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A new type of cytoplasmic male sterility in rye (*Secale cereale* L.): analysis of mitochondrial DNA

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Summary. Mitochondrial (mt) DNA of a new type of rye cytoplasm ('Gülzow', G) that induces cytoplasmic male sterility (CMS) was analyzed and compared with rye mtDNAs of different origins MtDNA of the G type was easily distinguishable from mtDNA of another CMS source, 'Pampa' (P) type, and from mtDNA of fertile lines with respect to restriction fragment patterns and hybridization with mitochondrial genes. The results of the molecular analyses indicate a close, but not identical relationship between the mtDNA of the G type cytoplasm and that of cv 'Pluto'.

Key words: *Secale cereale* – Cytoplasmic male sterility – Mitochondrial DNA

Introduction

The CMS-inducing cytoplasm detected in 'Pampa' (P) rye (Geiger and Schnell 1970) is currently the only cytoplasm that is being used commercially for hybrid seed production of rye. The inheritance of this type of CMS is rather complicated (Ruebenbauer et al. 1984). Adolf and Winkel (1985) developed a new CMS system in rye, which they designated as the 'Gülzow' (G) type. This G cytoplasm was originally found in rye cv 'Schlägler alt'. Male sterility in this system results from the G cytoplasm combining with one recessive nuclear allele (Melz and Adolf 1991). The cytoplasmic factor causing CMS in higher plants is generally assumed to be encoded in the mitochondrial genome (see Levings and Brown 1989), and rearrangements of the mtDNA seem to be primarily involved. In the T cytoplasm of maize, one of the best analyzed CMS systems, synthesis of an abnormal protein is likely to cause the CMS phenotype (Levings and Brown 1989). Tudzynski et al. (1986) have analyzed mtDNA of rye and reported differences between the restriction fragment patterns of mtDNAs of the P type and fertile lines. In this article we show that the G cytoplasm is different from the P type of rye with respect to mtDNA restriction fragment analysis and DNA hybridization patterns.

Materials and methods

MtDNAs of the following inbred lines and cultivars were analyzed: the P (CMS) and N (fertile: normal) lines of 'Pampa' rye; the G (CMS) and C (fertile: normal) lines of 'Gülzow' rye; cv 'Pluto' (Pl). Seedlings were grown under greenhouse conditions in the dark and harvested 8 days after sowing. MtDNA was isolated as described (Weihe et al. 1991) and purified by centrifugation through a CsCl-bisbenzimide gradient (Matthews and Widholm 1985). MtDNA digestion with restriction endonucleases followed the recommendations of the supplier (Amersham). Agarose gel electrophoresis, Southern blotting, radioactive labelling of probes, and hybridization were all performed according to standard procedures (Maniatis et al. 1982). The following clones were used for the hybridization analyses: pS1, internal 6.2-kb *Pst*I clone of plasmid S1 of *Zea mays*; *coxII*, 2.5-kb *Eco*RI clone of cytochrome c oxidase subunit II from *Zea diploerennis* (both obtained from C. S. Levings III, Raleigh); *coxIII*, 1.1-kb *Eco*RI/*Pst*I subclone of cytochrome oxidase subunit III; *cob*, 1.5-*Eco*RI subclone of apocytochrome B from *Oenothera berteriana* (obtained from A. Brennicke, Berlin).

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Results and discussion

Figure 1 shows the restriction fragment patterns of rye mtDNAs of different origins. The two fertile lines, N and C, could not be distinguished by the restriction of their mtDNAs with *Eco*RI (Fig. 1a) or with a number of other restrictions, including double digests with *Hin*dIII and *Xho*I (results not shown). We thus conclude that the normal (fertile) lines contain the same or at least a very similar type of cytoplasm. In all further analyses, only the N cytoplasm and a commercial cultivar, 'Pluto', were included as the fertile lines. In contrast to the fertile lines of the two CMS systems, the two male-sterile cytoplasms, P and G, showed different mtDNA restriction frgment patterns (Fig. 1a–d). Considerable differences were revealed by

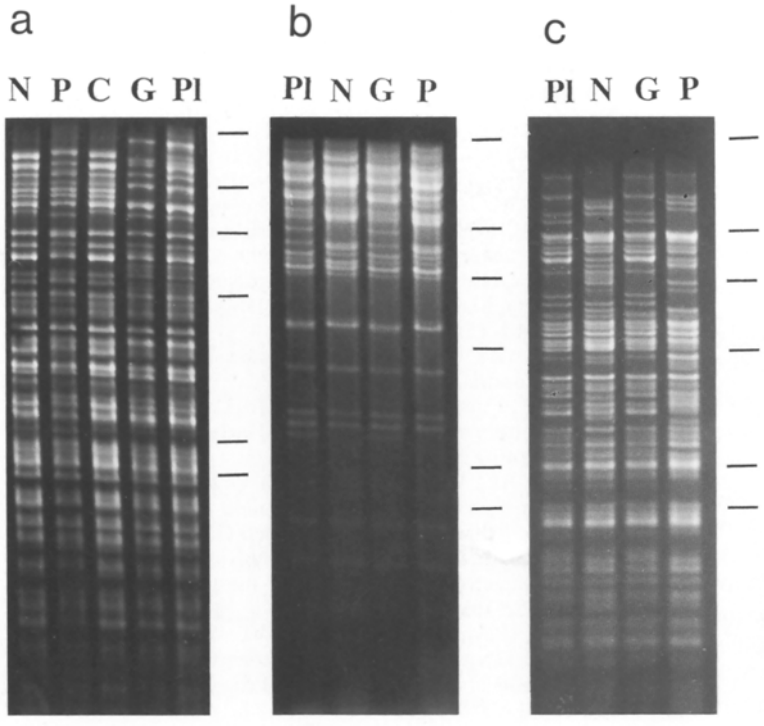

Fig. 1a–c. Restriction fragment patterns of mtDNA from rye inbred lines. **a** *Eco*RI, **b** *Sal*I, **c** *Hin*dIII. *N* fertile line of 'Pampa' rye, *P* CMS line of 'Pampa' rye, *C* fertile line of 'Gülzow' rye, *G* CMS line of Gülzow' rye, *Pl* cv 'Pluto'. *Horizontal bars* indicate positions of fragments of *Hin*dIII-digested lambda DNA (23.1, 9.4, 6.7, 4.4, 2.3, and 2.0 kb)

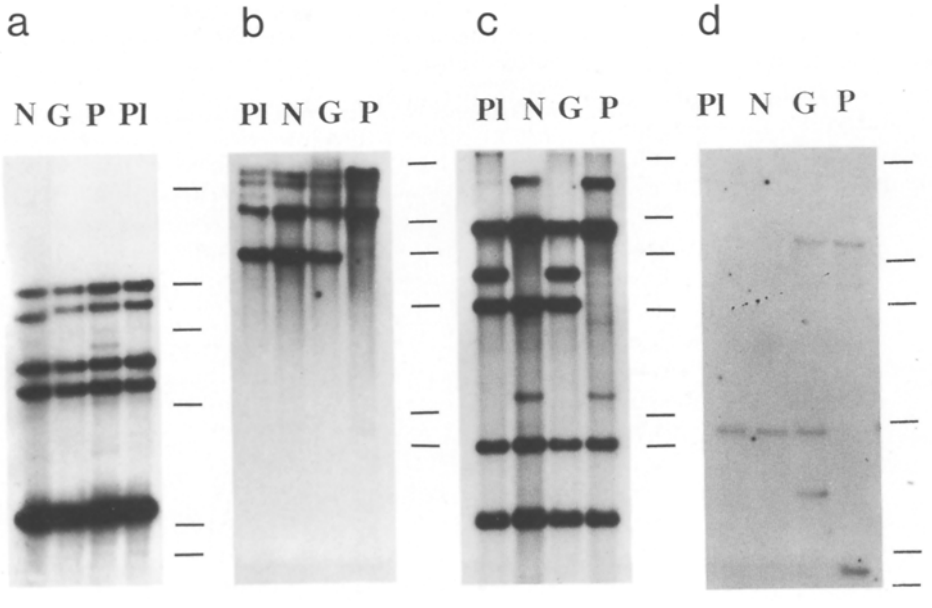

Fig. 2a–d. Hybridization of mitochondrial gene probes with rye mtDNA. DNA was restricted with *Eco*RI (**a**), *Sal*I (**b**), *Hin*dIII (**c**), and *Xho*I (**d**). Filters a–c were hybridized with a 'composite' probe consisting of *coxII*, *coxIII* and *cob*; filter d was probed with pS1. *Horizontal bars* indicate positions of fragments of *Hin*dIII-digested lambda DNA (23.1, 9.4, 6.7, 4.4, 2.3, and 2.0 kb). *Lanes* as in Fig. 1

the four restriction endonucleases used in the experiments, indicating that the G type CMS system is in fact based on a unique cytoplasmic source. The comparison of all of the restriction patterns shows a remarkable similarity between the G type mtDNA and that of (fertile) cv 'Pluto'; minor differences could only be found in the higher molecular weight range of the *Eco*RI pattern, while restriction with *Sal*I, *Bam*HI, and *Hin*dIII resulted in completely identical patterns. These data indicate a close relationship between the two cytoplasms. On the other hand, the mtDNA of the P type seems to be more closely related to the mtDNA of the fertile N cytoplasm than to the male-sterile G type. We have also used cloned mitochondrial genes as probes for the detection of rearrangements of mtDNA sequences in the different cytoplasms (Fig. 2). A 'composite' probe consisting of cloned mitochondrial genes *coxII*, *coxIII*, and *cob* was employed in Southern hybridizations with restricted mtDNA. The remarkable similarity between the mtDNA of the G type and that of cv 'Pluto' was confirmed by the hybridization patterns which were identical (Fig. 2a–c). In contrast, the G and P type CMS cytoplasms could be distinguished by the presence of hybridizing fragments of different sizes in each of the three Southern experiments (Fig. 2a–c). Tudzynski et al. (1986) detected fragments of rye mtDNA that was homologous to the linear S1 plasmid from CMS-S maize mitochondria (Pring et al. 1977). All of the cytoplasms investigated by us also showed hybridization signals with this sequence. The sequence homologous to the S1 plasmid was found on different fragments in the two CMS cytoplasms and N type cytoplasm. This hybridization also revealed a difference between the mtDNA of the G type and 'Pluto'. The S1 plasmid contains an open reading frame (ORF) encoding a protein with homology to a number of DNA polymerases from phages and yeast mitochondria (Kuzmin and Levchenko 1987). The sequence homology observed in rye mtDNA is due to the presence of this same ORF (T. Dohmen and P. Tudzynski, personal communication). Whether this sequence in fact codes for a functional DNA polymerase in rye mitochondria is still uncertain.

Our data prove that the G type cytoplasm represents a new, unique source of CMS in rye that is different from the 'Pampa' type, thus supporting the results of genetic analyses. The cytoplasm of the G type is closely related to that contained in cv 'Pluto'.

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