DNA markers in selection of restorer forms with CMS Pampa in rye

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INTRODUCTION

The phenomenon of cytoplasmic male sterility based on Pampa cytoplasm (Geiger, Schnell, 1970) is used in winter rye hybrid breeding. The sterilizing activity of this cytoplasm is the result of incompatibility between nuclear and mitochondrial genomes. In CMS Pampa system, the main problem is the restoration of pollen fertility that requires nuclear genes dispersed along rye chromosomes. The strongest genes have been mapped to 4R and 1R chromosomes (Miedaner et al., 2000). The molecular markers towards pollen fertility genes should be strongly linked/associated with them to avoid putative genetic drag effects. Preferentially, they should differentiate wide pool of materials. Recently, we have identified DArT markers tightly linked with fertility restoration genes (unpublished). Some of those markers were successfully converted into PCR specific conditions and proved to be useful for marker assisted backcross selection within a limited gene pool. The aim of the study was to perform preliminary marker assisted selection of polish restorer lines and the introduction of the 4R Rf gene from available sources.

MATERIAL AND METHODS

Seven different genotypes represented by rye restorer lines (SO 23R/09, SR 13, WM 11R, WM 19R, WM 30R, WM 34R, WM 35R) with Pampa cytoplasm and lacking 4R QTL were used. The restorer lines were crossed with donor form that contained 4R fertility restoration gene and followed by successive backcrossing resulting in BC3F1 plants. Backcrosses were controlled by PCR-based markers evaluated based on DArT sequences linked to 4R (namely, D-7, D-10 and D-11) and 1R (D-4 and D-23) QTLs.

PCR was carried out in 10µl of reaction mix containing 1x PCR buffer, 2.5mM MgCl2, 0.4mM dNTPs, 0.15pM F and R primers, 5 ng of nuclear DNA and 0.0125U HotStarTaq DNA polymerase using the following thermal profile: [95°C – 15min] [94°C – 30s; 65°C Ramp -0.7°C – 30s; 72°C – 60s]x12 [94°C – 30s; 56°C – 30s; 72°C – 60s]x29 [72°C – 10 min] [5°C ∞]. Electrophoresis on agarose gels verified the presence of markers. Obtained results were converted to a binary matrix.

RESULTS

The amplification of molecular markers (D-7, D-10 and D-11) based on backcrosses resulting from SO 23R/09, SR 13, WM 11R, WM 19R, WM 30R, WM 34R and WM 35R genotypes in 39, 15, 1, 11, 7, 107 and 18 lines, respectively suggested that the 4R QTL was transferred and preserved in BC3F1 lines. Similarly, amplification of the D-4 and D-23 markers among BC3F1 genotypes that originated from SO 23R/09, SR 13, WM 19R, WM 30R, WM 34R and WM 35R lines (they were present in 7, 3, 2, 9, 27 and 2 lines, respectively) could be interpreted as the presence of 1R QTL. The BC3F1 WM 11R genotype missed markers to 1R QTL. In case of SO 23R/09, SR 13, WM 11R, WM 19R, WM 34R and WM 35R BC3F1 genotypes, the 1R QTL was present in parallel to 4R one. The WM 30R BC3F1 genotype missed markers to the 4R QTL whereas those to 1R one were successfully amplified (Table 1).

Figure 1. Illustration of electrophoretic separation of D-7 marker linked to the 4R QTL. K1 – acceptor; K2 – donor of the 4R QTL; lines 3-46 reflect BC3F1 genotypes that originated from SO 23R/09 (1-9 lanes); WM 11R (10); SR 13 (11-13); WM 19R (14-16); WM 30R (17-18); WM 34R (19-42) and WM 35R (43-46) restorer lines lacking 4R QTL.

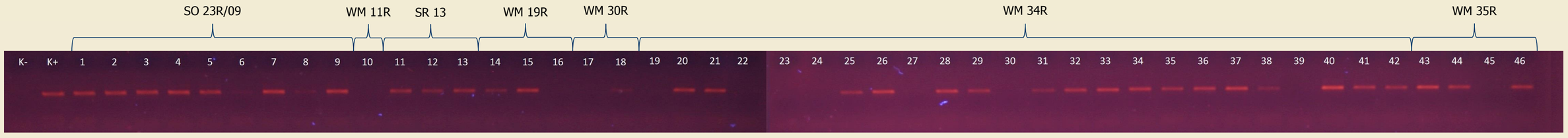

Figure 2. Illustration of electrophoretic separation of D-11 marker linked to the 4R QTL. K1 – acceptor; K2 – donor of the 4R QTL; lines 3-46 reflect BC3F1 genotypes originated from SO 23R/09 (1-9 lanes); WM 11R (10); SR 13 (11-13); WM 19R (14-16); WM 30R (17-18); WM 34R (19-42) and WM 35R (43-46) restorer lines lacking 4R QTL.

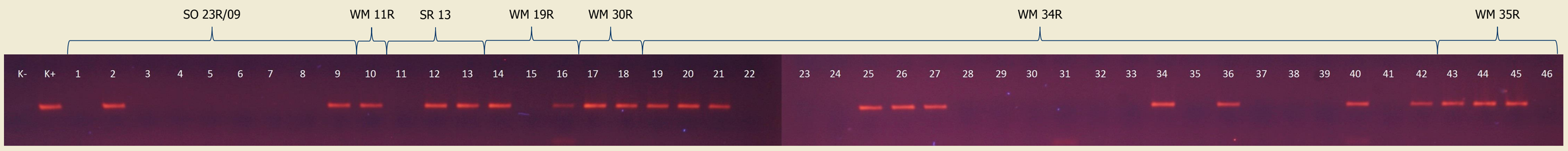

Figure 3. Illustration of electrophoretic separation of D-23 marker linked to the 1R QTL. K1 – acceptor; K2 – donor of the 4R QTL; lines 3-46 reflect BC3F1 genotypes originated from SO 23R/09 (1-9 lanes); WM 11R (10); SR 13 (11-13); WM 19R (14-16); WM 30R (17-18); WM 34R (19-42) and WM 35R (43-46) restorer lines lacking 4R QTL.

Table 1. Arrangement of data on the presence of specific DArT based markers within analyzed BC3F1 materials.

		Number of BC3F1 lines carrying 1R/4R QTLs		
Restorers crossed with line carrying 4R Rf QTL	Number of BC3F1 lines	1R	4R	1R/4R
SO 23R/09	42	7	39	7
SR 13	15	3	15	3
WM 11R	1	0	1	0
WM 19R	14	2	11	2
WM 30R	10	9	7	6
WM 34R	112	27	107	27
WM 35R	18	2	18	2
TOTAL	212	50	198	47

SUMMARY

1.DArT based markers proved to be useful for backcrossing of the 4R QTL to restorer lines with a broad genetic background.

2.Based on markers towards 4R Rf QTL the QTL was transferred to seven restorer lines initially missing that gene.

3.BC3F1 lines (corresponding to SO 23R/09, SR 13, WM 19R, WM 34R, WM 35R and WM 11R genotypes) most likely have 4R QTL but may miss 1R Rf QTL.

4.Three BC3F1 lines (WM 30R genotype) seem to have 1R but miss 4R QTL. A set of BC3F1 lines was evaluated carrying 1R and 4R Rf QTLs.

5.It is necessary to confirm the presence of the QTLs within the lines via analysis of F2 mapping populations.

References:
Geiger, H. H.; Schnell, F. W. Cytoplasmic male sterility in rye (Secale cereale L.). Crop Science, 1970, 10.5: 590-593.
Miedaner, T., et al. Mapping of genes for male-fertility restoration in 'Pampa' CMS winter rye (Secale cereale L.). Theoretical and Applied Genetics, 2000, 101.8: 1226-1233.

The project is funded by MRiRW – POSTĘP BIOLOGICZNY W PRODUKCJI ROŚLINNEJ, nr 21