

## Dynamics of *Vulmar/VulMITE* group of transposable elements in *Chenopodiaceae* subfamily *Betoideae*

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**Abstract** Transposable elements are important factors driving plant genome evolution. Upon their mobilization, novel insertion polymorphisms are being created. We investigated differences in copy number and insertion polymorphism of a group of Mariner-like transposable elements *Vulmar* and related *VulMITE* miniature inverted-repeat transposable elements (MITEs) in species representing subfamily *Betoideae*. Insertion sites of *these* elements were identified using a modified transposon display protocol, allowing amplification of longer fragments representing regions flanking insertion sites. Subsequently, a subset of TD fragments was converted into insertion site-based polymorphism (ISBP) markers. The investigated group of transposable elements was the most abundant in accessions representing the section *Beta*, showing intra-specific insertion polymorphisms likely resulting from their recent activity. In contrast, no unique insertions were observed for species of the genus *Beta* section *Corollinae*, while a set of section-specific insertions was observed in the genus *Patellifolia*, however, only two of them were polymorphic between *P. procumbens* and *P. webbiana*. We hypothesize that *Vulmar* and *VulMITE* elements were inactivated in the section *Corollinae*, while they remained active in the section *Beta* and the genus *Patellifolia*. The ISBP markers generally confirmed the insertion patterns

observed with TD markers, including presence of distinct subsets of TE insertions specific to *Beta* and *Patellifolia*.

**Keywords** Beta · Insertion polymorphism · Mariner · Miniature inverted repeat transposable elements · *Patellifolia* · Sugar beet

### Introduction

Transposable elements (TEs) constitute a significant portion of plant genomes. Their content in *Angiospermae* genomes varies from ca. 10% in *Arabidopsis* (*Arabidopsis* Genome Initiative 2000) to ca. 85% in maize genome (Schnable et al. 2009). TEs are capable of changing their localization in the genome and their activity may alter gene structure and regulatory functions or cause genome rearrangements (Bennetzen 2000). On the basis of their mechanism of transposition, TEs are divided into two classes. Class I elements include retrotransposons which transpose via an RNA intermediate. Subsequently, the RNA is reverse-transcribed and the DNA copy is integrated into a new genomic location. The ‘copy and paste’ transposition is replicative and each transposition event leads to increase of the TE copy number. In contrast, Class II elements—DNA transposons, are mobilized on the basis of a ‘cut and paste’ mechanism, i.e. their mobilization requires physical excision from a donor site and reintegration into a new acceptor site. Autonomous elements contain functional open reading frame(s) that encode the products required for transposition. Deletions in the coding regions are typical for non-autonomous elements, which nevertheless may be activated *in trans*, provided that the required transposition machinery encoded by a related autonomous element is present (Wessler 2006).

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Autonomous elements and their non-autonomous derivatives are grouped into families defined by DNA sequence conservation (Wicker et al. 2007). TE families differ in the TE copy number, which can be exceptionally high for some retrotransposon families. Also, a particular group of TEs, called miniature inverted-repeat transposable elements (MITEs) can reach hundreds to thousands copies per haploid genome (Feschotte et al. 2002a). MITEs are small, up to 600 bp, DNA transposons lacking any coding capacity. They were identified in several plant species, including maize (Bureau and Wessler 1992), rice (Bureau and Wessler 1994a, b), *Arabidopsis* (Casacuberta et al. 1998, Feschotte et al. 2003), carrot (Grzebelus and Simon 2009), and *Medicago* (Grzebelus et al. 2009). Because of the shared structural features, it was proposed that MITEs were reminiscent of non-autonomous elements. It is likely that the high copy number of MITEs is an effect of their *in trans* activation by related autonomous elements (Feschotte et al. 2002b).

A *Vulmar/VulMITE* group of TEs identified in *Beta* could be an example of such interrelationship. *Vulmar1* is a TE belonging to the *Tc1/mariner* superfamily and a representative of the *Vulmar* family. *Vulmar1* is 3,909 bp-long, non-autonomous due to frameshifts in the transposase gene. However, many open reading frames coding for *mariner*-like transposases were identified in beet, some of them likely representing autonomous elements, including those from the *Vulmar* family (Jacobs et al. 2004). Menzel et al. (2006) reported on three MITE families, named *VulMITE* I, II, and III, present in *B. vulgaris* and related to the *Vulmar* elements. While *VulMITE* I were typical MITEs with length of ca. 300 bp, the other two families were ca. 1 kb-long. Sequence similarity of the MITEs and *Vulmar1* was restricted to less than 200 bp 5' and 3' terminal regions. Menzel et al. (2006) also indicated that insertions of *VulMITE* I elements were polymorphic in *Beta*, resulting from transposition events, likely in the course of beet domestication.

A successful transposition event results in a novel insertion polymorphism. It can be detected by a range of techniques, including transposon display (TD). Transposon display is a modification of the AFLP system, in which subterminal fragments of transposons are used as target sequences. MITE-based transposon display was used to detect insertion polymorphisms in maize (Casa et al. 2000), rice (Park et al. 2003, Jiang et al. 2003, Kwon et al. 2006), and carrot (Grzebelus and Simon 2009). TE insertion polymorphisms can be applied to elucidate interspecific evolutionary relationships (Ray 2007). In order to investigate such relationships in *Betoideae*, we used TD approach to study *Vulmar/VulMITE* insertion polymorphisms.

Historically, all investigated species, representing family *Chenopodiaceae/Amaranthaceae*, were grouped in the

genus *Beta*, which was divided into four sections Ulbrich (1934). The section *Procumbentes*, grouping *B. procumbens*, *B. patellaris*, and *B. webbiana* was eventually removed from the genus *Beta* and the species were included in the genus *Patellifolia* (Scott et al. 1977). According to the current USDA/ARS NPGS GRIN taxonomy ([www.ars-grin.gov/cgi-bin/npgs/html/gnlist.pl?2303](http://www.ars-grin.gov/cgi-bin/npgs/html/gnlist.pl?2303), August 17, 2011), both *Beta* and *Patellifolia* are classified in subfamily *Chenopodioideae*, tribe *Beteae*. However, Hohmann et al. (2006) placed them in subfamily *Betoideae*, and provided molecular evidence for further division of *Betoideae* into *Beteae* comprising all *Beta* species, and a sister group, *Hablitziaceae*, including *Patellifolia*. Within *Beteae*, two sections are recognized—section *Beta*, comprising *B. vulgaris* and *B. macrocarpa*, and section *Corollinae*, comprising *B. corolliflora*, *B. lomatogona*, *B. macrorrhiza*, *B. trygina*, and *B. nana* (Kadereit et al. 2006).

Here, we describe differences in the copy number and insertion polymorphism of *Vulmar/VulMITE* transposons among *Beta* and *Patellifolia* species, likely reflecting differences in evolutionary dynamics of these elements.

## Materials and methods

### Plant material and DNA extraction

Two male sterile and two corresponding maintainer (Owen type) sugar beet breeding stocks (KHBC Straszaków, Poland), together with four wild *Beta* species, i.e. *B. vulgaris* subsp. *maritima* (section *Beta*), *B. corolliflora*, *B. lomatogona*, *B. macrorrhiza* (section *Corollinae*), and two *Patellifolia* species, i.e. *P. procumbens*, and *P. webbiana*, were used for *Vulmar/VulMITE* transposon display (VMTD). Each accession was represented by two plants. Insertion site-based polymorphism (ISBP) markers were applied to the above materials and few additional sugar beet and beetroot stocks (Table 1). DNA was extracted with DNeasy Mini Kit (Qiagen) as described in the manufacturer protocol.

### *Vulmar/VulMITE* transposon display (VMTD)

Approximately 300 ng of DNA samples were digested in 37°C for 3 h in 10 µl reaction mixture with 5 u *NdeI* (Fermentas). *NdeI*-compatible splinkerette adaptors were ligated to the restriction fragments according to the protocol of Grzebelus et al. (2007). Pre-amplification was performed with a primer VMtd1 (5' GCCTAGGAG TCCGTTTTAATCAC 3'), specific to 5' subterminal region of *Vulmar1* and *VulMITE* elements (Menzel et al. 2006), and with an adaptor-specific primer tdP1 (5' CGAATCC TAACCGTTCGTACGAGAA 3') in 10 µl reaction

**Table 1** List of plant materials used for transposon display (TD) and insertion site-based polymorphism (ISBP)

Code	Group	Genus	Section	Species	Stock	Origin	Number of analyzed plants	
							TD	ISBP
203O	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	O 02 203	KHBC Straszaków, Poland	–	3
203S	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	S 02 203		–	3
02O	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	O 02 1312		2	3
02S	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	S 02 1312		2	3
01O	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	O 001 073		2	3
01S	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	S 001 073		2	3
1061Ms	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	S04 1061 Ms		–	3
1061Dp	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	S04 1061 Dp		–	3
786Ms	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	S04 786 Ms		–	3
786Dp	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (sugar beet)	S04 786 Dp		–	3
A79A	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	AR79A	Univ. of Agriculture in Krakow, Poland	–	3
A79B	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	AR79B		–	3
279A	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	279A	Univ. of Wisconsin-Madison, USA	–	3
279B	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	279B		–	3
411A	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	W411A		–	2
411B	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	W411B		–	3
218A	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	218A		–	2
218B	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	218B		–	3
391A	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	391A		–	1
391B	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	391B		–	3
357A	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	357A		–	2
357B	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	357B		–	3
Ac	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	Action F1	Bejo Zaden, the Netherlands	–	3
As	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	Astar F1	KHiNO Polan, Poland	–	3
Gl	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. vulgaris</i> (beetroot)	Polglob F1		–	3
Bvm	<i>Beteae</i>	<i>Beta</i>	<i>Beta</i>	<i>B. v. subsp. maritima</i>	–	BAZ Braunschweig, Germany	2	3
Bl	<i>Beteae</i>	<i>Beta</i>	<i>Corollinae</i>	<i>B. lomatozona</i>	–	IHAR Bydgoszcz, Poland	2	3
Bm	<i>Beteae</i>	<i>Beta</i>	<i>Corollinae</i>	<i>B. macrorhiza</i>	–		2	3
Bc	<i>Beteae</i>	<i>Beta</i>	<i>Corollinae</i>	<i>B. corolliflora</i>	–		2	3
Pp	<i>Hablitzieae</i>	<i>Patellifolia</i>	–	<i>P. procumbens</i>	–	BAZ Braunschweig, Germany	2	3
Pw	<i>Hablitzieae</i>	<i>Patellifolia</i>	–	<i>P. webbiana</i>	–		2	3

mixture containing 0.5  $\mu$ M of each primer, 0.5 U of Taq polymerase (Fermentas), 250  $\mu$ M dNTP, and 1.5 mM MgCl<sub>2</sub> in the PCR buffer provided by the manufacturer. The following cycling conditions were applied: initial denaturation at 94°C/3 min., then 40 cycles of 94°C/30 s, 55°C/30 s, and 68°C/60 s, final elongation at 68°C/5 min. The reaction mixture was diluted 1:20 and used as a template in selective amplification. Selective amplifications were performed with a nested *Vulmar1*- and *VulMITE*-specific primer VMtd2 (5' CTAGGAGTCCGTTTTAAT CACAATG 3') and a nested adaptor-specific primer tdN-deI(NN) (5' TCCAACGAGCCAAGGTATGNN 3'), where 'NN' stands for the two selective nucleotides attached to the 3' end. Eight combinations of the two selective nucleotides were used, i.e. AC, AG, CA, CT, GA, GT, TC, and

TG. The reaction was set up in 10  $\mu$ l volume containing 0.5  $\mu$ M of each primer, 0.5 U of Taq polymerase (Fermentas), 1.5 mM MgCl<sub>2</sub>, 250  $\mu$ M dNTP, and 1xPCR buffer. Cycling conditions for selective amplification were set according to Grzebelus et al. (2007). TD amplicons were separated in 1% agarose gels and visualized with ethidium bromide.

#### Cloning and sequencing of PCR products

Target bands were cut from gels and purified with WIZARD SV Gel and PCR Clean-Up System (Promega) following the instructions provided by the manufacturer. Purified DNA fragments were ligated into pGem-T (Promega), cloned, and sequenced using GenomeLab DTCS

Quick Start Kit (Beckman Coulter) and separated in CEQ 8000 (Beckman Coulter) according to the manufacturer protocol. Sequences were analyzed with BioEdit (Hall 1999). Fourteen sequences representing flanking regions of TE insertions longer than 200 bp were deposited in GenBank (accession numbers: JF521550–JF521563).

#### Site-specific PCR

In order to convert *VMTD* polymorphisms into ISBP markers, primers anchored in the region flanking TE insertion sites were designed using Primer3 for the sequenced *VMTD* fragments (Supplementary Table 1). These primers were used for PCR in combination with the *VMtd2* primer. DNA amplification was carried out in 10  $\mu$ l reaction mixture containing 0.5  $\mu$ M of each primer, 0.5 U of Taq polymerase (Fermentas), 250  $\mu$ M dNTP, 2 mM  $MgCl_2$ . The following cycling conditions were applied: initial denaturation at 94°C/2 min., then 35 cycles of 94°C/30 s, 53 or 58°C/30 s, and 68°C/2 min., final elongation at 68°C/5 min. Products were separated in 1% agarose gels and visualized with ethidium bromide.

#### Analysis of genetic diversity

Presence or absence of *VMTD* bands was scored as 1 or 0, respectively and a binary matrix was created. Pairwise genetic distances were calculated as proposed for binary data by Huff et al. (1993), i.e. any comparison with the same state yielded a value of 0, while different states yielded a value of 1. Analysis of molecular variance (AMOVA) was performed with two sources of variation—stocks and plants. Test for statistical significance of components was based on 1,000 permutations. Principal Coordinates Analysis (PCoA) was performed using a Distance-Standardized method. All calculations were carried out in GeneAlex 6.3 (Peakall and Smouse 2006).

## Results

#### *Vulmar/VulMITE* transposon display

Eight combinations of selective nucleotides produced the total of 96 *VMTD* bands. The number of fragments ranged from 7 to 16, for selective nucleotide combinations AC and GA, respectively (Table 2). The average number of bands per one reaction was 11.9, only slightly lower than that obtained using a similar system based on *Krak* MITEs in carrot (Grzebelus and Simon 2009). Our protocol was a modification of the latter TD system. We replaced *MseI*—a four-cutter, with *NdeI* which is a six-cutter, which allowed production of longer TD fragments which were separated

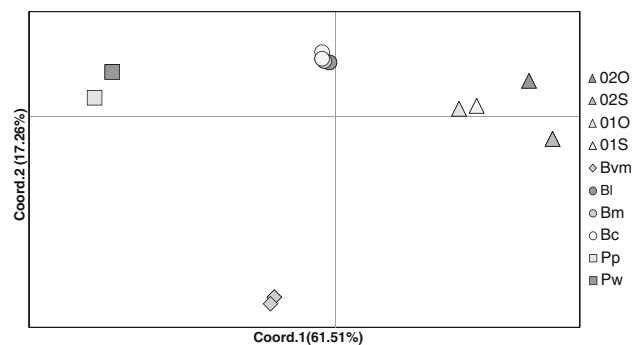
**Table 2** *Vulmar/VulMITE* transposon display fragments produced with eight selective nucleotide combinations

Selective nucleotides	Product size range (bp)	<i>VMTD</i> products				Total
		Polymorphic	Monomorphic			
AC	240–450	7	100%	0	0%	7
AG	150–650	9	82%	2	18%	11
CA	200–610	13	100%	0	0%	13
CT	140–500	13	100%	0	0%	13
GA	220–900	16	100%	0	0%	16
GT	150–650	14	93%	1	7%	15
TC	130–550	11	100%	0	0%	11
TG	150–600	10	100%	0	0%	10
Total	130–900	93	97%	3	3%	96

on agarose gels. The original TD protocol yielded products that were on average ca. 100 bp-long (Grzebelus and Simon 2009), while the average length of *VMTD* products was ca. 350 bp. Nearly all *VMTD* fragments (97.8%) were polymorphic among the investigated species.

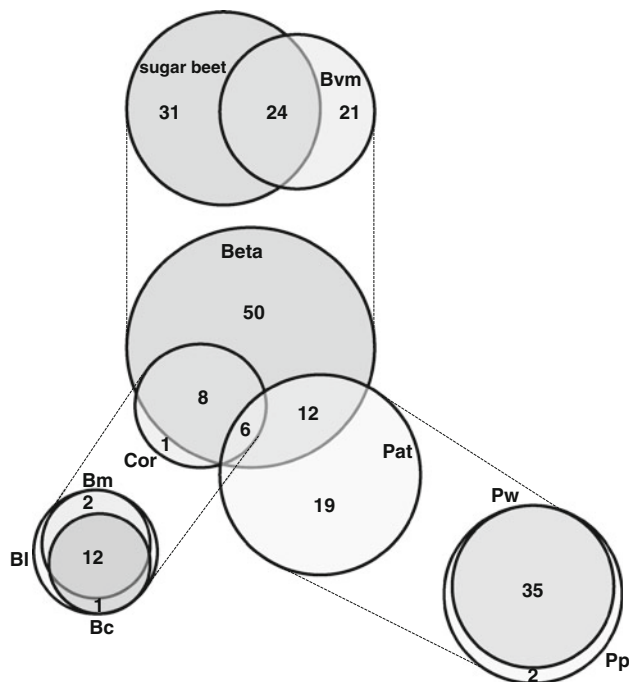
#### Distribution of *Vulmar/VulMITE* insertions in *Betoideae*

AMOVA partitioned 99% of molecular variance to differences among stocks, while only the remaining 1% could be attributed to the intra-stock variability. On the basis of the observed insertion polymorphism, the investigated *Betoideae* species could be divided into four distinct groups representing (1) sugar beet, (2) *B. vulgaris* subsp. *maritima*, (3) section *Corollinae*: *B. corolliflora*, *B. lomatogona*, *B. macrorhiza*, and (4) the genus *Patellifolia* (Fig. 1). For the wild species, the results were consistent with the current taxonomy of *Betoideae*, while separate positions of the cultivated and the wild *B. vulgaris* could be attributed to mobilization of the TEs, as discussed below.



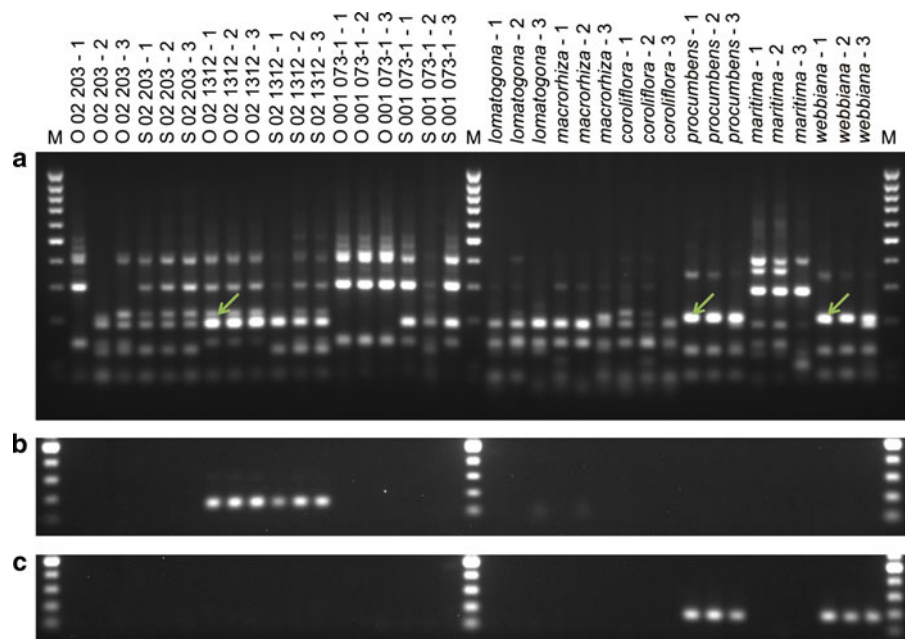
**Fig. 1** Principal coordinates analysis (PCoA) of *Betoideae* based on *VMTD* polymorphisms. Two individuals represent each stock. Individuals with identical *VMTD* scores occupy the same position on the graph. Plant stock codes are given according to Table 1

Species belonging to section *Corollinae* were characterized by a low number of identified insertions, all but one of them being shared with species representing other sections. A much higher number of insertions, i.e. 50 and 19, were



**Fig. 2** Graph illustrating relationships among the investigated *Beta* species with regard to the number of shared and unique *VulMITE I* insertions. Section *Beta* [Beta]—sugar beet, *B. vulgaris* subsp. *maritima* [Bvm], section *Corollinae* [Cor]—*B. corolliflora* [Bc], *B. lomatogona* [Bl], *B. macrorhiza* [Bm], and genus *Patellifolia* [Pat]—(*P. procumbens* [Pp], *P. webbiana* [Pw])

**Fig. 3** An example of co-segregation of the *VMTD* markers obtained with CT selective nucleotides and the derived ISBP markers Bv7 and Bp1. *VMTD* profile (a), arrows indicate localization of sequenced fragments. Amplification of ISBP markers: sugar beet-specific Bv7 (b) and *Patellifolia*-specific Pp1 (c), M molecular size marker



specific to section *Beta* and genus *Patellifolia*, respectively (Fig. 2). Of the 50 insertion sites present exclusively in the section *Beta*, 27 and 14 bands were unique to sugar beet and *B. vulgaris* subsp. *maritima*, respectively. In contrast, the two investigated *Patellifolia* species shared a set of 35 insertion sites, while only two TD bands were present in *P. procumbens* and absent in *P. webbiana*.

#### Conversion of *VMTD* polymorphisms into ISBP markers

To further elaborate the nature of polymorphisms identified by *VMTD* we sequenced 34 *VMTD* fragments, including nine groups (comprising two to five sequences) of co-migrating fragments amplified in plants from different accessions. Sixteen and seven fragments selected for sequencing originated from sugar beet and *B. vulgaris* subsp. *maritima*, respectively, while five and six were chosen for *Corollinae* and *Patellifolia*, respectively. Sequences of the co-migrating products always matched each other, so we obtained 20 sequences representing regions flanking *Vulmar/VulMITE* insertion sites. All sequenced TD fragments showed similarity to the terminal part of *Vulmar* and *VulMITE* immediately following the anchor site of the *VMtd2* primer, indicating that the fragments were specifically derived from regions bearing TE insertions.

Expected amplification products were obtained for 17 of the 20 sequenced insertion sites. Two PCRs produced complex banding patterns, likely indicating that the primer specific to the flanking region was anchored in the repetitive DNA, while the remaining one did not yield any products.

**Table 3** A summary of *Vulmar/VulMITE* ISBP polymorphisms identified in the genus *Beta*

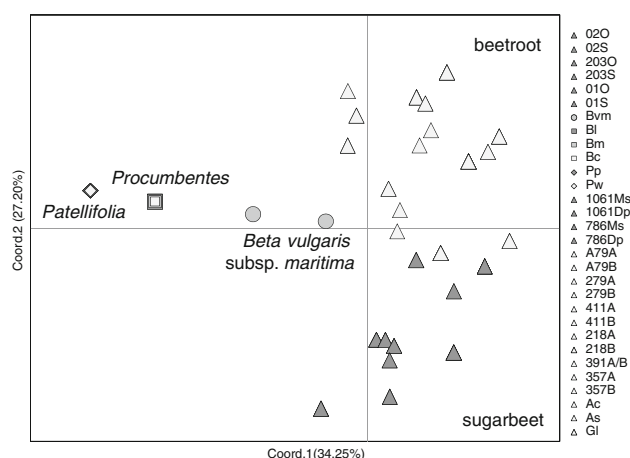
Marker code	Source	Sugar beet	Beetroot	<i>B. vulgaris</i> subsp. <i>maritima</i>	<i>Corollinae</i>	<i>Procumbentes</i>
Bv1	Sugar beet	Segregating	Absent	Absent	Absent	Absent
Bv4	Sugar beet	Segregating	Segregating	Absent	Absent	Absent
Bv6 <sup>a</sup>	Sugar beet/ <i>B. vulgaris</i> subsp. <i>maritima</i>	Segregating	Present	Present	Absent	Absent
Bv7	Sugar beet	Segregating	Segregating	Absent	Absent	Absent
Bv8	Sugar beet	Present	Segregating	Absent	Absent	Absent
Bv10	Sugar beet	Present	Segregating	Present	Absent	Absent
Bv11	Sugar beet/ <i>B. vulgaris</i> subsp. <i>maritima</i>	Segregating	Segregating	Segregating	Absent	Absent
Bv12	<i>Beta</i> spp.	Segregating	Segregating	Present	Present	Present
Bv13	Sugar beet	Segregating	Segregating	Absent	Absent	Absent
Bv14 <sup>a</sup>	Sugar beet/ <i>B. macrorhiza</i>	Present	Not tested	Present	Present	Present
Bv15	Sugar beet	Segregating	Not tested	Absent	Absent	Absent
Bvm3	<i>B. vulgaris</i> subsp. <i>maritima</i>	Absent	Absent	Segregating	Absent	Absent
Bvm5	<i>B. vulgaris</i> subsp. <i>maritima</i>	Absent	Absent	Present	Absent	Absent
Bvm8	<i>B. vulgaris</i> subsp. <i>maritima</i>	Absent	Not tested	Present	Absent	Absent
Pp1	<i>B. procumbens</i>	Absent	Absent	Absent	Absent	Present
Pp2	<i>B. procumbens</i>	Absent	Absent	Absent	Absent	Present
Pp3	<i>B. procumbens</i>	Absent	Not tested	Absent	Absent	Present

<sup>a</sup> ISBP markers showing segregation pattern not corresponding to that of the original TD product

Distribution of site-specific bands generally matched that of the original VMTD products (Fig. 3). In two cases (Bv6 and Bv14), a discrepancy between TD and ISBP scores was observed, likely resulting from scoring different TD bands of similar size as one product (Supplementary Table 2). Thirteen ISBP markers were used to screen a larger genepool representing cultivated beets. As expected, four of them, two originating from *Patellifolia* and two present exclusively in *B. vulgaris* subsp. *maritima*, were absent both in sugar beet and beetroot. The remaining nine ISBP markers segregated in the larger cultivated beet genepool (Table 3). Grouping of the investigated species based on the 13 ISBP polymorphisms resembled that obtained for the VMTD markers (Fig. 4). While both *Patellifolia* species and three representatives of *Procumbentes* showed distinct and uniform polymorphism patterns, a substantial diversity was observed in *B. vulgaris*. Wild *B. vulgaris* subsp. *maritima* differed from the cultivated materials, while sugar beet and beetroot formed two only marginally overlapping groups.

## Discussion

VMTD protocol allowed generation of longer fragments, which in turn facilitated the design of primers anchored in regions flanking *Vulmar/VulMITE* insertions. We used agarose gels for separation of the TD amplicons, which simplified the detection of polymorphisms. However, it came at a cost of a lower number of bands that could have



**Fig. 4** Principal coordinates analysis (PCoA) of *Betoideae* based on ISBP polymorphisms. Two to four individuals represent each stock. Individuals with identical ISBP scores occupy the same position on the graph. Plant stock codes are given according to Table 1

been identified in one reaction. Other MITE-based TD systems were more efficient and produced from 42 to 77 bands for *Heartbreaker* in maize (Casa et al. 2000) and 51 for *mPing* in rice (Naito et al. 2006). Sequences of selected VMTD fragments showed that all of them were derived from insertion sites. Thus, the technique allowed efficient identification of regions flanking TE insertions. The *DeMaster* TD system in carrot, similar to here reported VMTD, produced ca. 10% of non-specific fragments (Grzebelus et al. 2007).

It should be stressed that the observed *VMTD* polymorphism, especially on the interspecific level, may not result solely from TE insertion polymorphism, but also from single nucleotide polymorphisms in restriction sites or rearrangements of primer annealing sites. It is not possible to directly estimate the relative contribution of these factors to the overall level of the revealed interspecific differences. Two interrelated factors may have an effect on it, i.e. the distance between species, and the age of an insertion. In relatively distant species, common insertions that can be identified are old, thus there is more chance that the TE itself or the region flanking the insertion has been rearranged. As a result, the observed TD polymorphism may not reflect the actual TE presence/absence, but rather the inability to produce same-sized TD bands for both species. There were attempts to use TE-based platforms as tools for phylogenetic analysis (Nagy et al. 2006; Moisy et al. 2008), however, that problem was never addressed. On the other hand, all differences among the sugar beet stocks should in principle be attributed to the activity of the transposable elements, as transposition seems to be the most likely cause of TD polymorphisms in otherwise very similar genomes.

The data provided the opportunity to hypothesize about the activity of *Vulmar* and *VulMITE* in the course of evolution of *Betoideae*. We conclude that these elements were not active in section *Corollinae* and the shared insertions present in *B. corolliflora*, *B. lomatogona*, and *B. macrorrhiza* were already present in the common ancestor of all *Beta* species. In contrast, a substantial insertion polymorphism was observed in different sugar beet stocks, corroborating observations of Menzel et al. (2006). The highest copy number of the investigated elements was observed in sugar beet and *B. vulgaris* subsp. *maritima*. The identified intraspecific insertion polymorphism suggests that *Vulmar* and/or at least one of *VulMITE* families was active in these subspecies, most likely also in the period of beet domestication. Results reported by Menzel et al. (2006) suggest that *VulMITE I* family could be responsible for that recent burst of activity. Similar bursts of MITE activity was reported as an effect of rice domestication (Naito et al. 2006), while Grzebelus et al. (2009) reported on a possibility of periodical MITE mobilization in domesticated *M. truncatula*.

Some *Vulmar/VulMITE* elements were active also in *Patellifolia*, resulting in a subset of novel insertions not present in any *Beta* species. It has been debated if *P. procumbens* and *P. webbiana* should be regarded as separate species (Curtis 1968). In the present study both species shared almost the same set of insertion sites, which supported previous reports on a very limited level of interspecific variability between *P. procumbens* and *P. webbiana* (Wagner et al. 1989; Mita et al. 1991). In terms

of TE dynamics in *Patellifolia*, our results indicated that unlike *B. vulgaris*, their mobilization was much more limited, as there were very little differences between the two investigated species, *P. procumbens* and *P. webbiana* as compared to any pair of accessions of *B. vulgaris*. The divergence time of *Betoideae* and *Hablitzieae* was estimated to be 38.4–27.5 Mya (Hohmann et al. 2006), which suggests that the group of *Vulmar/VulMITE* elements consists of families that have been active in different evolutionary periods. Possibly, different families were active in *Beta* and *Patellifolia*, as we observed distinct subsets of insertions differentiating the two genera, which was confirmed by ISBP markers. This could be experimentally verified by cloning and sequencing full-length TEs from the diagnostic sites. Detailed investigation on the activity periods of *Vulmar* and *VulMITE* elements could help develop tools for TE insertion-based phylogenetic analysis of *Betoideae*. That strategy was successfully applied, e.g. the use of SINEs for studying phylogeny of primates (reviewed in Ray 2007). MITEs are a good source of phylogenetic information in plants, owing to their abundance and small size. However, as they derive from Class II, they are capable of excision and sequence verification of insertion sites, identifying footprints created upon excision might be required.

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