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Quantitative effects associated with male fertility restoration by the *Rf1* gene in sugar beet

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Introduction

In sugar beet plants the presence of the sterilizing cytoplasm (S-cytoplasm) impairs pollen production. This effect can be suppressed by the *Rf1* (*X*) gene identified by Owen (1945) and cloned by Matsuhira et al. (2012). The fertility restoring allele (*Rf1*/*X*) shows quadruplication of a sequence coding for an OMA1-like protein. Only one copy of this sequence is present in the maintainer allele (*rf1*/*x*). The purpose of this study was to check if fertility restoration by the *Rf1* gene is associated with accumulation of mRNAs encoding this OMA1-like protein.

Plant material

Four populations were included in the analysis – they segregated into male-sterile and male-fertile (restored) plants. In a given population either phenotypic class was represented by 4-6 plants. All these population resulted from crosses between a male-sterile plant and a candidate maintainer plant. The plant material was developed in KHBC Straszków, Poland.

Methods

Total cellular RNA was isolated from flower buds using commercially available kits with adsorption columns. The resulting RNA preparations were treated with DNase – e.g. from Turbo DNA-free Kit (Thermo Fisher Scientific). Reverse transcription was performed using First Strand cDNA Synthesis Kit (Thermo Fischer Scientific). For priming random hexamers were used. Real-time PCRs were prepared with the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fischer Scientific) and run in the QuantStudio 3 Real-Time PCR System with the following program:

initial denaturation	95°c – 10 min.	
denaturation	92°c – 45 sec.	
annealing	57°c – 45 sec. – fluorescence measurement	40 cycles
elongation	72°c – 2 min.	

Two primer pairs were used for the target gene (*Rf1*, they were designed for a conservative region of *Rf1* open reading frames) and one for the reference gene – *BvGAPDH* (Hoeft et al. 2018). Transcript accumulation was calculated using the comparative delta Ct method and male-sterile plants as calibrators.

End-point RT-PCR was performed according to Szklarczyk et al. (2000) on cDNAs used also for real-time PCR (see above). The primers were designed for a polymorphic microstallite sequence from the *Rf1* ORFs. The reaction products were resolved in a native 11 % polyacrylamide gel stained with ethidium bromide.

Results

In two of the analyzed populations - 3S 13 175 and MS 10L O77 - male-sterile and male-fertile (restored plants) did not show significant difference in accumulation of *Rf1* mRNA. In the other two populations male-fertile plants exhibited higher accumulation of these transcripts. In population 3S 18 40 the difference was approx. 2.5-fold and in case of population MS KRA 1537 the difference was approx. 10-fold. For each analyzed population accordant results were produced with both primer pairs for the target gene (Fig. 1).

Qualitative RT-PCR analysis revealed that male-sterile and restored plants produced different amplification profiles – in most cases the male-fertile plants exhibited the presence of a specific amplification product of approx. 140 bp (Fig. 2).

Our results indicate that fertility restoration is associated with the presence of a specific *Rf1* mRNA isoform which in some populations may increase the pool of *Rf1* transcripts in restored plants.

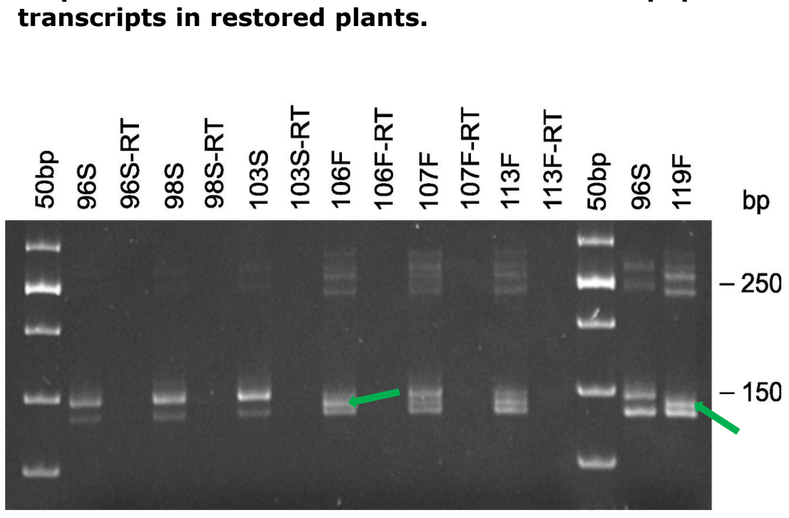

Figure 2. RT-PCR products obtained for male-sterile (S) and male-fertile (F) plants from population MS 10L 077. The primers spanned a microsatellite polymorphism from the *Rf1* open reading frames. Arrow – product specific for male-fertile plants. -RT – controls without reverse transcriptase. 50bp - 50 bp ladder, Guangzhou Dongsheng Biotech.

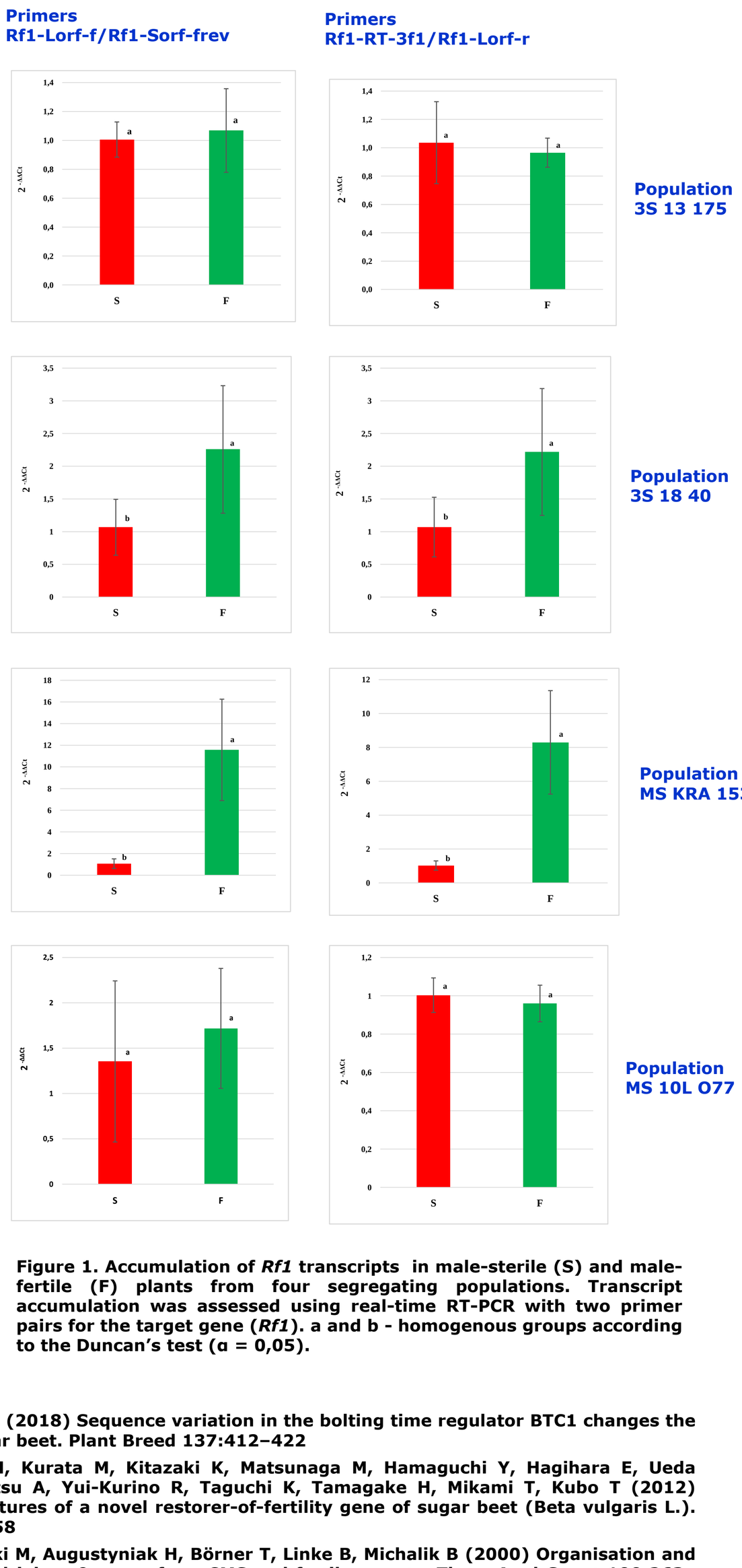

Figure 1. Accumulation of *Rf1* transcripts in male-sterile (S) and male-fertile (F) plants from four segregating populations. Transcript accumulation was assessed using real-time RT-PCR with two primer pairs for the target gene (*Rf1*). a and b - homogenous groups according to the Duncan's test (α = 0,05).

Literature

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Acknowledgements

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