Transposable elements in the genome of sugar beet

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Introduction

Sugar beet is one of the most important agricultural crops in Poland and a major raw material for the European sugar industry. Two high quality assemblies, namely the reference assembly RefBeet and a more contiguous assembly EL10, are currently available. Transposable elements (TEs) are mobile DNA segments that may significantly affect structure and function of the host genome in a number of ways and create novel genetic diversity.

Methods

We used the two sugar beet genome assemblies and specialized bioinformatic pipelines to identify TE families belonging to two groups – miniature inverted-repeat transposable elements (MITEs) and long terminal repeat retrotransposons (LTR-RTs), and to develop databases comprising consensus sequences representing each family. We PCR-verified insertional polymorphisms produced by two superfamilies of miniature inverted-repeat transposable elements, i.e. *Stowaways* and *hATs*.

Results

We identified ca. 45 and 47 thousand MITE copies in the RefBeet and EL10 assemblies, respectively, and classified them into 170 families, of which 69 were found independently in both genome assemblies. A *Stowaway* family, *BvSto36*, comprising more than 2000 copies in each reference genome, was the most numerous. The MITE families differed also with respect to the level of their insertional polymorphisms.

Ca. 3.5 and 7.5 thousand copies of full-length LTR-RT copies were retrieved from the RefBeet and EL10 assemblies. The difference likely reflected higher contiguity of the EL10 reference. We classified those LTR-RTs into the commonly recognized lineages of plant LTR-RTs. Of those, a *copia* lineage *BvSIRE*, and *gypsy* lineages *BvTekay*, *BvRetand* and *BvAthila* comprised few of the most abundant subfamilies In contrast, a *copia* lineage *BvAle* also comprised many copies, but they were divided into a large number of low-copy number subfamilies.

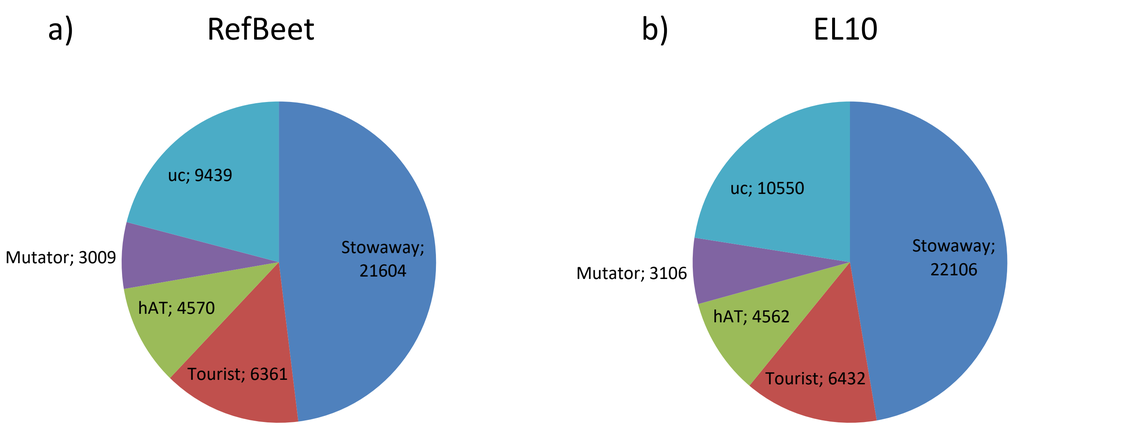

Fig 1. Proportions of MITE superfamilies identified in RefBeet (a) and EL10 (b) sugar beet genome assemblies

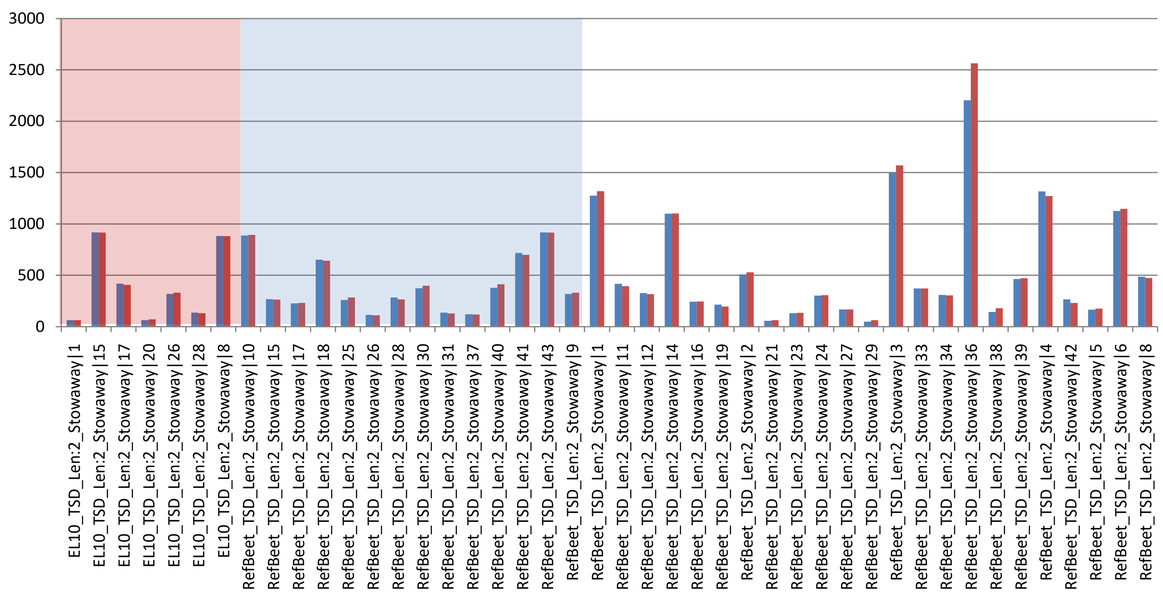

Fig 2. Copy number of *Stowaway* families residing in the sugar beet genome

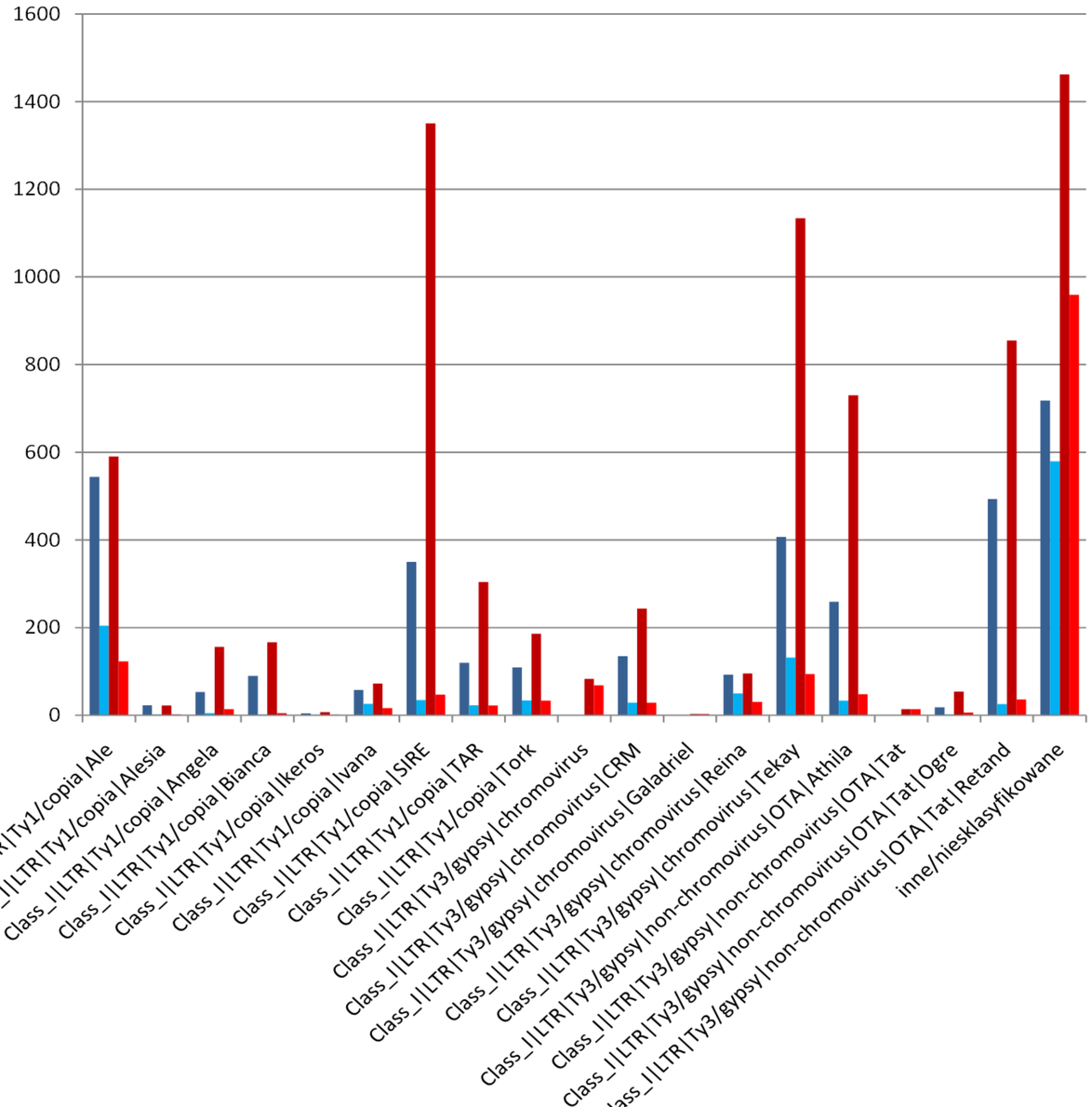

Fig 3. Number of LTR retrotransposons identified in RefBeet and EL10 sugar beet genome assemblies

Fig 4. Examples of insertional polymorphisms of *Stowaway* (a) and *hAT* (b) MITEs

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Expression analysis of the *Rf1* restorer gene in sugar beet

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Introduction

In sugar beet the *Rf1* gene is responsible for pollen fertility restoration to plants carrying the sterilizing (S-) cytoplasm. Without *Rf1* (or other restorer gene) such plants do not produce functional pollen due to defective performance of the mitochondria. The aim of our study was to check if the fertility restoration effect of *Rf1* is proportional to its transcript accumulation. Such possibility can be inferred from the quadruplicated character of the fertility restoring allele (Matsuhira et al. 2012).

Plant material

We analyzed two plant populations – 2S 1329 and 2S 1387 – obtained after crossing a male-sterile plant with a candidate maintainer plant (such plants are used for reproduction of male-sterile lines). Both populations segregated into male-sterile plants and plants exhibiting various levels of fertile (viable) pollen. The following phenotype categories were distinguished: MS – only sterile pollen grains, MS1 – less than 50% of viable pollen grains, MS2 – 50% of viable pollen grains, Fe – more than 50% of viable pollen grains. For each phenotypic class 2 – 9 plants were analyzed.

Methods

Total cellular RNA was isolated from flower buds using the method of Chomczyński and Sacchi (1987). After electrophoretic separation the RNA samples were blotted onto a nylon membrane and hybridized with a digoxigenin-labelled probe corresponding to a PCR-amplified fragment of one of the *Rf1* open reading frames. These procedures were based on recommendations from the DIG System User's Guide for Filter Hybridization (Boehringer Mannheim 1995) After detection the obtained hybridization signals were quantified using densitometry. Intensity of the obtained hybridization signals was normalized by dividing it by intensity of the upper (3.7 kb) rRNA band from the corresponding RNA gel. Statistical significance of observed differences in signal intensity was determined using the two-sided Student's t-test (α = 0.05).

Results

In plants from both populations the *Rf1* probe detected a transcript of 2.4 kb. The analyzed plants showed differences in accumulation of this mRNA.

In population 2S 1329 the plants with more than 50 % of viable pollen (male-fertile – Fe) displayed significantly higher accumulation of the *Rf1* mRNA than the remaining plants. Differences among other phenotypic classes (MS, MS1 and MS2) were not significant.

In population 2S 1387 male-sterile plants (with no viable pollen) had significantly lower accumulation of the analyzed transcript than the remaining plants. Differences among other phenotypic classes (MS1, MS2 and Fe) were not significant.

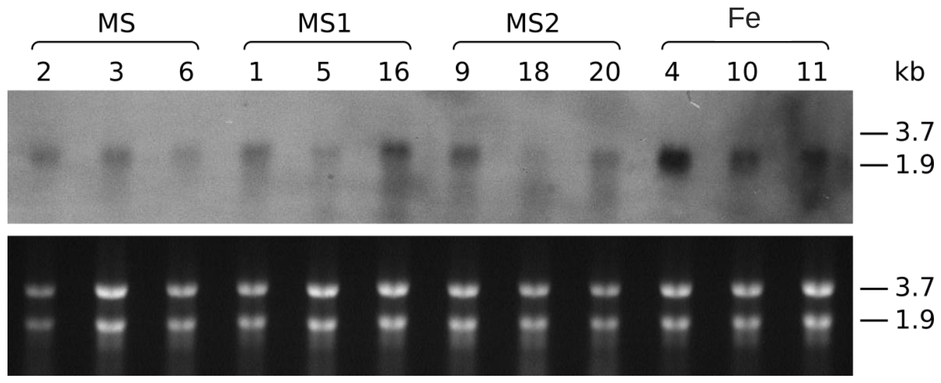

Figure 1. Hybridization signals obtained for flower bud RNAs after hybridization with the *Rf1* probe for plants from four phenotypic classes of population 2S 1329 (top). Corresponding RNA gel (bottom).

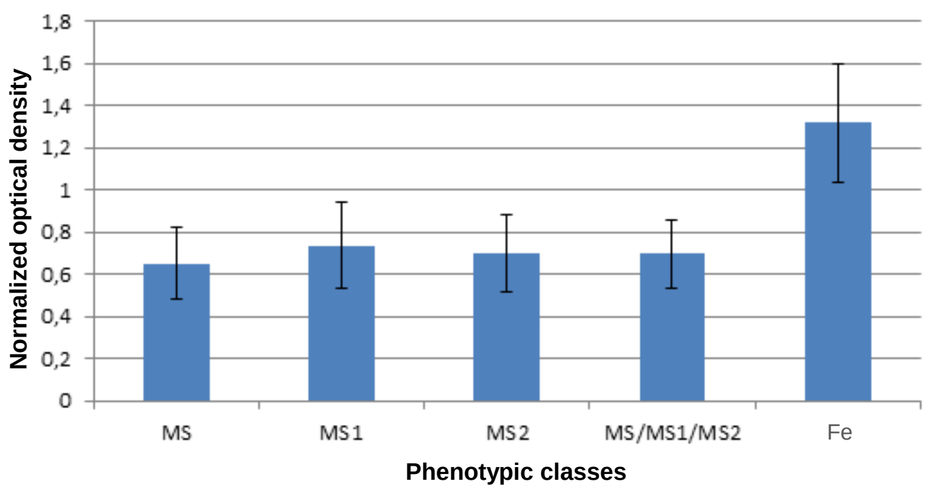

Figure 2. Arithmetic means of normalized optical density corresponding to *Rf1* mRNA accumulation in plants from four phenotypic classes in population 2S 1329. Error bars – standard error.

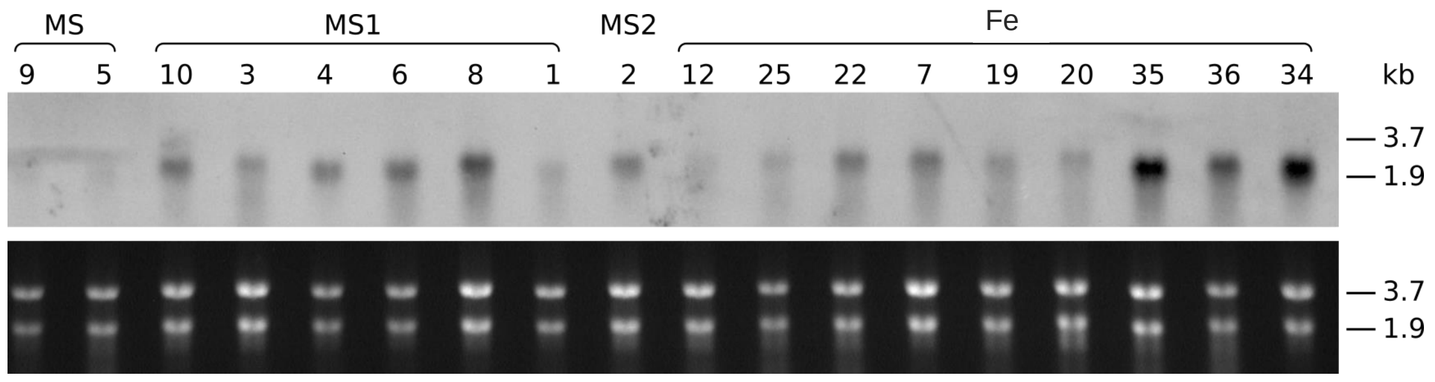

Figure 3. Hybridization signals obtained for flower bud RNAs after hybridization with the *Rf1* probe for plants from four phenotypic classes of population 2S 1387 (top). Corresponding RNA gel (bottom).

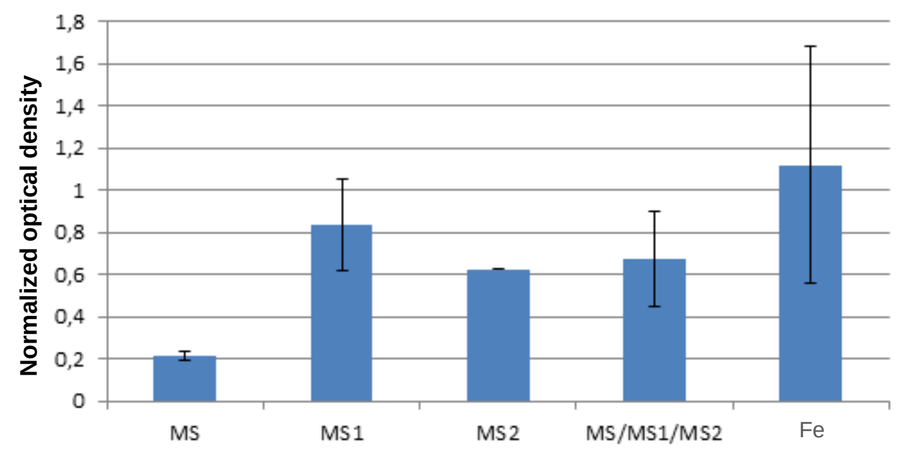

Figure 4. Arithmetic means of normalized optical density corresponding to *Rf1* mRNA accumulation in plants from four phenotypic classes in population 2S 1387. Error bars – standard error.

Conclusions

The obtained data indicate that there is a general correlation between accumulation of *Rf1* mRNA and expression of male fertility. In the case of population 2S 1387 for this conclusion we need to assume that the MS1 and MS2 (as well as fertile – Fe) plants carry the restorer allele.

Literature

Chomczyński P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

Matsuhira H, Kagami H, Kurata M, Kitazaki K, Matsunaga M, Hamaguchi Y, Hagihara E, Ueda M, Harada M, Muramatsu A, Yui-Kurino R, Taguchi K, Tamagake H, Mikami T, Kubo T (2012) Unusual and typical features of a novel restorer-of-fertility gene of sugar beet (*Beta vulgaris* L.). Genetics 192: 1347–1358

Acknowledgements

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