material obtained from sexual reproduction of *T. turcica* was easy to handle and showed great laboratory viability. Pseudothecia, asci and ascospores were identified in a relatively easy manner and their morphological structures corresponded exactly to those described by Luttrell (1958).

The number of asci and ascospores was variable, and in many crosses only the initial formation of the stroma of the pseudothecium was observed. Cross 20 x 30 showed the best production and was selected for the present study.

The monoascosporic crosses consisted of 7 ascospores selected at random from 3 different pseudothecia. The results are shown in Table II and permitted us to determine the compatibility group as follows: when a positive cross was detected, one of the parents was given indiscriminately the letter "A" and the other parent the letter "a". Thus the ascospores arbitrarily numbered 1, 3 and 7 from the same ascus belong to one group, and those numbered 2, 4, 5 and 6 to the opposite group. Of the remaining 9, nos. 8, 13, 14 and 16 belong to one group, and nos. 9, 10, 31 and 33 to the opposite group. No. 11 did not cross with any ascospore and remained undetermined.

Table II - Comparison of 16 monoascosporic isolates crossed in all possible combinations and resulting from the 20 x 30 cross of *T. turcica* in terms of pseudothecia production.

Compatibility group	Isolate	Reaction of the cross															
		a								A							
		1	3	7	8	13	14	16	2	4	5	6	9	10	22	33	
a	1	-	-	-	-	-	-	-	+	0	0	0	0	+	0		
	3	-	-	-	-	-	-	-	+	+	+	+	+	0	+	0	
	7	-	-	-	-	-	-	-	+	+	+	+	0	0	+	0	
	8	-	-	-	-	-	-	-	0	0	0	0	+	0	+	0	
	13	-	-	-	-	-	-	-	0	0	0	0	0	0	+	+	
	14	-	-	-	-	-	-	-	+	+	0	0	0	0	+	0	
A	16	-	-	-	-	-	-	-	0	0	+	0	+	0	+	0	
	2	+	+	+	0	0	+	0	-	-	-	-	-	-	-	-	
	4	0	+	+	0	0	+	0	-	-	-	-	-	-	-	-	
	5	0	+	+	0	0	0	+	-	-	-	-	-	-	-	-	
	6	0	+	+	0	0	0	0	-	-	-	-	-	-	-	-	
	9	0	+	0	+	0	0	+	-	-	-	-	-	-	-	-	
	10	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	
	22	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	
	33	0	0	0	0	+	0	0	-	-	-	-	-	-	-	-	

-, Absence of pseudothecia in comparisons of isolates from the same compatibility group.

+, Production of pseudothecia in comparison of isolates from groups of opposite compatibility.

0, Absence of pseudothecia in comparisons of isolates from groups of opposite compatibility.

The compatibility group of parents 20 and 30 was identified by backcrossing. The letters "A" and "a" assigned to the sexual compatibility group refer to the alleles genetically responsible for this characteristic.

Few ascospores were obtained from crosses 35 x 40 and 35 x 20: nos. 41, 42 and 43 from the former, and nos. 38 and 39 from the latter. Nos. 41, 43 and 38 were found to belong to one compatibility group, and nos. 32 and 39 to the opposite group.

DISCUSSION

In agreement with the literature, the present data show that monoconidial

and monoascosporic isolates can be crossed *in vitro*. The 1:1 ratio observed for the sex compatibility group among the seven ascospores obtained from the same ascus and from others selected at random agrees with the data reported by Luttrell (1958), Nelson (1959a) and Hamid and Aragaki (1975), and confirms the presence of one gene for the compatibility locus.

The quantitative differences measured through the strong or weak expression of the sexual reaction, i.e. through the variation in number of pseudothecia, asci and ascospores produced, indicate that secondary genes influence the expression of the compatibility locus. If this were not the case, all A x a combinations should show similar reactions when crossed under the same laboratory conditions.

The fact that the same isolate showed some differences when crossed with an isolate from a group of opposite compatibility and marked differences in the degree of compatibility suggests that several genes control this variation. The existence of other loci that control some steps in the reproductive process makes the compatibility locus a simple link in the reproductive chain.

According to Esser and Kuenen (1967), in many ascomycetes a series of genes is involved in the differentiation of sex organs and fructiferous bodies. On the other hand, in ascomycetes in general there are at least three specific stages of sexual reproduction during which genetic factors can inhibit development. Nelson (1970), in a study of sexual reaction in *Cochliobolus carbonum*, raised the following hypothesis for the role of secondary genes in the sex cycle: "sexual reproduction only operates if all biochemical pathways function in sequence". According to the same authors (Nelson, 1959b,c), the compatibility locus may consist of several genes which are transferred as a single unit during the divisions. The correct gene sequence for reproduction may be obtained by genetic recombination. The mechanism which interferes with the formation of perithecia between compatible isolates of *C. heterostrophus* may be controlled by a single gene independent of the compatibility locus.

In *T. turcica*, the presence of other genes intervening in the sex process may explain the behavior of heterokaryotic monoconidial isolates by disrupting the equilibrium of the number of genes responsible for an ideal response. Thus, on the basis of the known fact that sexual reproduction in Ascomycetes is a class characteristic, it is viable to propose the presence of secondary genes that act on the sexual reaction in *T. turcica* also.

RESUMO

Em vista da escassez de dados disponíveis na literatura, especialmente no Brasil, a respeito da genética do *Trichometasphaeria turcica*, um estudo comparativo de monoconídios e monoascosporos isolados do fungo foi conduzido para determinar a genética do padrão da compatibilidade sexual começando com cruzamentos *in vitro*.

A determinação exata do grupo de compatibilidade é dificultada pela grande heterogeneidade genética do núcleo presente nos conídios multinucleados.

O resultado de cruzamentos monoconidiais e os fatores físicos que devem operar para o sucesso do cruzamento são discutidos.

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