



SHORT COMMUNICATION

DIFFERENTIATION OF *DRECHSLERA GRAMINEA* ISOLATES BY CULTURAL CHARACTERISTICS AND SDS-PAGE

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SUMMARY

Isolates of *Drechslera graminea*, the causal agent of the barley leaf stripe disease, were obtained from phytopathological samples collected in 1998 from different regions of Syria and analyzed for differences in cultural characteristics and protein profiles as determined by a 10% sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Differences in mycelia growth and colour were noticed among isolates collected from different regions. On the other hand, protein polymorphisms (SDS-PAGE) were detected, and highly significant differences ($P < 0.001$) for band numbers were obtained among isolates. The protein profiles were highly repeatable, suggesting that the biochemical SDS-PAGE protein markers are suitable to detect variability among *D. graminea* isolates.

Key words: *Drechslera graminea*, Barley (*Hordeum vulgare* L.), SDS-PAGE.

Pyrenophora graminea Ito & Kuribayashi [anamorph *Drechslera graminea* (Rabenh. ex. Schlech. Shoem)] is a seed-borne pathogen which causes brown leaf stripe of barley (*Hordeum vulgare* L.), a disease responsible of crop yield reductions (Porta-Puglia *et al.*, 1986). The fungus survives on kernels as mycelium inside the parenchymatical cells of the pericarp. When the barley seeds germinate, the pathogen enters the plantlets through coleorhiza (Platenkamp, 1976). A conidium of this pathogen consists of several cells and their genetic content may differ (Smedegaard-Petersen, 1983).

Traditionally, morphological features have been used for estimating variability among pathogen isolates, but such features are often subject to environmental factors.

Moreover, this procedure requires some expertise since variation in morphological features among isolates occur (Jawhar *et al.*, 2000), and furthermore, it is a time consuming task when similar species are present in the same field. The discontinuous buffer system to fractionate proteins based on Laemmli (1970) sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) offers one approach for the above purpose (Payne *et al.*, 1981; Ahmad and McNeil, 1996). This technique has an extra advantage in providing a tool for species/varieties identification and in deducing taxonomic relationships (Panda *et al.*, 1986).

The aim of the present study was to characterize isolates of *D. graminea* collected from different regions of Syria, using morphological characteristics and SDS-PAGE protein markers.

Selection and designation of isolates. During a preliminary study, 15 isolates of *D. graminea* selected on the basis of cultural morphology and virulence (Arabi *et al.*, 2001) were used in this study (Table 1). These isolates were obtained from lesions on naturally infected barley leaves collected in 1998 from different regions of Syria. Barley leaf tissues showing necrosis and perinecrotic chlorosis were cut into pieces (5 x 10 mm) and sterilized in a 5% sodium hypochlorite solution (NaOCl) for 5 min. After immersion in sterile distilled water (three times for 5 min), leaf fragments were transferred to Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) and incubated for 8 days at $21 \pm 1^\circ\text{C}$ in dark.

Table 1. Isolates of *D. graminea* collected from different regions of Syria.

Isolate	Region
NE1, NE2, NE3, NE4, NE5	North-East
SW1, SW2, SW3, SW4, SW5	South-West
I1, I2, I3, I4, I5	ICARDA*

* ICARDA: International Center of Agriculture Research in the Dry Areas, Aleppo, Syria.

Cultural characteristics assessment. Comparison of fungal growth was carried out by measuring the diameter of 3 or 4 colonies per isolate after 8 days culture on PDA at 21±1°C, and colony colour was recorded using an empirical scale from 1 (white) to 5 (black) (Table 2). Statistical analyses to evaluate correlation between geographical origin and cultural characteristics of isolates were performed using Statview computer package.

Table 2. SDS-PAGE bandnumbers, mycelia growth, after 8 days culture and isolates colour of 15 of *D. graminea*.

Isolate	Mycelia growth (cm)	Mycelia colour	Band no.
NE1	5.8	5 ^a	4
NE2	6.1	4	1
NE3	5.8	4	2
NE4	4.0	4	7
NE5	5.4	5	3
SW1	5.0	2	6
SW2	5.1	3	5
SW3	5.8	2	7
SW4	5.6	3	3
SW5	5.3	3	2
I1	3.8	1	3
I2	3.1	2	2
I3	3.4	1	5
I4	3.4	2	6
I5	4.3	2	5
LSD	0.9		

^a (1): white, (2): white gray, (3): gray, (4): gray black and (5): black.

Protein extraction. Mycelia were harvested from 15 isolates (Table 1) after 18 days culture on PDA media according to preliminary testing. 40 mg from actively growing mycelium are suspended in 1 ml of an extraction buffer that contained 2% (W/V) SDS, 5% (V/V) 2-mercaptoethanol, 0.001% (W/V) pyronin, 10% (V/V) glycerol and 0.063 M Tris-HCl (pH 6.8). Samples were left for 90 min at room temperature and shaken every 15 min. Then they were placed in a boiling water bath for 2 min and allowed to cool and centrifuged (Eppendorf microcentrifuge) for 5 min at 14,000 rpm.

SDS-PAGE. Protein extracts (15 µl) were placed into each slot of a vertical slab gel electrophoresis unit (160 x 180 x 0.75mm, Hoefer SE-600). A constant current of 25 mA was used to run two gels for 3.5 h. The procedure was performed according to Payne *et al.*

(1981) using a 5% stacking gel (pH 6.8) and a 10% running gel (pH 8.8). The Canadian wheat cultivar Marquis was included as a reference to estimate molecular weights as suggested by Ng and Bushuk (1987).

Gels were stained for 24 h with 0.02% Coomassie blue R250, 5% ethanol, and 6% Trichloroacetic acid (TCA). After 5 hours destaining with 6% (TCA), gels were immersed in water, photographed, air dried for 24 hours and kept.

SDS-PAGE electrophoresis runs were repeated five times for all isolates and have similar results. Subunits were scored as presence (1) and absence (0). Unweighted Pair Group Means Analysis (UPGMA) of the STATISTICA computer package was used.

Highly significant differences were observed among geographical origin and mycelia growth ($P < 0.001$). North-East (NE) and South-West (SW) isolates after 8 days culture showed the highest in mycelia growth followed ICARDA (I) isolates (Table 2). Isolate NE2 and I2 showed the highest and lowest mycelia growth after 8 days of growth (6.1 and 3.1 cm respectively) (Table2). Different colony colours were also observed among isolates after 8 days of growth on PDA medium. However, colony colour of North-East was black, whereas the South-West and ICARDA isolates were gray to white (Table 2).

A high level of protein polymorphism was detected among isolates of different origin, and the number of protein subunits varied between 1 and 7 (Table 2, Fig. 1). Band numbers ranged from 1 to 7 for NE and SW isolates and 2 to 6 for I isolates. Most of protein subunits ranged between 60 and 150 kD (Fig. 1). When protein subunits were analyzed using cluster analysis of UPGMA, the isolates clustered in distinct groups (Fig. 2). ICARDA isolates appeared to have a slightly greater diversity than other regions. Noteworthy, no significant correlations were obtained between number of subunits and all other cultural characteristics.

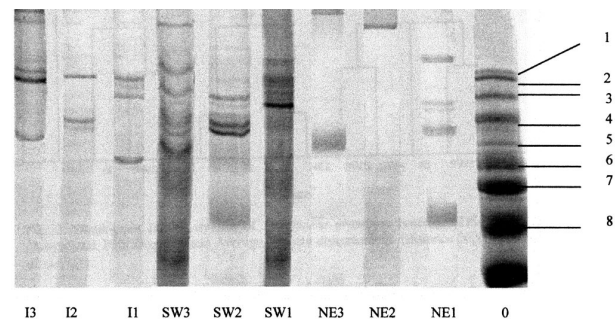


Fig. 1. SDS-PAGE of 9 isolates of *P. graminea*. 0: Canadian wheat variety Marquis (Marker), where; 1≈145, 2≈112, 3≈98, 4≈83, 5≈60, 6≈54, 7≈50 and 8≈45 KD (Ng and Bushuk, 1987). NE: North-East Syria, SW: South-West Syria, I: ICARDA.

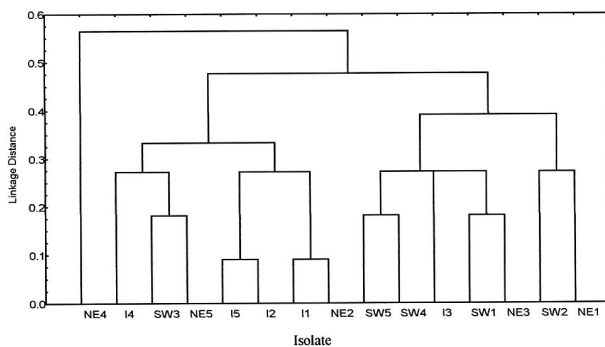


Fig. 2. Dendrogram for 15 isolates of *D. graminea* based on SDS-PAGE data. Unweighted Pair Group Mean Average, Percent disagreement. [distance (x_i, y_i) = (Number of $x_i \neq y_i$)/ i].

In the results of this study, the isolates of *D. graminea* could be distinguished based on culture characters and SDS-PAGE marker. Both methods suggest that there were high differences among the isolates of this pathogen collected from different origin from Syria. Protein profiles illustrated that the identification of *D. graminea* isolates based on physiological and morphological data alone was not possible due to the similarities of the morphological characters among the isolates. Leung *et al.* (1993) attributed genetic variability within the pathogen population to several factors such as genetic change (mutation or recombination) or migration from other geographic areas. In addition, the seasonal nature of agricultural plant production may cause large fluctuations in the population sizes of both the host and the pathogen leading to a genetic drift (Burdon, 1992).

Morphological characters such as colony colour may be subjected to environmental variation and so a biochemical approach was used to help identify isolates. Therefore, they may not always be an accurate way of identification unless the morphological statistics are based on a large population taken from diverse origins and environments (Smedegaard-Petersen, 1983). No correlation between band number obtained by SDS-PAGE and mycelia growth and colour was observed. This suggests that SDS-PAGE technique can be used to further facilitate characterization of the *D. graminea* isolates in different regions. On the other hand, the dendrogram revealed that most isolates in each group based on protein profiles belonged to different regions. This may be due to the fact that the spores of this fungus are able to spread over hundreds of kilometres either through transportation of infected seeds or through the winds.

Our results could support results of Stevens *et al.*, 1996 and Jawhar *et al.*, 2000 that SDS-PAGE markers

can provide additional information to the RAPD data, revealing other types of polymorphism, and that this data can help in better clarification of the genetic structure of *D. graminea* isolates.

In conclusion, cultural characteristics, though important, are not sufficient to characterize *D. graminea* isolates and further biochemical and molecular markers are needed to characterize these isolates accurately. Although our sample size was limited, the results, although preliminary, indicated wide diversity in the population structure of the pathogen in Syria.

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