Isozyme and Amplified Fragment Length Polymorphisms from *Cephalosporium maydis* in Egypt

Kurt A. Zeller,[1] James E. Jurgenson,[2]
Elhamy M. El-Assiuty[3] and John F. Leslie[1,*]

Isoenzyme and amplified fragment length polymorphisms (AFLP) variation within a set of 48 isolates of *Cephalosporium maydis* was characterized. These isolates included ten cultures that have served as standards in the Egyptian maize resistance breeding program and 38 additional strains collected from 11 governates in Egypt during the 1997 growing season. Eight isozymes also were tested, but only five (acid phosphatase, fumerase, glucose-4-phosphate isomerase, isocitrate dehydrogenase, and malate dehydrogenase) produced identifiable bands and all five of these enzymes were monomorphic. Sixty-eight AFLP primer-pair combinations were used and 865 bands were scored, of which 288 (33%) were polymorphic and could be used to discriminate four distinct subgroups, or lineages. Representatives from only two of the four lineages are included in the set of ten strains that has been used to challenge new lines in the Egyptian maize breeding program. From among these 68 primer-pair combinations, we identified a set of four AFLP primer-pairs that were strongly correlated (Pearson's $r > 0.85$) with the full data set that can be used as markers to determine the distribution of these lineages and to identify new lineages in field populations.
KEY WORDS: Clonal populations; corn; genetic diversity; isozymes; late wilt; maize; stalk rot.

INTRODUCTION

Late wilt of maize, caused by *Cephalosporium maydis* Samra, Sabet & Hingorani, is economically the most important fungal disease of maize in Egypt (33,35-37). *Cephalosporium maydis* is a soilborne vascular wilt pathogen that penetrates root tissue and colonizes the xylem (32). Less commonly, this pathogen also can be seedborne (13), and may occasionally cause seed rot or pre-emergence damping-off under heavy inoculum pressure (34). In infested fields, up to 80% of the susceptible plants may become infected, and yield losses may approach 40% (33,37). Previous studies of this organism have focused on morphological and physiological descriptions of the fungus (*e.g.* 31), pathogenic variability (*e.g.* 30), cultural control (*e.g.* 1,15,37), and the identification of resistant maize germplasm (*e.g.* 14). No sexual stage has been described for *C. maydis*, and there are no published reports on its genetic variation or population structure.

Contribution no. 99-357-J from the Kansas Agricultural Experiment Station, Manhattan, KS. Received Sept. 26, 1999; received in final form Dec. 23, 1999; http://www.phytoparasitica.org posting Feb. 2, 2000.
[1] Dept. of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506-5502, USA. *Author for correspondence [Fax: +1-785-5322414; e-mail: jfl@plantpath.ksu.edu].
[2] Dept. of Biology, University of Northern Iowa, Cedar Falls, IA 50614, USA.
[3] Maize and Sorghum Diseases Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

To this point, researchers have worked with relatively few strains of *C. maydis* (14,30). However, considerable differences in virulence have been reported even among these few strains. Differences in virulence within field populations of *C. maydis* also are poorly characterized. The development of resistant maize lines is the only economically feasible control for late wilt, and resistance appears to be controlled by at least three major genes (12,38). To increase the effectiveness of the maize breeding program, it is necessary to obtain a better understanding of this fungus's pathogenic limitations and variations, and any correlated genetic variation.

Most recent assessments of fungal pathogens have used multi-locus markers to characterize populations. Analyses of vegetative compatibility have proven useful in characterizing pathogenic populations of *Fusarium* (*e.g.* 11,23) and *Verticillium* (*e.g.* 4,41). Multi-locus molecular markers, including restriction fragment length polymorphisms (RFLPs) (*e.g.* 8,11,22,24,28,45), and PCR-based random amplified polymorphic DNAs (*e.g.* 5,7,19,26), also have proven useful in characterizing some populations of fungal pathogens. Recently, another PCR-based methodology, amplified fragment length polymorphism (AFLP) (43), has been used to characterize fungal populations (*e.g.* 16,25).

AFLPs can provide complex marker profiles with no prior cloning or sequence data, and it is possible to generate a very large number of markers (as many as 40–50 per primer pair depending upon genome size and the primers selected) much more quickly than is possible with typical RAPD or RFLP analyses. Due to the large number of possible primer pairs, in our case 256, and the fact that not all primer pairs are equally informative, identifying the genetically most informative primer pairs is a critical first step in the application of AFLPs as markers for population analyses.

Our objectives in this study were to identify: (i) molecular markers to describe genetic diversity within *C. maydis*, and (ii) a subset of no more than five AFLP primer pairs that could be used as a practical marker system for population-size samples. Portions of this work have been published previously in abstract form (46,47).

MATERIALS AND METHODS

Strains: A representative set of 48 strains was tested for isozyme (Table 1) and AFLP polymorphisms. This set included ten strains (10782–10791) that have been in culture and used routinely in resistance screening nurseries in Egypt since the early 1980s. Also analyzed were 38 additional strains collected in 1997 from symptomatic maize in 11 Egyptian governates: El-Behira (1 – 10792), Kafer El-Shiekh (4 – 10793, 10795-10797), Gharbya (5 – 10799-10803), Dakahlya (3 – 10808, 10810, 10811), Minoufya (6 – 10812-10817), Giza (2 – 10824, 10825), Beni-suef (5 – 10826-10830), Menia (5 – 10832-10836), Assiut (2 – 10838, 10839), Sohag (4 – 10840-10843), and Qena (1 – 10844). The sample, presumably random, is large enough to be 95% certain of detecting all strain types present in the population at a frequency of at least 6%.

Isozyme analyses: The isolates were incubated at 25°C for 7–10 days on complete medium (CM) slants and then transferred to petri dishes containing minimal medium plus vitamins (6). After 2–3 weeks of growth, mycelia were harvested, crude protein extracts

for isozyme analyses were prepared, and soluble enzymes resolved as described by Huss *et al.* (18).

DNA isolation: Flasks of liquid CM were inoculated with two or three small pieces of colonized medium cut from a 4–5-day-old culture of *C. maydis*, and then placed in the dark at room temperature (21–24°C) without shaking for 2–3 days to initiate growth into the liquid medium. The flasks were transferred to a rotary shaker (150 rpm) for an additional 3–5 days of incubation at 25°C. Mycelia were collected by filtration (Isotropic Milk Filters, Schwartz Manufacturing Co., Two Rivers, WI, USA), blotted dry with paper towels, and stored at -20°C until needed for DNA isolation. DNA was isolated with a cetyltrimethyl ammonium bromide (CTAB) procedure modified from that of Murray and Thompson (29), as described by Kerényi *et al.* (21).

AFLPs: AFLPs were generated essentially as described by Vos *et al.* (43). We digested approximately 100 ng of DNA with 2 units each of *Eco*RI and *Mse*I (New England Biolabs, Beverly, MA, USA) according to manufacturers' instructions, and ligated these DNAs to *Eco*RI (an equimolar mixture of *Eco*RI oligo-1, CTCGTAGACTGCCTACC and *Eco*RI oligo-2, AATTGGTACGCAGTC, 5 pmole/μl) and *Mse*I adapters (an equimolar mixture of *Mse*I oligo-3, GACGATGAGTCCTGAG and *Mse*I oligo-4, TACTCAGGACTCAT, 50 pmole/μl) with T4 DNA ligase (New England Biolabs). These DNA fragments were then pre-amplified and amplified as described by Vos *et al.* (43). The *Eco*RI primers in the final amplification mixes were end-labeled with γ^{33}P and fragments were separated in 6% polyacrylamide gels (Long Ranger, FMC, Rockland, ME, USA). Gels were dried and exposed to autoradiography film (Classic Blue Sensitive, Molecular Technologies, St. Louis, MO, USA) for 2–5 days at room temperature to identify DNA bands.

To analyze AFLP profiles, the presence or absence of bands across the set of 48 strains was manually scored for each of the 68 primer-pair combinations (Table 2). Bands of the same molecular size in different individuals were assumed to be identical. The presence or absence of each band was determined for each individual. Each band was treated as a single independent locus with two alleles and unresolved bands or missing data were scored as ambiguous.

Clustering: The pooled data from all tested primer pairs were analyzed for evidence of subclustering among the 48 isolates. Isolate relatedness was evaluated with the Unweighted Pair Grouping by Mathematical Averages (UPGMA), Neighbor-Joining (NJ), and Maximum Parsimony (MP) subroutines of PAUP 4.0* (42). Bootstrap analyses (1000 iterations) were conducted on the resulting UPGMA, NJ and MP networks to assess support for any resulting subgroupings.

Identifying marker subsets of AFLP primer pairs: One of our objectives was to identify a small set of AFLP primer pairs that could effectively discriminate any significantly divergent subclusters. The pairwise distance matrix resulting from all 68 AFLP primer-pair combinations was compared to the eight separate distance matrices produced by pooling data from primer pairs that shared a common *Eco*RI primer, *e.g.* the nine primer pairs that included *Eco*RI+AA. Pearson's product moment (r) correlations were

calculated with SAS (v 6.11 for PC, SAS Institute, Cary, NC, USA) between these eight distance matrices and the overall data matrix to determine which *Eco*RI-primer subset had the highest correlation to the entire data set.

After identifying the *Eco*RI-primer subset with the highest correlation to the overall data set, we serially removed data from one primer-pair combination at a time from that *Eco*RI-primer subset data pool. We identified critical primer-pair combinations that, when their data were removed from the analysis, disrupted the unity of any previously strongly supported subclusters. The data from these critical primer-pair combinations were pooled and used to generate new pairwise distance estimates. Correlations between these distance estimates and those from the overall pooled data were compared by again calculating Pearson's product moment correlations as described above. We also conducted UPGMA, NJ, and MP analyses with bootstrap resamplings for these data subsets, and correlation analyses among each potentially diagnostic subset of primers as outlined above for the pooled data from all 68 primer pairs.

RESULTS

Isozymes: Eight isozymes were assayed for activity and polymorphism (Table 1). No activity was detected for alcohol dehydrogenase, phosphoglucomutase, and triose-phosphate isomerase, in a preliminary screening of isolates 10782–10792, so the other 38 strains for these enzymes were not assayed. Activity for acid phosphatase, fumerase, glucose-4-phosphate isomerase, isocitrate dehydrogenase, and malate dehydrogenase was detected in at least one of the two buffer systems; however, no polymorphic bands were detected among the 48 samples. Thus, while isozymes may be useful as species-level diagnostic markers for *C. maydis*, no intra-specific variation was detected that could be used to characterize populations of this pathogen.

TABLE 1. Isozymes and buffer systems assayed (after ref. 18)

Enzyme	Abbreviation	E.C. no.	Buffer[z]	No. of bands[y]
Acid phosphatase	ACP	3.1.3.2	M/S6	1/1
Alcohol dehydrogenase	ADH	1.1.1.1	S6	No activity detected
Fumerase	FUM	4.2.1.2	M/S6	1/1
Glucose-4-phosphate isomerase	GPI	5.3.1.9	S6	1
Isocitrate dehydrogenase (NADP)	IDH	1.1.1.42	M	1
Malate dehydrogenase	MDH	1.1.1.37	M/S6	4/1
Phosphoglucomutase	PGM	2.7.5.1	M/S6	No activity detected
Triose-phosphate isomerase	TPI	5.3.1.1	S6	No activity detected

[z] M = Morpholine buffer system; S6 = Soltis 6 buffer system.
[y] Number of bands seen in morpholine buffer is given before the slash; the number of bands in the Soltis 6 buffer is given following the slash.

AFLPs: A total of 865 AFLP bands were scored from amplifications with 68 primer-pair combinations; 56% of the bands (484) were invariant among all 48 isolates. A little more than 10% (93) of the bands were polymorphic in a 47:1 ratio, and were unique characters to single isolates. An additional 288 bands (33%) were polymorphic, but not unique to a single isolate. This last set of polymorphic bands should be phylogenetically informative.

TABLE 2. Pearson's product moment correlations (r) between AFLP primer-pair data subsets and the complete data set from 68 AFLP primer pairs

Primer pairs[z]		r^y
*Eco*RI[x]+	*Mse*I[w]+	
AA	Pool[v]	0.893
AA	CC, CA, CG & GA	0.858
AA	CC, CA & GA	0.854
AA	CC, CG & GA	0.828
AA	CC, CA & CG	0.810
AA	CA, CG & GA	0.770
TG	Pool[u]	0.857
TT	Pool[t]	0.840
AT	Pool[s]	0.814
AG	Pool[v]	0.811
TC	Pool[v]	0.794
AC	Pool[v]	0.766
TA	Pool[v]	0.743

[z] One *Eco*RI primer and one *M*seI primer were used for each amplification reaction.
[y] All correlations are highly significant ($P < 0.0001$).
[x] Non-selective *Eco*RI primer sequence is AGA CTG CGT ACC AAT TC, followed by the two base pairs indicated.
[w] Non-selective *Mse*I primer sequence is GAT GAG TCC TGA GTA A, followed by the two base pairs indicated.
[v] Pool is AA, AC, AG, AT, CA, CC, CG, CT, and GA.
[u] Pool is AC, AG, AT, CC, CG, CT, and GA.
[t] Pool is AA, AC, AG, AT, CC, CG, CT, and GA.
[s] Pool is AA, AC, AG, AT, CA, CG, CT, and GA.

Average similarity among isolates based on UPGMA clustering of the pooled data was greater than 89%, with minimum and maximum similarities between isolates of 77% and 99%, respectively. UPGMA clustering separated the isolates into four strongly supported subgroups, each containing 7–19 of the strains (Fig. 1A). Average pairwise similarity among isolates within each cluster was generally greater than 95%, whereas similarity between clusters ranged from 91% to 92% (Fig. 1A). Bootstrap support of these clusters was strong in UPGMA (89–100%) (Fig. 1A), NJ (100%; data not shown) and MP networks (100%; data not shown). Some subclusters also occur within each of the four main clusters (Fig. 1A).

Identification of marker subsets of AFLP primer pairs: Pearson's correlations between the full data set and the eight *Eco*RI-primer pools ranged from 0.743 to 0.893 (Table 2), indicating that the data subsets are strongly correlated to the overall data ($P < 0.0001$ in all cases). The *Eco*RI+AA pool had the highest correlation coefficient (r = 0.893).

Data from five of the nine *Eco*RI+AA-primer pairs could be removed individually from the *Eco*RI+AA pool without disrupting the clustering topology established in the analysis of the complete data set. Removal of the data from the *Mse*I+CC primer alone, or the combined data from either the primer pairs *Mse*I+CA and *Mse*I+CG, or *Mse*I+CA and *Mse*I+GA, shifted one, or more, of the isolates to a location in the UPGMA tree outside of their prior strongly supported clade(s) (data not shown). The data were pooled from all possible combinations of three and four of these *Mse*I-primers and then the clustering

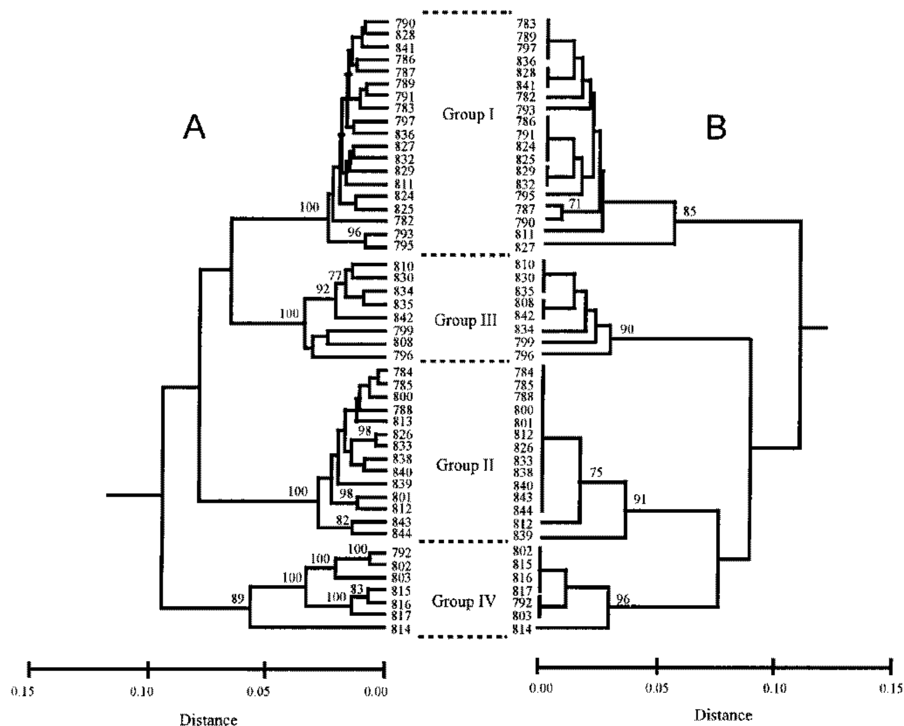

Fig. 1. UPGMA (unweighted pair grouping by mathematical averages) relatedness among the 48 tested *Cephalosporium maydis* isolates based on pooled data from *(a)* 68 AFLP primer pairs, and *(b)* four selected primer pairs (*Eco*RI + AA with *M*seI + CA, *M*seI + CC, *M*seI + CG and *M*seI + GA). The analysis separates the 48 isolates into four strongly supported clusters (Groups I through IV, as indicated). Bootstrap support (1000 iterations) for clusters receiving greater than 70% support is indicated. For clarity, only the last three digits of the five-digit strain number are used; thus, strain 10791 is listed in the figure as 791.

and correlation analyses were run as above (Table 2). Correlations between four of the five pooled data sets and the overall data pool produced $r > 0.800$ (Table 2). Two of the data subsets (*Eco*RI+AA with *Mse*I+CA, *Mse*I+CC, *Mse*I+GA and *Eco*RI+AA with *Mse*I+CA, *Mse*I+CC, *Mse*I+CG, *Mse*I+GA) had $r > 0.850$, a value higher than that of any of the other larger *Eco*RI+NN data pools, except for that of the *Eco*RI+TG pool (Table 2). Average similarity among the 48 samples with this data subset was approximately 88%, with minimum and maximum similarities of 68% and 100%, respectively. UPGMA (Fig. 1B), MP and NJ analyses (data not shown) also divided the isolates into the same clusters as observed in the analysis of the complete data set, although bootstrap support for these groupings was decreased (Fig. 1B), as expected with a reduced data set.

DISCUSSION

Many types of molecular markers have been used to characterize genetic diversity in fungi (see refs. 2 and 27 for reviews). The utility, repeatability and efficiency of the AFLP technique have led to its widespread application for the analysis of genetic

diversity in plants (*e.g.* 17), bacteria (*e.g.* 20) and fungi (*e.g.* 16,25). In our study, and in that of Gonzalez *et al.* (16), correlations between subsets of AFLP data appear to be relatively high, an improvement over many analyses conducted with other anonymous marker systems, *e.g.* RAPDs (16,40). In addition, the large number of useful AFLP markers (nearly 300 in our study) permits a more robust statistical analysis and, presumably, represents a greater coverage and dispersal of markers across the genome.

Based on AFLP polymorphisms, we have identified at least four distinct subgroups within *C. maydis* from Egypt. The distinctiveness of the four subgroups is strongly supported by bootstrapping (Fig. 1A). Isozyme patterns for five enzymes (Table 1) from these strains were monomorphic and were not useful as indicators of population subdivision. The lack of isozyme polymorphism suggests that these isolates all are closely related and probably belong to the same species, thereby confirming conclusions reached previously on the basis of morphology alone. This conclusion also is consistent with the 89% average similarity between AFLP profiles among the 48 strains that we examined.

One set of four AFLP primer pairs and a second set of three primer pairs both produce estimates of distance that are strongly correlated (r = 0.858 and 0.854, respectively) to distance estimates based on the pooled data from 68 primer pairs (Table 2). Not surprisingly, the clustering networks produced with the four primer-pair subset separated the isolates into the same four subgroups as the much larger data set (Figs. 1A and 1B), although bootstrap support for separation of these subgroups was diminished when only the subset data were used. These sets of three and four primer pairs should provide useful markers for identifying new and existing genetic subdivisions within more extensive collections of *C. maydis* now being isolated. If the existing subdivisions are confirmed in this larger sample, then the conserved and polymorphic bands from these AFLP profiles may be useful in the development of PCR diagnostics for this species and for its primary subgroups.

There is no detectable correlation between governate and pathogen subgroup, although our sample size is small for such an analysis. However, a more general grouping does suggest some pattern. Isolates from subgroups I, II and III were all collected from Lower, Middle and Upper Egypt. However, isolates from subgroup IV have so far been recovered only from the Nile delta region of Lower Egypt (a region that encompasses more than one governate). Additional sampling will be required to determine if this preliminary observation is indicative of geographic subdivision of this population.

Only two of the four subgroups, I and II, are represented in the ten isolates (10782–10791) that have been used in Egyptian pathogenicity analyses and maize breeding trials. These isolates are not inclusive of the total potential genetic diversity within *C. maydis* from Egypt. Although the strains in this historic set are known to vary in virulence (30,32), it remains to be determined whether the two additional subgroups that we have identified differ significantly from those already known in either virulence or host specificity. If differences in pathogenicity are correlated with subgroup, then the pathogenic potential of *C. maydis* has probably been underestimated. Pathogenic limitations have been observed for subgroups and for genetically separated populations of several other plant pathogenic fungi, including clonal lineages within *Magnaporthe grisea* infecting rice (24,44,45), and among geographically separated populations of *Colletotrichum lindemuthianum* on common bean (39). However, such correlations are not found in all host/pathogen combinations. Similar analyses have been made of populations

of *Colletotrichum graminicola* that cause sorghum anthracnose (3), but in these analyses the pathogen populations could not be readily subdivided into pathogenic subgroupings, even though there appears to be a relatively limited number of molecular genetic lineages. At least in the case of *M. grisea,* this combination of molecular genetic and pathogenic data has led directly to improved strategies for developing and deploying resistant rice cultivars (45). If pathogenic variation is correlated with subgroups in *C. maydis*, then a similar breeding and deployment strategy could be developed to counter this important pathogen. Studies of aggressiveness of the four different lineages are in progress in both field and greenhouse settings. In preliminary greenhouse studies, the four *C. maydis* lineages differ in their relative abilities to infect a series of eight maize differentials (9), and in the ability competitively to colonize plants (10).

In conclusion, AFLP markers were developed that distinguish four subgroups within Egyptian populations of *C. maydis*. The distribution of these subgroups does not appear to be uniform throughout the country, and not all have been included in the inoculum prepared to challenge new lines in the maize breeding program. The results of this study will be used by us to provide a more robust challenge for the Egyptian maize breeding program, to determine if economically important pathogenicity traits are correlated with molecular genetic lineage, to delineate the geographic distribution of the known subgroups in a larger and more diverse population sample, and to identify additional rare or geographically limited subgroups.

ACKNOWLEDGMENTS

This research was supported in part by the Agricultural Technology – Utilization and Transfer (ATUT) Program (USAID – Cairo) and the Kansas Agricultural Experiment Station. We thank A. Beyer, A. Clouse and A. Hanson for technical assistance.

REFERENCES

1. Abd-el-Rahim, M.F., Sabet, K.A., El-Shafey, H.A. and El-Assiuty, E.M. (1982) Chemical control of the late-wilt disease of maize caused by *Cephalosporium maydis. Agric. Res. Rev., Egypt* 60:31-49.
2. Anderson, J.B. and Kohn, L.M. (1995) Clonality in soilborne plant-pathogenic fungi. *Annu. Rev. Phytopathol.* 33:369-391.
3. Casela, C.R., Ferreira, A.S., Zeller, K.A. and Levy, M. (1995) Pathotype variation in the sorghum anthracnose fungus: a phylogenetic perspective for resistance breeding. *in:* Leslie, J.F. and Frederiksen, R.A. [Eds.] Disease Analysis through Genetics and Biotechnology: Interdisciplinary Bridges to Improved Sorghum and Millet Crops. pp. 257-276. Iowa State University Press, Ames, Iowa, USA.
4. Chen, W.D. (1994) Vegetative compatibility groups of *Verticillium dahliae* from ornamental woody plants. *Phytopathology* 84:214-219.
5. Chen, X., Line, R.F. and Leung, H. (1993) Relationship between virulence variation and DNA polymorphism in *Puccinia striiformis. Phytopathology* 83:1489-1497.
6. Correll, J.C., Klittich, C.J.R. and Leslie, J.F. (1987) Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77:1640-1646.
7. Dobinson, K.F., Patterson, N.A., White, G.J. and Grant, S. (1998) DNA fingerprinting and vegetative compatibility analysis indicate multiple origins for *Verticillium dahliae* race 2 tomato isolates from Ontario, Canada. *Mycol. Res.* 102:1089-1095.
8. Drenth, A., Tas, I.C.Q. and Govers, F. (1994) DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *Eur. J. Plant Pathol.* 100:97-107.
9. El-Assiuty, E.M., El-Shafey, H.A., Ismael, A.-S., Fahmy, Z.M. and El-Abedein, A.Z. (1998) Pathogenic variation in *Cephalosporium maydis. Phytopathology* 88:S25.
10. El-Assiuty, E.M., Ismael, A.M., Zeller, K.A. and Leslie, J.F. (1999) Relative colonization ability of greenhouse-grown maize by four lineages of *Cephalosporium maydis* from Egypt. *Phytopathology* 89:S23.

11. Elias, K.S., Zamir, D., Lictman-Pleban, T. and Katan, T. (1993) Population structure of *Fusarium oxysporum* f.sp. *lycopersici*: restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility group is an indicator of evolutionary origin. *Molec. Plant-Microbe Interact.* 6:565-572.
12. El-Itriby, H.A., Khamis, M.N., El-Demerdash, R.M. and El-Shafey, H.A. (1984) Inheritance of resistance to late wilt (*Cephalosporium maydis*) in maize. *Proc. 2nd Mediterranean Conf. on Genetics* (Cairo, Egypt), pp. 29-44.
13. El-Shafey, H.A., Abd-el-Rahim, M.F., Abd-el-Azim, O.Z. and Abd-el-Hamid, M.S. (1976) Carry-over of maize stalk-rot fungi in seed. *Agric. Res. Rev., Egypt* 54:29-42.
14. El-Shafey, H.A., El-Shorbagy, F.A., Khalil, I.I. and El-Assiuty, E.M. (1988) Additional sources of resistance to the late-wilt disease of maize caused by *Cephalosporium maydis. Agric. Res. Rev., Egypt* 66:221-230.
15. El-Shafey, H.A., Mahmoud, S.A.Z., El-Brolousy, M., El-Azhary, T.M. and Ibrahim, T.F. (1990) Biological control of maize late-wilt disease by inoculation with *Bacillus subtilis* and manuring. *Egypt. J. Appl. Sci.* 5:182-191.
16. Gonzalez, M., Rodriguez, M.E.Z., Jacabo, J.L., Hernandez, F., Acosta, J., Martinez, O. and Simpson, J. (1998) Characterization of Mexican isolates of *Colletotrichum lindemuthianum* by using differential cultivars and molecular markers. *Phytopathology* 88:292-299.
17. Hill, M., Witsenboer, H., Zabeau, M., Vos, P., Kesseli, R. and Michelmore, R. (1996) PCR-based fingerprinting using AFLPs as a tool for studying genetic relationships in *Lactuca* spp. *Theor. Appl. Genet.* 93:1202-1210.
18. Huss, M.J., Campbell, C.L., Jennings, D.B. and Leslie, J.F. (1996) Isozyme variation in the *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola*). *Appl. Environ. Microbiol.* 62:3750-3756.
19. Jacobson, K.M., Miller, O.K., Jr. and Turner, B.J. (1993) Randomly amplified polymorphic DNA markers are superior to somatic incompatibility tests for discriminating genotypes in natural populations of the ectomycorrhizal fungus *Suillus granulatus. Proc. Natl. Acad. Sci. USA* 90:9159-9163.
20. Janssen, P., Coopman, R., Huys, G., Swings, J., Bleeker, M., Vos, P., Zabeau, M. and Keraters, K. (1996) Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. *Microbiology* 142:1881-1893.
21. Keréyi, Z., Zeller, K., Hornok, L. and Leslie, J.F. (1999) Standardization of mating-type terminology in the *Gibberella fujikuroi* species complex. *Appl. Environ. Microbiol.* 65:4071-4076.
22. Kistler, H.C., Momol, E.A. and Benny, U. (1991) Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum. Phytopathology* 81:331-336.
23. Leslie, J.F. (1993) Fungal vegetative compatibility. *Annu. Rev. Phytopathol*. 31:127-150.
24. Levy, M., Correa-Victoria, F.J., Zeigler, R.S., Xu, S. and Hamer, J.E. (1993) Genetic diversity of the rice blast fungus in a disease nursery in Colombia. *Phytopathology* 83:1427-1433.
25. Majer, D., Mithen, R., Lewis, B., Vos, P. and Oliver, R.P. (1996) The use of AFLP fingerprinting for the detection of genetic variation in fungi. *Mycol. Res.* 100:1107-1111.
26. Meijer, G., Megnegneau, B. and Linders, E.G.A. (1994) Variability for isozyme, vegetative compatibility and RAPD markers in natural populations of *Phomopsis subordinaria. Mycol. Res.* 98:267-276.
27. Milgroom, M.G. (1996) Recombination and the multilocus structure of fungal populations. *Annu. Rev. Phytopathol.* 34:457-477.
28. Milgroom, M.G., Lipari, E.S. and Powell, W.A. (1992) DNA fingerprinting and analysis of population structure in the chestnut blight fungus *Cryphonectria parasitica. Genetics* 131:297-306.
29. Murray, M.G. and Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8:4321-4325.
30. Sabet, K.A., Samra, A.S. and Khalil, I.K. (1966) Variation in *Cephalosporium maydis*, the cause of the late wilt disease. *in:* Samra, A.S. and Sabet, K.A. [Eds.] Investigations on Stalk-Rot Diseases of Maize in U.A.R. pp. 46-93. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.
31. Sabet, K.A., Samra, A.S. and Mansour, I.M. (1966) Late-wilt of maize and a study of the causal organism. *in:* Samra, A.S. and Sabet, K.A. [Eds.] Investigations on Stalk-Rot Diseases of Maize in U.A.R. pp. 8-45. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.
32. Sabet, K.A., Zaher, A.M., Samra, A.S. and Mansour, I.M. (1970) Pathogenic behavior of *Cephalosporium maydis* and *C. acremonium. Ann. Appl. Biol.* 66:257-263.
33. Samra, A.S. and Sabet, K.A. (1966) An introductory note. *in:* Samra, A.S. and Sabet, K.A. [Eds.] Investigations on Stalk-Rot Diseases of Maize in U.A.R. pp. 1-7. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.
34. Samra, A.S., Sabet, K.A. and Abd-el-Rahim, M.F. (1966) Seed transmission of stalk-rot fungi and effect of seed treatment. *in:* Samra, A.S. and Sabet, K.A. [Eds.] Investigations on Stalk-Rot Diseases of Maize in U.A.R. pp. 94-116. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.

35. Samra, A.S., Sabet, K.A. and Hingorani, M.K. (1962) A new wilt disease of maize in Egypt. *Plant Dis. Rep.* 46:481-483.
36. Samra, A.S., Sabet, K.A. and Hingorani, M.K. (1963) Late wilt disease of maize caused by *Cephalosporium maydis. Phytopathology* 53:402-406.
37. Samra, A.S., Sabet, K.A., Kamel, M. and Abd-el-Rahmin, M.F. (1971) Further Studies on the Effect of Field Conditions and Cultural Practices on Infection with Stalk-rot Complex of Maize. Arab Republic of Egypt, Min. of Agriculture, Plant Protection Dept., Bull. no. 2.
38. Shehata, A.H. and Salem, A.M. (1972) Genetic analysis of resistance to late-wilt of maize caused by *Cephalosporium maydis. Sabrao J.* 4:1-5.
39. Sicard, D., Michalikis, Y., Dron, M. and Neema, C. (1997) Genetic diversity and pathogenic variation of *Colletotrichum lindemuthianum* in three centers of diversity of its host, *Phaseolus vulgaris. Phytopathology* 87:807-813.
40. Spooner, D.M., Tivang, J., Nienhuis, J., Miller, J.T., Douches, D.S. and Contreras, M.A. (1996) Comparison of four molecular markers in measuring relationships among the wild potato relatives *Solanum* section *Etuberosum* (subgenus *Potatoe*). *Theor. Appl. Genet.* 92:532-540.
41. Strausbaugh, C.A., Schroth, M.N., Weinhold, A.R. and Hancock, J.G. (1992) Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. *Phytopathology* 82:61-68.
42. Swofford, D.L. (1998) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4.0*. Sinauer Associates, Sunderland, MA, USA.
43. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995) AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407-4414.
44. Zeigler, R.S., Tohme, J., Nelson, R.J., Levy, M. and Correa-Victoria, F. (1994) Lineage exclusion: A proposal for linking blast population analysis to resistance breeding. *Proc. Int. Symp. on Rice Blast Disease* (International Rice Research Institute, Los Baños, the Philippines), pp. 267-292.
45. Zeigler, R.S., Cuoc, L.X., Scott, R.P., Bernardo, M.A., Chen, D.H., Valent, B. and Nelson, R.J. (1995) The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. *Phytopathology* 85:443-451.
46. Zeller, K.A., Jurgenson, J.E. and Leslie, J.F. (1998) Genetic variation in *Cephalosporium maydis* from Egypt. *Phytopathology* 88:S103.
47. Zeller, K.A., Jurgenson, J.E. and Leslie, J.F. (1999) AFLP markers reveal genetic variation in Egyptian populations of *Cephalosporium maydis. Fungal Genet. Newsl.* 46(suppl):94.