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Wheat chromosome 2D carries genes controlling the activity of two DNA-degrading enzymes

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Abstract DNA-degrading enzymes of 24.0 kDa and 27.0 kDa were observed to have different activities in two common wheat (*Triticum aestivum* L.) cultivars, 'Wichita' and 'Cheyenne'. A substrate-based SDS-PAGE assay revealed that these two enzymes were much more active in 'Wichita' than in 'Cheyenne'. Genes controlling the activities of these two enzymes were localized on chromosome 2D by testing DNA-degrading activities in reciprocal chromosome substitution lines between 'Wichita' and 'Cheyenne'. While the allele on 'Wichita' chromosome 2D stimulated the activities of the 24.0- and 27.0-kDa enzymes in Cheyenne, the allele on 'Cheyenne' chromosome 2D did not reduce the activities of the 24-kDa and 27-kDa enzymes in 'Wichita'. Whether these genes code for the DNA-degrading enzymes themselves or for factors that regulate the enzyme activities remains unknown.

Key words *Triticum aestivum* · DNase · Nuclease · Cytogenetics · Gene mapping

Introduction

DNA-degrading enzymes can be nucleases that digest both RNA and DNA molecules, or deoxyribonucleases (DNases) that can only degrade DNA (Wilson 1982). Several DNA-degrading enzymes have been reported in common wheat (Hanson and Fairly 1969; Chevrier and Sarhan 1980; Blank and McKeon 1989; Yen and Baenziger 1993). Negatively photoregulated DNA-degrading activities have also been observed to be closely associated with wheat leaf senescence (Blank and McKeon 1989). However, none of the loci controlling the DNA-degrading activity has been genetically or physically mapped. This lack of information on the genetic and physical locations of these loci is a major limitation to our understanding, manipulation, and isolation of their respective genes.

In order to map the loci that encode or regulate the activities of DNA-degrading enzymes, the activities of individual enzymes need to be distinguished, and the genetic polymorphism at these loci need to be identified. Both of these goals can be achieved by investigating DNA-degrading activities in crude plant extracts with a substrate-based SDS-PAGE assay (Yen and Green 1991; Yen and Baenziger 1993). This is done by studying DNA-degrading activity band patterns in SDS-PAGE gels cast with highly polymerized DNA molecules (Blank and McKeon 1989; Yen and Green 1991; Yen and Baenziger 1993). These bands result from *in situ* digestion of DNA molecules by electrophoretically separated DNA-degrading enzymes and thus represent the existence of corresponding enzymes. The objectives of the investigation presented in this communication were (1) to identify polymorphism of DNA-degrading activities in the two cultivars, and (2) localize the loci controlling the identified polymorphic enzyme activities to specific chromosomes.

Materials and methods

Common wheat (*Triticum aestivum* L., 2n = 6X = AABBDD) cvs 'Chinese Spring' (CS), 'Wichita' (WI), and 'Cheyenne' (CNN) and a complete set of 42 reciprocal chromosome substitution lines between WI and CNN were used in this study. Independently developed duplicates of D genome chromosome substitution lines were also used to investigate the possible heterogeneity in genetic background in our repeated experiments (Law 1966; Yen and Baenziger 1992). Substitution lines are designated by the abbreviation for the background parent, and the substituted chromosome is designated in parentheses. For example, a 'Wichita' chromosome substituted into 'Cheyenne' is designated CNN(WIxx), where xx indicates the substituted chromosome, such as 1A, 2D, etc. The development of these lines has been previously described (Zemetra et al. 1986; Berke et al. 1992a). These reciprocal chromosome substitution lines were kindly provided by Dr. R. Morris and are maintained at the University of Nebraska.

The preparation of crude plant extracts and the electrophoresis and activity staining of wheat DNA-degarding enzymes were done as previously described by Yen and Baenziger (1993). Protein content was assayed

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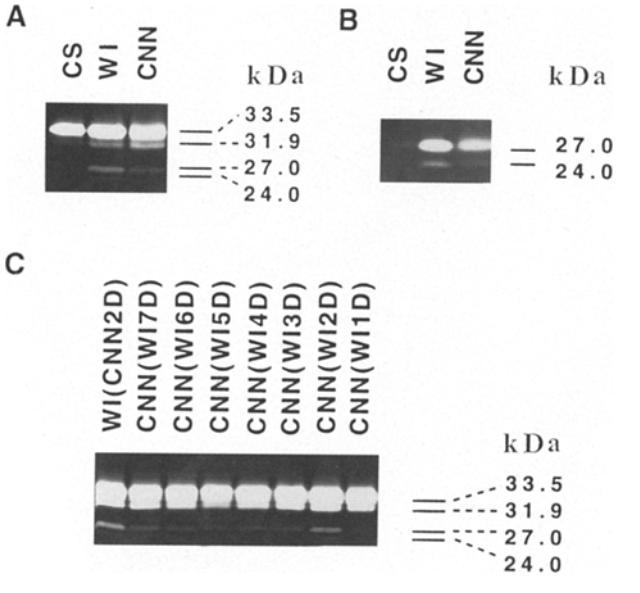
according to Bradford (1976). The BIO-RAD Protein II and mini protein II electrophoresis systems were used. Separating gels were cast with 0.5 mg/ml highly polymerized single-stranded fish sperm DNA (US Biochemical Co). To insure the repeatability of our results, multiple gels were loaded with crude plant extract samples from (1) the same extractions (repeating the gel procedure), (2) different extractions of the same plants (avoiding tissue-specific results and sampling errors), or (3) different extractions from different plants (avoiding plant-to-plant variation). The estimate of apparent molecular mass was based on the mobilities of the enzymes relative to those of the prestained protein markers as specified by the supplier (Bethesda Research Laboratories) and reflected the mobilities of these enzymes in the absence of reducing agents. All of the results were visually scored and summarized based on gels from separate experiments.

Results and Discussion

Four DNA-degrading activity bands were observed in the gels loaded with extracts of wheat cvs 'Chinese Spring', 'Wichita', and 'Cheyenne' (Fig. 1A, B). The appearance and intensity of these bands were generally consistent in our repeated experiments. These bands represent DNA-degrading enzymes that have an apparent molecular mass of 24.0, 27.0, 31.9, and 33.5 kDa, respectively. The 27.0-, 31.9-, and 33.5-kDa enzymes have been reported by Yen and Baenziger (1993) in 'Chinese Spring'. The appearance of the 24.0-kDa enzyme, which was not observed by Yen and Baenziger (1993), may be explained by our use of a lower concentration of DNA in the gels (0.5 mg/ml for this study versus 0.6 mg/ml for the previous study).

Fig. 1A–C Band patterns of DNA-degrading enzyme activities in wheat cultivars and intercultivar substitution lines. **A** Cultivars 'Chinese Spring' (*CS*), 'Wichita' (*WI*), and 'Cheyenne' (*CNN*). Each line was loaded with 60 µg total protein. Gel was run in a BIO-RAD Mini Protein II cell. **B** Cultivars 'Chinese Spring' (*CS*), 'Wichita' (*WI*), and 'Cheyenne' (*CNN*). Each line was loaded with 180 µg total protein. Gel was run in a Bio-RAD Protein II cell. **C** Wichita D-genome chromosome substitutions in 'Cheyenne' [*CNN (WI1D ~ CNN (WI7D)*] and 'Cheyenne' chromosome 2D substitution in 'Wichita' [*WI (CNN2D)*]. Gel was run in a BIO-RAD Mini Protein II cell. Each line was loaded with 80 µg total protein



By casting less DNA in the gel, we reduced the background staining density and thus increased the assay sensitivity for this enzyme, which showed weak activity under our assay conditions. The 27-kDa band in 'Chinese Spring' and the 24-kDa band in 'Cheyenne' were not visible when approximately 50-100 µg of total protein per lane (our normal loading amount) was loaded (Fig. 1A), but they did appear when the loading amount was tripled (Fig. 1B). The 24-kDa band was not visible in 'Chinese Spring' even if the plant extract loaded was tripled (Fig. 1B).

Our assay revealed that the 24- and 27-kDa DNA-degrading activities were much higher in 'Wichita' than in 'Cheyenne' (Fig. 1A, B). This difference in enzyme activity provided an opportunity to map the respective loci genetically and physically. As a first step toward these goals, a complete set of 42 reciprocal chromosome substitution lines between 'Wichita' and 'Cheyenne' were assayed for the activities of these two enzymes. The results showed that the 27-kDa enzymes were active, though at different levels, in all of the WI (CNNxx) and CNN (WIxx) substitution lines [only data for CNN (WI1 ~ 7D) and WI (CNN2D) are shown in Fig. 1C]. The activity of the 24-kDa enzyme was observed in all of the WI (CNNxx) lines and CNN (WI2D). In addition, the activity of the 24-kDa enzyme, though very weak, was still detectable in all of the remaining CNN (WIxx) lines, as in 'Cheyenne' (Fig. 1B), when the amount of plant extracts loaded was tripled (data not shown). However, the activities of the 24- and 27-kDa enzymes were much higher in the WI (CNNxx) lines than in the CNN (WIxx) lines, except for the CNN (WI2D) in which these two enzymes were as active as in 'Wichita' (Fig. 1C). The effect of 'Wichita' chromosome 2D in the 'Cheyenne' genetic background indicated that this chromosome carries a gene or genes that are responsible for the activity difference of the 24- and 27-kDa enzymes between 'Wichita' and 'Cheyenne'.

While CNN (WI2D) showed increased activities of the 24- and 27-kDa enzymes, WI (CNN2D) showed no such loss of DNA-degrading activities. Our results demonstrate an unidirectional effect of reciprocal chromosome substitutions. Similar cases in reciprocal substitution analyses have been reported for winter wheat heading date by Zemetra et al. (1986), for winter wheat lodging by Al-Qaudhy et al. (1988), and for winter wheat yield, and yield components and their stability by Berke et al. (1992a, b). All of the reciprocal substitution lines used carry a cytoplasm from 'Chinese Spring' (Gill et al. 1963; Morris et al. 1966), and the possible cytoplasmic effect was, therefore, eliminated. The anomalous behavior of WI (CNN2D) could also be explained by incorrect substitution caused by a univalent shift during the derivation of this line. However, this possibility was extremely low because of the test-crossing with CS ditelosomic lines during the derivation (R. Morris, personal communication) and by our study of the independantly-made duplicate lines (data not shown). Al-Qaudhy et al. (1988) and Berke et al. (1992a) suggested that the unidirectional effects observed in their reciprocal chromosome substitution analysis might be caused by the epistatic interaction of genes on different chromosomes. Considering that the activities of the 24- and 27-kDa enzymes are weak yet detectable in 'Cheyenne' and all of the CNN (WIxx) lines, it is

likely that the 'Wichita' 2D genes are the major, but not the only, contributors to the 24- and 27-kDa enzyme activities. Alleles that contribute much less to these activities than the 'Wichita' 2D alleles may also exist on 'Cheyenne' chromosome 2D. In addition, homoeologous loci with minor contributions may also exist on other wheat chromosomes. The additive effect of all these minor contributors could possibly make up for the loss of the 'Wichita' 2D genes in WI(CNN2D). Whether these WI2D genes code for the 24- and 27-kDa enzymes themselves or for their regulators remains unknown.

Previous studies have shown a unidirectional effect of 'Wichita' chromosome 2D. When substituted into 'Cheyenne', this chromosome significantly increased seed weight (Berke et al. 1992a) and the stability of the grain test weight (Berke et al. 1992b) and decreased plant height and basal-internode length (Al-Qaudhy et al. 1988). No significant effect of 'Cheyenne' chromosome 2D, however, was observed in any of these reciprocal substitution analyses. These unidirectional effects of 'Wichita' chromosome 2D in 'Cheyenne' background may be governed by the same genetic mechanism, or the 24- and 27-kDa DNA-degrading enzymes have some role in the performance of these agronomic traits. Alternatively, chromosome 2D may be polymorphic at many loci between 'Wichita' and 'Cheyenne'. These effects of chromosome 2D warrant further study.

Our previous study of DNA-degrading enzymes was done in 'Chinese Spring' (Yen and Baenziger 1993). Therefore, 'Chinese Spring' was used in this study as a control. It is interesting that the activities of the 24- and 27-kDa enzymes were either undetectable or very weak in 'Chinese Spring' (Fig. 1B). 'Chinese Spring' chromosome 2D ditelosomic lines are available. Also, it now possible to generate a series of chromosome 2D deletions (Werner et al. 1992). Therefore, we have an opportunity to further understand, and genetically and physically map the genes responsible for the high activities of the 24- and 27-kDa enzymes by studying the segregating telosomic or deletion populations derived from the hybrids of CNN(WI2D) with the detelosomic or deletion lines of 'Chinese Spring' chromosome 2D.

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