Theor Appl Genet (1986) 72:743–747

Association of an isozyme locus and strawbreaker foot rot resistance derived from *Aegilops ventricosa* in wheat

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Received April 12, 1986; Accepted May 22, 1986
Communicated by A. R. Hallauer

Summary. Thirty lines from a cross between VPM/Moisson 421 and Selection 101 were used in the study to determine whether strawbreaker foot rot resistance derived from *Aegilops ventricosa* was associated with an allele for endopeptidase. The progeny examined for foot rot lesions represented F_2 derived F_5 lines and enzyme assays were done on F_6 seedlings. The results indicate that the wheat and 'VPM/Moisson 421' endopeptidase alleles are distinctly different. The endopeptidase allele frequencies of 30 lines were compared with strawbreaker foot rot resistance as measured by the lesion severity index. The results demonstrate a close association between the gene for strawbreaker foot rot resistance and the endopeptidase allele derived from *Ae. ventricosa.*

Key words: Isozyme – Wheat breeding – Foot rot lesions – Eyespot – *Aegilops ventricosa*

Introduction

Limited resistance to strawbreaker foot rot (eyespot) caused by *Pseudocercosporella herpotrichoides* (Fron) exists in *Triticum aestivum* L. One cultivar that has proven to have some resistance is 'Cappelle-Desprez' from France. The highest level of resistance occurs in an alien grass species, *Aegilops ventricosa* Tausch which is also designated as *Triticum ventricosum* Ces. Some of the increased level of resistance observed in *Ae. ventricosa* has been introduced into a wheat line, 'VPM-1' (Maia 1967). Although 'VPM-1' has increased resistance to strawbreaker foot rot, it also exhibits a significant decrease in yield. Therefore, efforts were initiated to use 'VPM-1' to obtain resistant cultivars without the large decrease in yield. From these efforts the cultivar 'Roazon' was developed (Doussinault et al. 1983).

The resistance of 'Cappelle-Desprez' was shown to be caused by a gene on chromosome 7A (Law et al. 1975). The resistance of 'Roazon' has been shown to be caused by a gene located on chromosome 7D (Jahier et al. 1979). Gale et al. (1984) suggest that the gene for strawbreaker foot rot resistance was located on the distal end of chromosome 7DL, since an isozyme marker for α-amylase previously located near the centromere segregated independently of the resistant gene.

A biochemical marker which is closely linked to resistance to strawbreaker foot rot would be valuable for breeding programs designed to incorporate resistance since the present assays for resistance are tedious and not always reliable. Isozyme loci are excellent biochemical markers since they are usually codominantly inherited, do not show pleiotropic effects, and rarely exhibit epistasis.

The isozyme loci for endopeptidase are located on chromosomes 7AL, 7BL, and 7DL (Hart and Langston 1977). The endopeptidase loci on chromosome 7AL and 7BL have been shown to be approximately 42 map units from the centromere (McMillin 1977; McMillin and Tuleen 1977).

This paper examines the relationship between an isozyme locus for endopeptidase and the reaction to strawbreaker foot rot of 30 wheat lines from a cross between a 'VPM-1' derived parent and foot rot susceptible parent. The lines were examined to determine whether endopeptidase could be used as a marker for resistance.

Materials and methods

Genetic stocks

Seeds of 'VPM-1' and 'Roazon' were kindly supplied by G. Doussinault (National Institute of Agronomic Research, Le Rheu, France) and D. H. Smith (USDA-ARS, Small Grains Collection, Beltsville, MD). Seeds of 'Chinese Spring' were kindly supplied by E. R. Sears (University of Missouri).

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The 30 lines were chosen from a cross between 'VPM/Moisson 421' and 'Selection 101' (CI 13438) which had been inoculated with the foot rot fungus. 'VPM/Moisson 421' and 'Selection 101' are highly resistant and highly susceptible to strawbreaker foot rot, respectively. Groups of resistant, susceptible, and intermediate reaction types were represented without regard for the distribution of the population. A subjectively assigned foot rot severity lesion index was the criterion for classification of foot rot reaction of the wheat germplasm.

Electrophoresis

Seeds from F_5 lines were surface sterilized for 10 min with 5% sodium hypochlorite, washed twice with distilled water, and soaked for 24 h. The seeds were planted on germination paper and grown at 22 °C for nine days. Nine-day old etiolated shoots were homogenized in 0.025 M glycylglycine buffer, pH 7.4, using a mortar and pestle chilled on ice. The extracts were applied to 9 × 10 mm Gelman absorbent strips (No. 51291) and inserted into vertical slots cut into 12% starch gels (6% Connaught: 6% Sigma starch). The starch gels were prepared as previously described by Scandalios (1969). A Tris-citrate buffer system was used for electrophoresis. A Tris-citrate stock solution was prepared by dissolving solid citric acid into a 1 M solution of Tris until a pH of 7.0 was reached. The gel buffer consisted of 5 ml stock buffer to 295 ml distilled water while the tank buffer consisted of 50 ml of stock of buffer to 1 liter distilled water. Electrophoresis was conducted overnight at a constant voltage of 150 volts for 16–17 h. Following electrophoresis the gel was sliced into slabs 2 mm thick, placed in a tray and stained for endopeptidase activity using a modification of Melville and Scandalios (1972). The gel was stained for 2 h at 37 °C in a solution made up of 11.3 mg N-α-Benzoyl-DL-Arginine-LB-Naphthylamide (BANA) and 25 mg Black K Salt added to 0.1 M Tris-maleate-NaOH, pH 6.4. Gels were fixed in a solution of 1 : 5 : 5, glacial acetic acid : methanol : water.

Determination of strawbreaker foot rot resistance

The two parents and 70 progeny of the cross were sown in single rows 1.5 m in length and spaced 0.3 m apart. The test was planted 16 September 1982, near Pullman, WA, and replicated two times. The seeding rate was 7 g per row. Progeny were F_2 derived F_5 lines and had not been selected intentionally for other traits. The rows were inoculated on 23 November 1982, with the strawbreaker foot rot pathogen when plants had reached the 4 to 7 tiller stage. The inoculation technique was similar to the conidial suspension spray method described by Murray and Bruehl (1983). The concentration of the suspension was 2×10^5 conidia per milliliter. The suspension was applied directly onto the plants within each row with a hand sprayer.

At harvest the plants were cut 10 cm above the soil surface and the stubble or crown portion of the plants was removed from the soil. A foot rot lesion severity index was assigned by scoring 50 randomly chosen straws from several plants (approximately 20 plants) within each row. A rating scale consisting of 1 to 5 was used, where 1 = no eyespot lesions, 2 = shallow but definite lesion, 3 = deeper lesion, 4 = deep lesion with some pseudoparenchyma, and 5 = deep lesion with abundant pseudoparenchyma. This scale represents a slight modification of the one described by Bruehl and Machtmes (1985). The mean score over straws for each row was obtained by multiplying the number of straws in class 1 to 5 by 1, 2, 3, 4 and 5, respectively, summing their products and dividing by 50. The mean score of the 30 lines were used for comparisons with their EP-V1 allele frequencies.

Results

The initial study was conducted to determine whether wheat germplasm possessing the *Ae. ventricosa* endopeptidase allele displayed an altered zymogram pattern. 'VPM-1' has foot rot resistance derived from *Ae. ventricosa*. 'Roazon' and 'VPM/Moisson 421' also carry the foot rot resistance gene from *Ae. ventricosa*.

'Chinese Spring', 'Moisson', and 'Selection 101', do not possess the *Ae. ventricosa* gene for strawbreaker foot rot resistance. When the endopeptidase zymogram pattern observed in 'VPM-1', 'Roazon', and 'VPM/Moisson 421' was compared with the endopeptidase pattern observed in 'Chinese Spring', 'Moisson', and 'Selection 101', the *Ae. ventricosa* endopeptidase gene (*EP-V1*) could clearly be distinguished from the wheat gene (*EP-D1*) (Fig. 1). 'VPM-1', 'Roazon', and 'VPM/Moisson 421' expressed the *Ae. ventricosa* endopeptidase gene (*EP-V1*) and were classified as phenotype B while 'Chinese Spring', 'Selection 101', and 'Moisson' expressed the wheat endopeptidase gene (*EP-D1*) and were classified as phenotype A (Fig. 1). For example,

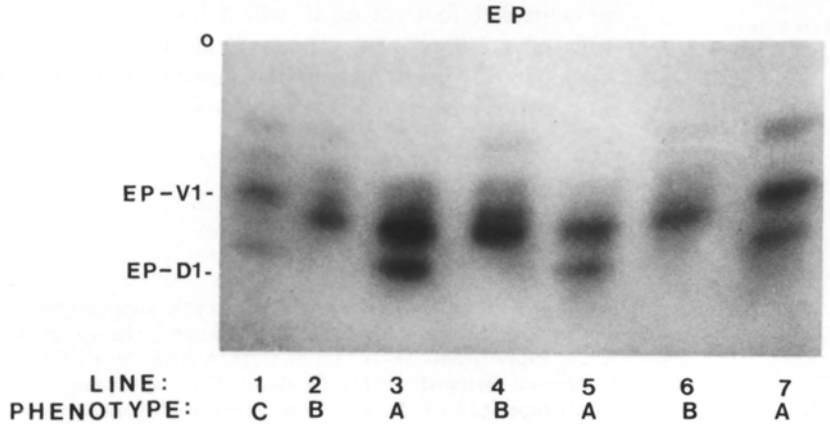

Fig. 1. Zymogram phenotypes of endopeptidase observed in *Aegilops ventricosa* and *Triticum aestivum*. Migration is anodal. 0 = point of sample insertion. *Line 1* = *Aegilops ventricosa*, *Line2* = 'VPM', *Line 3* = 'Selection 101', *Line 4* = 'VPM/Moisson 421', *Line 5* = 'Moisson', *Line 6* = 'Roazon', *Line 7* = 'Chinese Spring'. Phenotype A = presence of *EP-D1*, absence of *EP-V1*. Phenotype B = absence of *EP-D1*, presence of *ED-V1*. Phenotype C = *Ae. ventricosa*

'VPM/Moisson 421' exhibits no *EP-D1* but possesses *EP-V1* (Fig. 1). Since the *Ae. ventricosa* endopeptidase gene (*EP-V1*) was distinctly different from the wheat gene (*EP-D1*), endopeptidase variation could be analyzed in lines segregating for strawbreaker foot rot resistance.

Thirty lines had mean foot rot lesion indices that ranged from 1.30 to 4.41 with some lines representing enzyme classes 1, 2, 4 and 5 (Table 1). They were chosen for a blind study to determine whether endopeptidase could be used as a marker for strawbreaker foot rot resistance. At least 20 F_6 seeds for most lines were chosen at random to be examined electrophoretically. The zymogram phenotype was determined for each plant. Plants expressing phenotype A have only the wheat endopeptidase gene *EP-D1,* while plants expressing phenotype B have only the *Ae. ventricosa* gene, *EP-V1* (Fig. 2). The allele frequency of the wheat and *Ae. ventricosa* endopeptidase alleles was determined for each line based on all the seed examined. The lines were grouped into five phenotypic classes based on the endopeptidase allele frequencies. Class I were lines completely homozygous for the wheat allele, *EP-D1.* Class II were lines where *EP-D1* was much more frequent than the *Ae. ventricosa* allele, *EP-V1* (Fig. 2A). Class III was to be for lines with equal frequencies of *EP-D1* and *EP-V1* but none were detected among the 30 lines tested. Class IV were lines where *EP-V1* occurred at a higher frequency than *EP-D1.* Class V were lines homozygous for the *Ae. ventricosa* allele, *EP-V1* (Fig. 2B).

Table 1. Endopeptidase allele frequencies and strawbreaker foot rot indices for thirty lines of the cross 'VPM/Moisson 421'/'Selection 101'

Line no./parent	Endopeptidase phenotypic class	Frequency of *Aegilops allele, EP-V1*	Mean indices for resistance to strawbreaker foot rot
2406	I	0	3.20
2396	I	0	3.38
2397	I	0	3.28
2399	I	0	4.41
2405	II	0.025	3.93
2426	II	0.056	4.09
2464	II	0.071	3.21
2423	II	0.075	3.60
2418	II	0.136	2.22
2461	II	0.150	3.63
2438	II	0.167	3.76
2385	II	0.312	2.06
2458	IV	0.647	2.00
2437	IV	0.656	1.35
2459	IV	0.679	1.55
2448	IV	0.737	1.92
2433	IV	0.750	1.49
2383	IV	0.800	2.19
2460	IV	0.806	1.36
2452	IV	0.833	1.41
2379	IV	0.844	2.11
2395	IV	0.875	1.98
2412	IV	0.900	1.30
2425	IV	0.925	1.38
2447	IV	0.933	2.11
2422	V	1.0	1.48
2413	V	1.0	1.50
2427	V	1.0	2.02
2380	V	1.0	1.94
2414	V	1.0	1.54
'VPM/Moisson'	V	1.0	1.57 ± 0.13
'Selection 101'	I	0	3.91 ± 0.26

After the allele frequencies were determined for all the lines, the mean lesion indices for strawbreaker foot rot resistance were provided for two replications from Pullman, Washington.

The results conclusively revealed a close association between increased frequency of the *Ae. ventricosa* endopeptidase gene *EP-V1* and an increase in foot rot resistance (Table 1). Class I, homozygous for the wheat gene *EP-D1* has the lowest level of resistance to foot rot (the higher the score, the lower the resistance), while Class V, homozygous for the *Aegilops ventricosa* gene *EP-V1* has the highest resistance (Table 1). The simple correlation between the allele frequency of *EP-V1* and resistance to strawbreaker foot rot was highly significant ($r=0.867$, $P<0.001$) and clearly demonstrated that the frequency of the *Ae. ventricosa* allele is closely associated with strawbreaker foot rot resistance.

Discussion

The zymogram patterns of 'VPM-1', 'Roazon' and 'VPM/Moisson 421' were distinctly different from 'Chinese Spring', 'Selection 101', and 'Moisson' indicating that the *Ae. ventricosa* endopeptidase gene could be detected in breeding programs utilizing 'VPM-1', 'Roazon', or 'VPM/Moisson 421' for resistance to strawbreaker foot rot.

The high correlation between endopeptidase gene *EP-V1* and reaction to strawbreaker foot rot as measured by the lesion severity index was indicative that the endopeptidase gene is closely linked to the gene for foot rot resistance. The endopeptidase gene should be a useful marker for screening wheat selections for foot rot resistance. The coefficient of determination for predicting foot rot reaction from the frequency of the *EP-V1* allele was moderately high ($r^2=0.75$) for the 'VPM/Moisson 421'//'Selection 101' cross. The mean lesion severity indices and confidence intervals for the

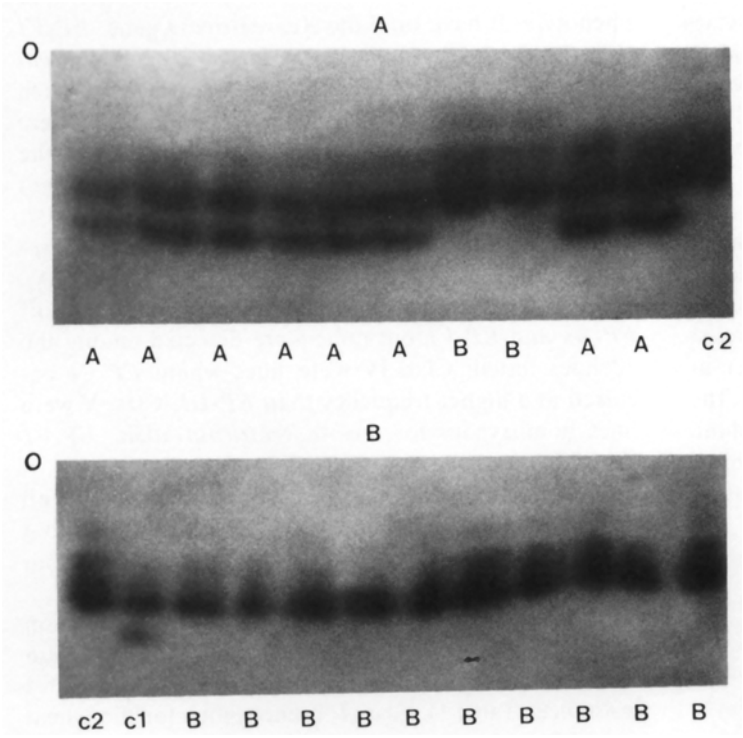

Fig. 2. **A** Zymogram phenotypes observed in plants of line 2461. Migration is anodal. 0 = point of sample insertion. C_2 = VPM control. **B** Zymogram phenotypes observed in plants of line 2413. Migration is anodal. 0 = point of sample insertion. C_2 = 'VPM' control, C_1 = 'Chinese Spring' control

susceptible and resistant parents were 3.91 ± 0.26 and 1.57 ± 0.13, respectively (Table 1). As shown in Table 1, if all lines in the endopeptidase phenotypic classes of I and II were discarded, no lines with lesion severity indices equivalent to the resistant parent would have been eliminated. Conversely, none of the lines in endopeptidase phenotypic classes IV and V were equivalent to the foot rot susceptible parent for lesion severity. Therefore, selection among progenies in endopeptidase phenotypic classes of IV and V should identify lines whose progeny have a high frequency of the 'VPM/Moisson 421'-derived gene for resistance to foot rot.

No class III lines were found in the present study. Of the thirty lines used in the study, about 10 lines each represented progeny which had the most resistant (1.30 to 1.55) and susceptible (3.20 to 4.41) lesion indices. There were 48 lines with intermediate lesion indices of 1.61 to 2.78. Only 10 lines with values of 1.92 to 2.22 were tested for zymogram phenotype. It is likely some class III lines would have been detected if the complete range of intermediate lines had been included. Furthermore, the wide range of frequencies of seedlings with the *EP-V1* and *EP-D1* alleles detected in lines of classes II and IV was not expected. Assuming that these classes comprise lines derived from F_2 plants which were heterozygous for the isozyme locus, the proportion of seedlings with each allele should be very similar by the F_6. Perhaps a sample of 20 seedlings was too small.

Since the assay for strawbreaker foot rot is tedious and not totally accurate, the use of the endopeptidase locus will be a valuable tool for breeding programs designed to incorporate strawbreaker foot rot resistance into commercial wheat cultivers.

Experiments are underway to determine the actual map distance between *EP-V1* and the resistant gene as well as to determine whether endopeptidase variation can be used as a marker for strawbreaker foot rot resistance derived from 'Cappelle-Desprez'.

Acknowledgement. The authors gratefully acknowledge the assistance of J. A. Pritchett, Agricultural Research Technician, USDA-ARS, Pullman, WA for his assistance in assessing foot rot lesion indices.

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