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Transposition and aggressiveness in a *Phaeosphaeria nodorum* sexual cross

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ABSTRACT

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Genetic segregation of aggressiveness, anonymous molecular markers and transposon DNA probes in a *Phaeosphaeria nodorum* sexual cross was studied. The progeny of a *P. nodorum* isolate with high aggressiveness to wheat (Sn26-1) and a *Phaeosphaeria* sp. from rye (Sn48-1) was evaluated. The magnitude of aggressiveness to cereals caused by two cross parents and their progeny was triticale > wheat > rye. Different levels of aggressiveness were detected in the progeny. Progeny 64-3 and 64-5 had equal or higher aggressiveness than two parental isolates. Low or no aggressiveness in the progeny 64-1, 64-2, and 64-4 was due to low or no sporulation in these cultures. Using RFLP fingerprinting with anonymous molecular markers and transposon DNA probes, six segregation patterns were found in the progeny, and the segregation occurred in both meiotic divisions during ascospore formation. Several asymmetric transposon-tagged bands were detected in the progeny with low aggressiveness and low sporulation. The possible association of transposon-tagged bands with pathological characters is discussed.

Key words : aggressiveness, molecular markers, *Phaeosphaeria* spp., RFLP, rye, *Stagonospora nodorum*, transposon, triticale, *Triticum aestivum* L., wheat

INTRODUCTION

The fungus *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (anamorph *Stagonospora nodorum* (Berk.) Castellani & E. G. Germano) is a foliar and glume pathogen of wheat (*Triticum aestivum* L.) as well as other cereals and grasses [23,45]. Isolates of *P. nodorum* with different levels of aggressiveness on various host plants have been reported [2,40,41,44]. Sexuality in fungi is proposed to be responsible for the prevalence of gene flow in the field population and potential evolution of new, highly aggressive isolates [4,6,18,24,28]. Recently, segregation of biological and pathological characters in an ascospore progeny has been reported [15]. In the previous work, PCR-based amplifications were used to analyze the segregation and recombination of numerous anonymous fragments in the sexual progeny of *P. nodorum* and *P. avenaria* f. sp. triticea [8]. No conclusions were made on the genes responsible for aggressiveness in *P. nodorum*.

Transposons are ubiquitous mobile segments of DNA that possess the ability to integrate, exit and reintegrate throughout the genome. The active movement of transposons can change gene expression, alter gene sequence, increase gene copy numbers, cause chromosome rearrangements and result in phenotypic instability [9,19,22,30]. Transposons have been used for studies of genetic segregation, diagnosis, gene tagging, evolution, and phylogenetic and population analyses of many fungal species [5,12,13,17,21,35,36]. Transposon *impala* from *Fusarium oxysporum* is able to transpose in other fungi, such as *Penicillium griseoroseum* and *Magnaporthe grisea* [11,49]. A new transposon-arrayed gene knockout system (TAGKO) has been used to investigate the amino acid oxidation pathway in the rice blast fungus (*Magnaporthe grisea*) and cereal Septoria leaf blotch pathogen (*Mycosphaerella graminicola*) [1,16]. Transposons have been divided into two main groups. Class I elements are retrotransposons that use a reverse transcriptase and move via a RNA intermediate. Class II elements are transposons with inverted terminal repeat (ITR) and use a transposase to move as a DNA element [13]. Both groups of fungal transposons have been identified in fungi using DNA fingerprinting of

cloned dispersed repetitive sequences [14,50]. Many other transposons, like Fot1 (*Fusarium oxysporum*), Vader (*Aspergillus niger* var. *awamori*), Flipper (*Botrytis cinerea*) and *Restless* (*Tolypocladium inflatum*), have been isolated as DNA fragments trapped into negatively selective genes involving nitrogen metabolism [3,10,20,25]. With this trapping approach, a class I retrotransposon, *Elsa*, and two class II ITR transposons, *Molly* and *Pixie*, have been isolated from spontaneous mutants of *P. nodorum* defective in the nitrate reductase (*NIA1*) gene [7,33,34]. The ascospores derived from a single ascus in the previous work [8] were used to study segregation and mobility of these transposon-tagged DNA fragments and their relation to aggressiveness in *P. nodorum*. Several new transposon-tagged bands were detected in low aggressive ascospore-derived cultures.

MATERIALS AND METHODS

Inoculation test

Eight *P. nodorum* ascospore-derived cultures from an ordered tetrad numbered sequentially (64-1 to -8) and two cross parents, Sn26-1 and Sn48-1, were used in this study [8] (Table 1). The wheat *P. nodorum* isolate, Sn26-1 with *MAT1-2* mating-type gene, was highly aggressive to wheat. The *Phaeosphaeria* sp. from rye, Sn48-1 with *MAT1-1* mating-type gene, was particularly aggressive to some rye cultivars (unpublished data). In comparison to wheat biotype *P. nodorum* isolates, the *Phaeosphaeria* sp. from rye (Sn48-1) contains a 4 nucleotide difference in the conserved α-box sequence of *MAT1-1* gene and 6 nucleotide substitutions in a ribosomal DNA internal transcribed spacer (ITS) region (GenBank accession numbers AY072933, U77362, AF322008 and AF321323). Two less aggressive *P. nodorum* isolates, 9074 and 9076, were used as controls [24]. Two moderately susceptible cultivars of wheat (*Triticum aestivum*) (LSU90518 and Pioneer2684), triticale (x *Triticosecale* Wittmack) (Florida201 and Sunland), and rye (*Secale cereale*) (Wrens96 and Wrens Abruzzi) were inoculated to determine pathogen aggressiveness. Six five- leaf seedlings in each pot were grown in a growth chamber controlled with 22/13℃ day/night temperatures, and a 16 h photoperiod with 21 klux light intensity. The *P. nodorum* inocula were prepared by growing cultures on V-8 Czapek-Dox agar at 18℃ under continuous cool white fluorescent light for 10-14 days. Pycnidiospores were suspended in sterile deionized water and filtered through sterile cheesecloth to remove mycelial fragments. The spore concentration was adjusted with the aid of a haemocytometer. A drop of Tween 20 was added to 50 ml inoculum as a surfactant prior to inoculation. The seedlings in each pot were sprayed with 3 ml of the pycnidiospore suspension (3 x 10^6 spores/ml). The plants were maintained in a high humidity environment for 72 h after inoculations. Fourteen days after inoculation, the fifth leaves were assessed for percentage of necrosis and scored using a 0-9 (resistant to susceptible) scale [37]. The inoculation test was repeated four times. Variance was analyzed with SAS PROC GLM procedure [39].

Table 1. Isolates of *Phaeosphaeria nodurum* used for genetic and pathogenic study

Isolates	Original hosts	Geographic location	Mating-type gene
Sn26-1	Winter wheat	Rzeszow, Poland	*MAT1-2*
Sn48-1	Winter rye	Boleslawiec, Poland	*MAT1-1*
64-1 to 64-8 [1]			
9074 [2]	Wheat	Gallatin, MT, USA	*MAT1-2*
9076 [2]	Wheat	Richland, MT, USA	*MAT1-2*
Sn27-1 [3]	Winter wheat	Sieradz, Poland	*MAT1-1*
Sn37-1 [3]	Winter wheat	Szelejewo, Poland	*MAT1-2*
Sn39-1 [3]	Winter wheat	Byszewy, Poland	*MAT1-1*
S-80-301 [3]	Triticale	Williamson, GA, USA	*MAT1-2*
S-74-20A [3]	Wheat	Griffin, GA, USA	*MAT1-1*

[1.] Progeny from a single ascus in a Sn26-1 x Sn48-1 cross
[2.] Isolates used for inoculation test only
[3.] Isolates used for cluster analysis only

DNA isolation and analyses

Growth of fungal cultures in a liquid medium and isolation of genomic DNA were performed essentially following the procedures described by Ueng [46]. Thirty-eight anonymous probes showing polymorphism in *P. nodorum* (SNWEs 1, 6, 16, 22, 35, 40, 43, 45, 48, 57, 60, 67, 74, 76, 78, 85, 92, 101, 119, 131, 152, 153, 161, 167, 171, 203, 210, 211, pSNS4, pSNLs13, 15, and pJSNs 3, 4, 27, 35, 42, 73, 121) were used for restriction fragment length polymorphism (RFLP) fingerprinting [28,46,47,48]. Genomic DNA (10 μg) from five *P. nodorum* isolates, two cross parents and eight ascospore-derived progeny was digested using 50 U *Eco*RI endonuclease enzyme at 37℃ overnight (Table 1). Gel resolution, membrane blotting, and hybridization with ^{32}P-labeled probes were as previously described. [46]. Cluster analysis was performed using the NTSYS-pc program, version 2.0 (Exeter Software, E. Setauket, NY).

For transposon fingerprinting, three partial transposon sequences identified from the wheat-biotype *P. nodorum* isolates were used as DNA probes [33,34]. The DNA fragments with 2,290 bp (*Cla*I/*Sac*I cuts) from pELSA-1 (GenBank accession number AJ277966), 1,522 bp (*Xho*I/*Acc*I cuts) from pMOLLY-2 (GenBank accession number AJ488502), and 663 bp from pPIXIE-5 (nucleotide #314-#977) (GenBank accession number AJ488503) were isolated. Genomic DNA (20 μg) from 2 cross parents and 8 ascospore-derived progeny was digested using 85 U ScaI endonuclease enzyme at 37℃ overnight. Based on the sequence data, these three *P. nodorum* transposons do not contain an endonuclease site digested by this enzyme. Gel resolution, membrane blotting, and hybridization with ^{32}P-labeled probes were as previously described [38,46].

RESULTS

Inoculation test

Based on analyses of variance (ANOVA), a significant difference in host plant susceptibility, pathogen aggressiveness and pathogen-plant interaction was found. With two cultivars of each cereal used in this study, the magnitude of aggressiveness caused by 9074, 9076, Sn26-1, Sn48-1 and seven ascospores was triticale (3.63) > wheat (2.79) > rye (1.83). Within ascospore-derived progeny, 64-3 and 64-5 were similar in aggressiveness to the two respective parental isolates, Sn26-1 and Sn48-1 (Table 2). In particular, 64-5 had a high aggressiveness on three cereals (wheat, triticale and rye) tested. Three cultures, 64-6, 64-7 and 64-8, were less aggressive than two parental isolates. Control isolates, 9074 and 9076, were less aggressive than all of the above. The low disease severity exhibited by cultures 64-2 and 64-4 was a result of poor pycnidiospore production in the inocula, only 10^3 - 10^4 pycnidiospores per ml could be used for inoculation sprays. Apparently culture 64-1 lost the ability to sporulate after subculturing. Even with repeated attempts by inoculating and isolating from wheat leaves, 64-1 did not sporulate on the culture media and no infection data could be obtained.

RFLP fingerprinting

Most of the anonymous *P. nodorum* DNA probes produced 1-3 RFLP bands in DNA hybridization assays. Eight probes, namely SNWEs 45, 167, 203, 210 and pJSNs 3, 4, 35, 73, showed multiple banding patterns. Of the 38 anonymous probes used, only six (SNWEs 57, 92, 131, 171 and pJSN 27, 42) gave the same RFLP fingerprint patterns in the two cross parents, Sn26-1 and Sn-48-1. With 16 probes (SNWEs 6, 16, 22, 35, 43, 45, 48, 60, 74, 119, 167, 203, 211, pSNL15, and pJSNs 35, 121), 28 unique RFLP banding patterns not seen in six wheat-biotype *P. nodorum* isolates were detected in Sn48-1 (Fig. 1). In cluster analysis, the similarity between six *P. nodorum* isolates and Sn48-1 was 0.425 indicating their genetic relatedness is not close (Fig. 2). Eight ascospore progeny derived from a single ascus in the cross Sn26-1 x Sn48-1 could be grouped into four subsets, and each subset was composed of two adjacent ascospores with highly genetic similarity, excepting 64-5/64-6 twin ascospores (Fig. 3). Bands were found to be absent in RFLP pattern of 64-6 using the five DNA probes, SNWEs 6, 45, 167, 203 and pJSN4, as compared with 64-5. Segregation of anonymous DNA probes in eight ascospores followed a Mendelian frequency of 4:4, and was grouped to six patterns

Table 2. Comparison of aggressiveness toward wheat, triticale, and rye by *Phaeosphaeria nodorum* isolates Sn26-1, Sn48-1 and their derived progeny

Isolate	Wheat		Triticale		Rye		All
	Pioneer2684	LSU90518	Florida201	Sunland	Wrens Abruzzi	Wrens96	
9074	1.6 b-e	0.9 e	1.0 e	0.6 g	0.5 d	0.5 d	0.8 d
9076	2.8 a-d	3.0 bc	3.1 cd	1.8 efg	1.5 bcd	1.0 d	2.0 c
Sn26-1	4.1 a	5.1 a	5.4 ab	5.4 ab	2.4 abc	2.5 abc	4.2 a
Sn48-1	1.9 b-e	5.0 a	6.4 a	4.4 abc	2.9 ab	3.6 a	4.0 a
64-1	----	----	----	----	----	----	----
64-2	0.4 e	1.1 de	0.7 e	0.7 fg	0.4 d	0.5 d	0.7 d
64-3	3.6 ab	4.6 ab	5.8 ab	5.9 a	2.8 abc	2.8 ab	4.3 a
64-4	0.9 de	0.9 e	1.2 de	2.4 def	1.7 a-d	0.8 d	1.3 d
64-5	4.5 a	4.6 ab	6.0 ab	5.5 a	3.2 a	3.6 a	4.6 a
64-6	3.3 abc	3.3 bc	5.1 abc	3.4 cde	1.7 a-d	2.0 bcd	3.1 b
64-7	2.8 a-d	2.5 cd	4.8 abc	3.8 bcd	1.3 cd	1.2 cd	2.8 bc
64-8	1.3 cde	3.1 bc	4.0 bc	2.4 def	1.8 a-d	1.6 bcd	2.3 bc

Means in columns followed by the same letter are not significantly different according to Duncan's New Multiple Range Test (P=0.05).

Table 3. Segregation of anonymous DNA probes in eight ascospores from a single ascus in a *Phaeosphaeria* cross, Sn26-1 x Sn48-1

Numbers of bands	Progeny								Meiotic division
	1	2	3	4	5	6	7	8	
23	b [1]	b	b	b	a	a	a	a	1st
10	a	a	a	a	b	b	b	b	1st
9	a	a	b	b	b	b	a	a	2nd
4	b	b	a	a	a	a	b	b	2nd
5	a	a	b	b	a	a	b	b	2nd
9	b	b	a	a	b	b	a	a	2nd

[1.] The letters 'a' or 'b' indicated the restriction fragment length polymorphism patterns are the same as the parental isolates, Sn26-1 and Sn48-1, respectively.

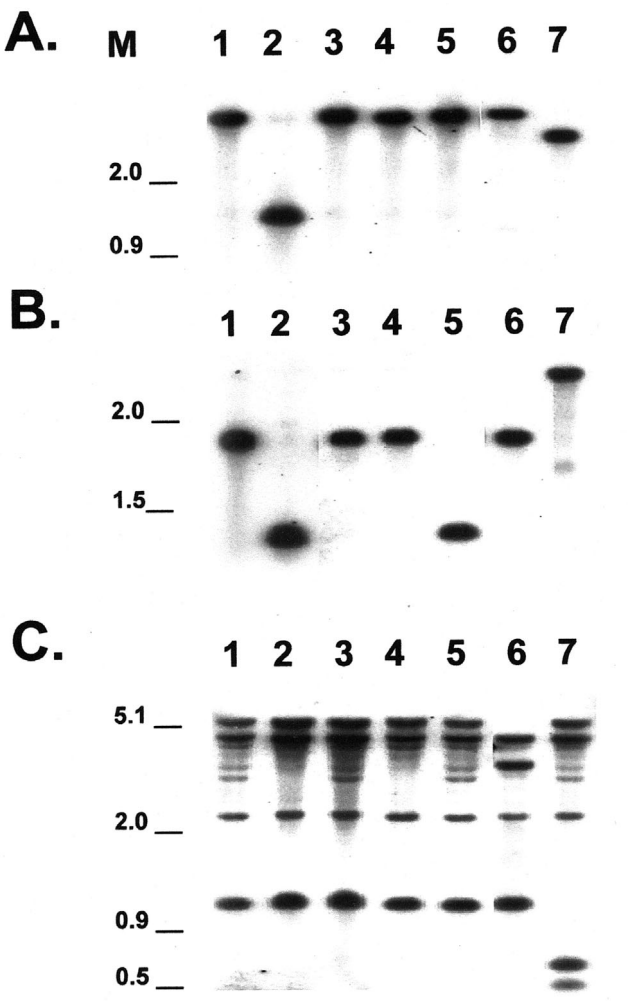

Fig. 1. Restriction fragment length polymorphism patterns (RFLP) in *Phaeosphaeria nodorum* and a rye *Phaeosphaeria* sp. Six wheat-biotype *P. nodorum* isolates (1 = Sn27-1, 2 = S-74-20A, 3 = S-80-301, 4 = Sn37-1, 5 = 39-1 and 6 = Sn26-1) and one rye *Phaeosphaeria* sp. (7 = Sn48-1) were compared by DNA hybridization using three anonymous DNA probes (A = SNWE22, B = SNWE35 and C = SNWE167). M indicates the λDNA/*Hin*dIII/*Eco*RI molecular markers in kilo base pairs (kb).

(Table 3). These data suggest that the segregation occurred in the first and second meiotic division during ascospore formation.

Based on RFLP fingerprinting, polymorphism in two cross parents, Sn26-1 and Sn48-1, were identified using hybridization with three transposon probes (Fig. 4). Four RFLP bands found in two cross parents were not found in their progeny. Most of transposon- tagged banding patterns followed the Mendelian frequency of 4:4 and were similar in each of four ascospore subsets. These results suggest that segregation of transposon- tagged fragments occurred in meiotic divisions. Nevertheless, several new bands in some progeny were not present in two cross parents. A band with different size was *Elsa*-tagged in 64-3, and 64-4, *Molly*-tagged in 64-5 and *Pixie*-tagged in 64-2 and 64-5 (Fig. 4). Also one *Molly*-tagged band shown in 64-1 was absent in 64-2, but two bands were presented in 64-2 with lower mobility (Fig. 4B). Transposon mobilization might occur during the cell division and spore formation.

DISCUSSION

With the detached wheat leaf segments assays, a wide range of aggressiveness and sporulating capability in the *P. nodorum* progeny from two sexual crosses were detected [15]. Even though no significant correlation between necrosis and sporulation on leaf lesions and cultured medium could be found, the same level of aggressiveness was detected in most of the ascospore subset cultures [15]. In this study, aggressiveness toward three different cereals was transferred to the progeny (Table 2). One of the factors possibly associated with low aggressiveness seemed to correlate with the ability to sporulate. One important biological character affecting *P. nodorum* aggressiveness is reported to be the variability of asexual sporulation in fungal cultures [42]. However, the fungal aggressiveness in two individual ascospore subset cultures (64-3/-4 and 64-5/-6) was significantly different. An ascospore subset (300.7/.8) from the cross M300 x A/5 with different sporulating ability was also observed [15]. In an early study by Rapilly et al. [32], the 4 pairs of twin ascospores from a *P. nodorum* sexual reproduction identified by cultural characteristics were not distinguishable based on their aggressiveness. The mechanism for elaboration of different aggressiveness between twin ascospores is unknown. Nevertheless, the pycnidiospores derived from a single ascospore were found to be heterogeneous [32].

Genetic segregation of anonymous probes determined by RFLP primarily followed the Mendelian 4:4 frequency and occurred in two meiotic divisions but seldom in mitosis. Since the number of probes and ascospores used here was limited, neither irregular nor aberrant segregation could be identified. In order to detect the RFLP bands associated with aggressiveness in the future, more DNA probes and more progeny from a cross should be analyzed.

The transposon *impala* is reported to be active in the asexual stage of *Fusarium oxysporum* [10], and the pSTL70 probe appears to be mobile in *Mycosphaerella graminicola* during both sexual and asexual reproduction [14]. Based on RFLP fingerprinting, many transposon-tagged bands in *P. nodorum* progeny segregated in a Mendelian fashion (Figure 4). These integrated transposons apparently were not actively mobilized during the sexual stage. Loss of mobility can be explained by the detection of duplicated sequences in the genome and subsequently silencing with hypermutation and DNA methylation during the heterokaryon stage [26,27,43]. Nevertheless, the appearance of the asymmetric RFLP bands in ascospore subset cultures indicates an active movement of

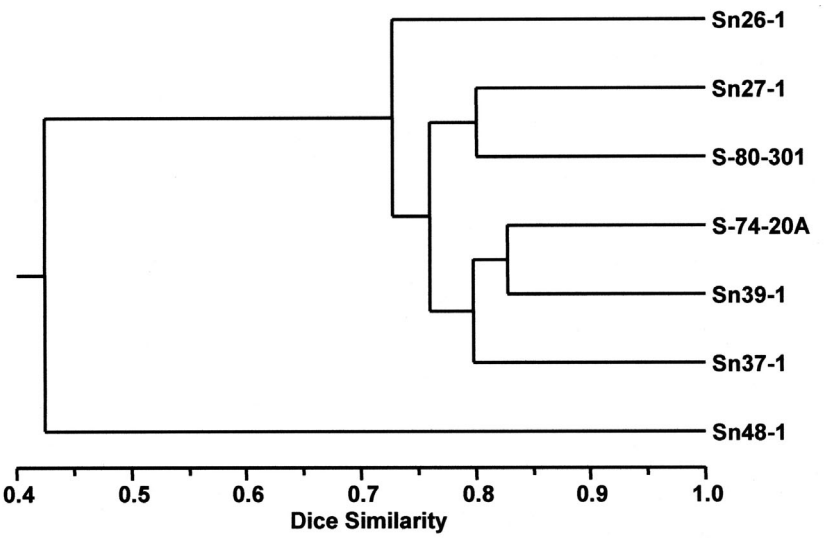

Fig. 2. Cluster analysis of six *Phaeosphaeria nodorum* isolates and the rye *Phaeosphaeria* sp. based on dice similarity of 60 DNA bands produced by hybridization with 38 anonymous probes.

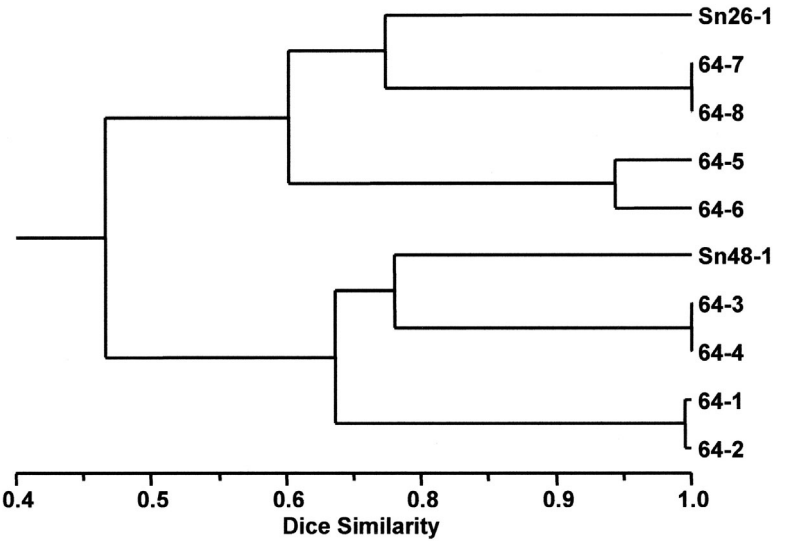

Fig. 3. Cluster analysis of 8 ascospore-derived progeny (64-1 to -8) and two *Phaeosphaeria* cross parental isolates (Sn26-1 and Sn48-1) based on dice similarity of 60 DNA bands produced by hybridization with 38 anonymous probes.

these transposons during post-meiotic mitosis and/or during vegetative growth.

It has become possible to clone the genes responsible for fungal pathogenicity using a transposon as a tag. The ability of transposons to inactivate genes involved in pathogenicity has been demonstrated with transposon *impala* in two different fungal pathogens. About 3.5% of *impala*-impaired revertants of *Fusarium oxysporum f. sp. melonis* showed either a no wilt symptom or delayed symptom development on the host plant [29]. In the other research, *Magnaporthe grisea* was transformed with *impala*, and a DNA fragment containing *ORP1* gene from a nonpathogenic mutant (Rev77) was isolated [49]. The identity of this *ORP1* gene is unknown and reported to be essential for host leaf penetration. A preliminary attempt to isolate genes involved in the *P. nodorum*- wheat interaction by transposition was successful [31]. Two pathogenicity genes encoding a peptide transporter (heterotrimeric G-protein α subunit) and a mannitol-1-phosphate dehydrogenase were reported.

From the results of pathological segregation in sexual crosses reported by Halama [15], the molecular element (s) responsible for aggressiveness seems to be polygenic and difficult to determine in *P. nodorum*. Two UV-induced parental strains (M270 and M300) with medium and low aggressiveness used in the crosses with a highly aggressive isolate (A/5) were believed to be pleiotropic mutants, and the genes associated with fungal aggressiveness and sporulation was unknown [15].

Even though several new transposon-tagged bands were detected in our study, their correlation with fungal

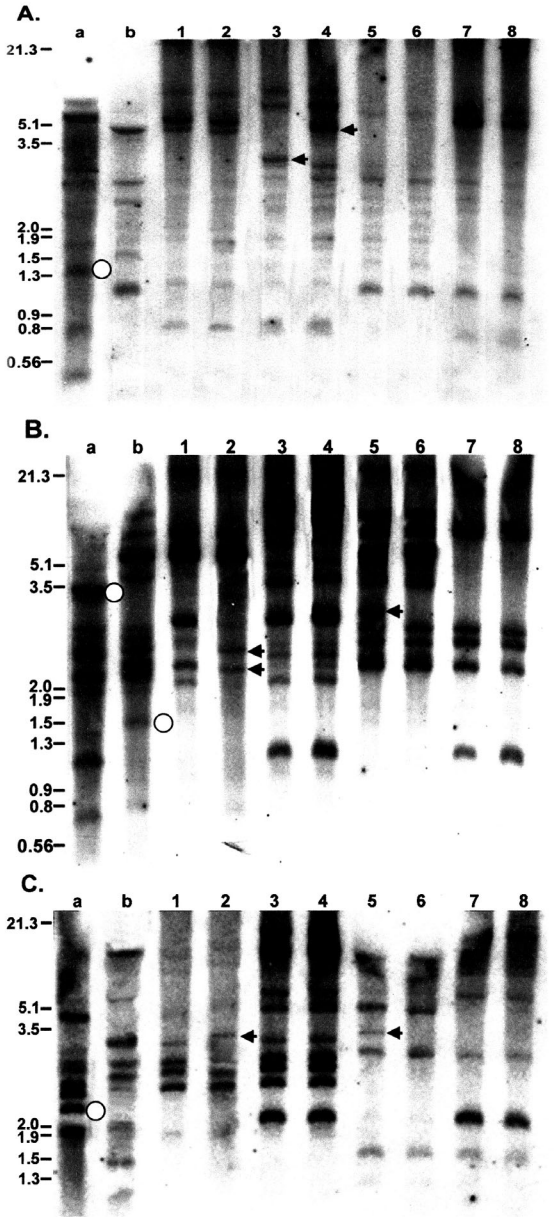

Fig. 4. DNA fingerprinting of two *Phaeosphaeria* cross parental isolates (Sn26-1 = a, Sn48-1 = b) and 8 ascospore-derived progeny (64-1 to −8 = 1 −8). Partial fragments of transposons, Elsa (A), *Molly* (B) and *Pixie* (C) were used as probes in hybridization. Arrows (◄–) indicate the asymmetric transposon-tagged bands. Open circles (O) indicate the bands present in parents are not shown in the progeny.

aggressiveness is only a speculation. Low aggressiveness in 64- 2 and 64-4, and high aggressiveness in 64-5 might not be related to any transposition activity as indicated in RFLP. Random genetic tagging by active transposons during sexual cross, asexual production and/or vegetative growth may not necessarily affirm the changes of fungal aggressiveness. In order to prove the molecular basis of aggressiveness in fungal pathogens, the association of attenuating disease severity and sporulating capability with silencing of a particular gene by transposon tagging needs to be studied in the future.

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LITERATURE CITED

1. Adachi, K., Nelson, G. H., Peoples, K. A., Frank, S. A., Montenegro-Chamorro, M. V., DeZwaan, T. M., Ramamurthy, L., Shuster, J. R., Hamer, L., and Tanzer, M. M. 2002. Efficient gene identification and targeted gene disruption in the wheat blotch fungus *Mycosphaerella graminicola* using TAGKO. Curr. Genet. 42:123-127.
2. Allingham, E. A., and Jackson, L. F. 1981. Variation in pathogenicity, virulence, and aggressiveness of *Septoria nodorum* in Florida. Phytopathology 71:1080-1085.
3. Amutan, M., Nyyssönen , E., Stubbs, J., Diaz-Torres, M. R., and Dunn-Coleman, N. 1996. Identification and cloning of a mobile transposon from *Aspergillus niger* var. *awamori*. Curr. Genet. 29:468-473.
4. Arseniuk, E., Góral , T., and Scharen, A. L. 1998. Seasonal patterns of spore dispersal of *Phaeosphaeria* spp. and *Stagonospora* spp. Plant Dis. 82:187-194.
5. Capy, P., Vitalis, R., Langin, T., Higuet, D., and Bazin, C. 1996. Relationships between transposable elements based upon the integrase-transposase domains: is there a common ancestor? J. Mol. Evol. 42:359-368.
6. Caten, C. E., and Newton, A. C. 2000. Variation in cultural characteristics, pathogenicity, vegetative compatibility and electrophoretic karyotype within field populations of *Stagonospora nodorum*. Plant Pathol. 49:219-226.
7. Cutler, S. B., Cooley, R. N., and Caten, C. E. 1998. Cloning of the nitrate reductase gene of *Stagonospora* (*Septoria*) *nodorum* and its use as a selectable marker for targeted transformation. Curr. Genet. 34:128-137.
8. Czembor, P. C., and Arseniuk, E. 2000. Segregation and recombination of PCR based markers in sexual progeny of *Phaeosphaeria* species. Mycol. Res. 104:919-926.
9. Daboussi, M. J. 1997. Fungal transposable elements and genome evolution. Genetica 100:253-260.
10. Daboussi, M. J., Langin, T., and Brygoo, Y. 1992. Fot1, a new family of fungal transposable elements. Mol. Gen. Genet. 232:12-16.
11. de Queiroz, M. V., and Daboussi, M. J. 2003. *Impala*, a transposon from *Fusarium oxysporum*, is active in the genome of *Penicillium griseoroseum*. FEMS Microbiol. Lett. 28:317-321.

12. Farman, M. L. 2002. *Pyricularia grisea* isolates causing gray leaf spot on perennial ryegrass (*Lolium perenne*) in the United States: relationship to *P. grisea* isolates from other host plants. Phytopathology 92:245-254.
13. Finnegan, D. J. 1989. Eukaryotic transposable elements and genome evolution. Trends Genet. 5:103-107.
14. Goodwin, S. B., Cavaletto, J. R., Waalwijk, C., and Kema, G. H. J. 2001. DNA fingerprint probe from *Mycosphaerella graminicola* identifies an active transposable element. Phytopathology 91:1181-1188.
15. Halama, P., Skajennikoff, M., and Dehorter, B. 1999. Tetra analysis of mating type, mutations, esterase and aggressiveness in *Phaeosphaeria nodorum*. Mycol. Res. 103:43-49.
16. Hamer, L., Adachi, K., Montenegro-Chamorro, M. V., Tanzer, M. M., Mahanty, S. K., Lo, C., Tarpey, R. W., Skalchunes, A. R., Heiniger, R. W., Frank, S. A., Darveaux, B. A., Lampe, D. J., Slater, T. M., Ramamurthy, L., DeZwaan, T. M., Nelson, G. H., Shuster, J. R., Woessner, J., and Hamer, J. E. 2001. Gene discovery and gene function assignment in filamentous fungi. Proc. Natl. Acad. Sci. USA 98:5110-5115.
17. Hua-Van, A., Langin, T., and Daboussi, M. J. 2001. Evolutionary history of the *impala* transposon in *Fusarium oxysporum*. Mol. Biol. Evol. 18:1959-1969.\
18. Keller, S. M., McDermott, J. M., Pettway, R. E., Wolfe, M. S., and McDonald, B. A. 1997. Gene flow and sexual reproduction in the wheat glume blotch pathogen *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*) Phytopathology 87:353- 358.
19. Kempken, F. 1999. Fungal transposons: from mobile elements towards molecular tools. Appl. Microbiol. Biotechnol. 52:756-760.
20. Kempken, F., and Kück , U. 2000. Tagging of a nitrogen pathway-specific regulator gene in *Tolypocladium inflatum* by the transposon *Restless*. Mol. Gen. Genet. 263:302- 308.
21. Kempken, F., and Windhofer, F. 2001. The *hAT* family: a versatile transposon group common to plants, fungi, animals, and man. Chromosoma 110:1-9.
22. Kidwell, M. G., and Lisch, D. 1997. Transposable elements as sources of variation in animals and plants. Proc. Natl. Acad. Sci. USA 94:7704-7711.
23. King, J. E., Cook, R. J., and Melville, S. C. 1983. A review of Septoria diseases of wheat and barley. Ann. Appl. Biol. 103:345-373.
24. Krupinsky, J. M. 1997. Aggressiveness of *Stagonospora nodorum* isolates obtained from wheat in the northern Great Plains. Plant Dis. 81:1027-1031.
25. Levis, C., Fortini, D., and Brygoo, Y. 1997. Flipper, a mobile Fot1-like transposable element in Botrytis cinerea. Mol. Gen. Genet. 254:674-680.
26. Margolin, B. S., Garrett-Engele, P. W., Stevens, J. N., Fritz, D. Y., Garrett-Engele, C., Metzenberg, R. L., and Selker, E. U. 1998. A methylated *Neurospora* 5S rRNA pseudogene contains a transposable element inactivated by repeat-induced point mutation. Genetics 149:1787-1797.
27. Martienssen, R. A., and Colot, V. 2001. DNA methylation and epigenetic inheritance in plants and filamentous fungi. Science 293:1070-1074.
28. McDonald, B. A., Miles, J., Nelson, L. R., and Pettway, R. E. 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. Phytopathology 84:250-255.
29. Migheli, Q., Steinberg, C., Davière , J. M., Olivain, C., Gerlinger, C., Gautheron, N., Alabouvette, C., and Daboussi, M. J. 2000. Recovery of mutants impaired in pathogenicity after transposition of Impala in Fusarium oxysporum f. sp. melonis. Phytopathology 90:1279-1284.
30. Oliver, R. 1991. Transposons in filamentous fungi. In Proceedings of the EMBO- Workshop, "Molecular biology of filamentous fungi", Stahl U and Tudzynski P (eds.), VCH: Weinheim, New York, Basel, Cambridge, p.3-11.
31. Oliver, R. P., Solomon, P. S., Tan, K. C., Wilson, T. J. G., Lee, R., and Parker, K. 2003. Molecular dissection of the *Stagonospora nodorum*-wheat interaction. 8th International Congress of Plant Pathology, Christchurch, New Zealand, p.194.
32. Rapilly, F., Skajennikoff, M., Halama, P., and Touraud, G. 1992. La reproduction sexuee et l'agressivité de *Phaeosphaeria nodorum* Hedj (= *Septoria nodorum* Berk). Agronomie 12 :639-649.
33. Rawson, J. M. 2000. Transposable elements in the phytopathogenic fungus *Stagonospora nodorum*. Ph.D. thesis, The University of Birmingham, England.
34. Rawson, J. M., Cutler, S. B., and Caten, C. E. 1999. Isolation and characterisation of a retrotransposon in the phytopathogenic fungus *Stagonospora nodorum*. Abstract #88. The 20th Fungal Genetics Conference, Pacific Grove, CA.
35. Rosewich, U. L., and Kistler, H. C. 2000. Role of horizontal gene transfer in the evolution of fungi. Annu. Rev. Phytopathol. 38:325-363.
36. Rubin, E., Lithwick, G., and Levy, A. A. 2001. Structure and evolution of the *hAT* transposon superfamily. Genetics 158:949-957.
37. Saari, E. E., and Prescott, J. M. 1975. A scale for appraising the foliar intensity of wheat diseases. Plant Dis. Reptr. 59:377-380.
38. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY.
39. SAS Institute Inc. 2001. SAS/STAT User's Guide. Version 8, North Carolina.
40. Scharen, A. L., and Eyal, Z. 1983. Analysis of symptoms on spring and winter wheat cultivars inoculated with

different isolates of *Septoria nodorum*. Phytopathology 73:143- 147.
41. Scharen, A. L., Eyal, Z., Huffman, M. D., and Prescott, J. M. 1985. The distribution and frequence of virulence genes in geographically separated populations of *Leptosphaeria nodorum*. Phytopathology 75:1463-1468.
42. Scharen, A. L., and Krupinsky, J. M. 1970. Cultural and inoculation studies of *Septoria nodorum*, cause of glume blotch of wheat. Phytopathology 60:1480-1485.
43. Selker, E. U. 1997. Epigenetic phenomena in filamentous fungi: useful paradigms or repeat-induced confusion? Trends Genet. 13:296-301.
44. Skajennikoff, M., and Rapilly, F. 1989. Variability of pathogenicity in *Septoria nodorum* Berk. (= *Leptosphaeria nodorum* Mull.). Agronomie 9:693-670.
45. Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press, New York.
46. Ueng, P. P., Bergstrom, G. C., Slay, R. M., Geiger, E. A., Shaner, G., and Scharen, A. L. 1992. Restriction fragment length polymorphisms in the wheat glume blotch fungus, *Phaeosphaeria nodorum*. Phytopathology 82:1302-1305.
47. Ueng, P. P., and Chen, W. 1994. Genetic differentiation between *Phaeosphaeria nodorum* and *P. avenaria* using restriction fragment length polymorphisms. Phytopathology 84:800-806.
48. Ueng, P. P., Cunfer, B. M., Alano, A. S., Youmans, J. D., and Chen, W. 1995. Correlation between molecular and biological characters in identifying the wheat and barley biotypes of *Stagonospora nodorum*. Phytopathology 85:44-52.
49. Villaba, F., Lebrun, M. H., Hua-Van, A., Daboussi, M. J., and Grosjean-Cournoyer, M. C. 2001. Transposon *impala*, a novel tool for gene tagging in the rice blast fungus *Magnaporthe grisea*. Mol. Plant-Microbe Interact. 14:308-315.
50. Yeadon, P. J., and Catcheside, D. E. A. 1995. *Guest*: a 98 bp inverted repeat transposable element in *Neurospora crassa*. Mol. Gen. Genet. 247:105-109.

摘 要

翁 溥[1,5]、E. Arseniuk[2]、B. M. Cunfer[3]、Q. J. Song[4]. 2003. 小麥葉枯病菌 (*Phaeosphaeria nodorum*) 雜交子代的基因轉位與致病毒力分析. 植病會刊12:149-156. ([1.] 美國農業部分子植物病理研究室；[2.] 波蘭植物育種及馴化研究所植物病理學系；[3.] 美國喬治亞大學植物病理學系；[4.] 中國南京農業大學農藝學系；[5.] 聯絡作者：電子郵件uengp@ba.ars.usda.gov；傳眞：+0021-301-504-5449)

本研究利用致病毒力 (aggressiveness) 的遺傳分離、未命名的分子標記 (anonymous molecular markers) 及轉位子 (transposon) 等核酸探針分析小麥葉枯病菌 (*Phaeosphaeria nodorum*) 的雜交子代。評估的主要對象是對小麥具高致病力的葉枯病菌 Sn26-1 菌株與來自裸麥的 *Phaeosphaeria* sp. Sn26-1 菌株交配後的子代。此二親本菌株及其雜交子代對不同穀類的致病力強弱依序爲：黑小麥> 小麥 >裸麥。本研究發現各子代間的致病力有不相同程度的差異。子代 64-3 與 64-5 菌株的致病力相等於或強於其二親本菌株，然而子代 64-1、64-2 及 64-4 菌株則因在培養中產孢量低或不產孢而致病力低或無致病力。利用未命名的分子標記及轉位子等核酸探針分析所得之 RFLP，結果顯示子代菌株中共有六種指紋圖譜，且其分離現象係發生在兩次減數分裂形成子囊孢子的過程。在致病力低及產孢量低的子代菌株中也出現數個非對稱的轉位子片段，文中也將討論轉位子片段與病理特性之可能關係。

關鍵詞：小麥、小麥葉枯病菌、分子標記、致病力、限制片段長度多型性 (RFLP)、黑小麥、裸麥、轉位子