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BRIEF COMMUNICATION

Mitochondrial minisatellite polymorphisms in fodder and sugar beets reveal genetic bottlenecks associated with domestication

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Abstract

Historically, sugar beets were selected from fodder beets. We used mitochondrial minisatellite loci to analyze cytoplasmic genetic diversity in fodder beet and sugar beet. Among the 8 sugar beet accessions examined we identified 3 multi-locus haplotypes. These 3 haplotypes were a subset of 5 haplotypes identified among the 29 fodder beet accessions examined. All but one haplotype in fodder beet comprised, in turn, a subset of 12 haplotypes identified previously in leaf beets. Such apparent decreases in cytoplasmic genetic diversity must result from genetic bottlenecks associated with domestication and the ensuing breeding processes. We also detected the haplotype associated with the male-sterile Owen cytoplasm of sugar beet in the fodder beet gene pool. Furthermore, the presence of a 39 kDa protein associated with the Owen cytoplasm was confirmed in two fodder beet plants by Western blot analysis. These results lead us to speculate that the Owen cytoplasm may have originated in fodder beet, from which sugar beet was derived.

Additional key words: *Beta vulgaris,* cytoplasmic male sterility, haplotypes, Owen cytoplasm, tandem repeats.

There are four major cultivated beets (Lange *et al*. 1999): leaf beet (*Beta vulgaris* L. ssp. *vulgaris* convar. *cicla* (L.) Alef.) is grown for leaves and has not appreciably swollen tap root; garden beet (*B. vulgaris* L. ssp. *vulgaris* var. *vulgaris*) has swollen hypocotyl and is mostly utilized as vegetable and in the canning industry; fodder beet (*B. vulgaris* L. ssp. *vulgaris* var. *rapacea* Koch) has large swollen hypocotyl and root and is used to feed livestock; and sugar beet (*B. vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) has swollen root that is used as an important source of sugar. These crops and their wild relatives are all members of one primary gene pool, *Beta vulgaris* L. (Bartsch and Ellstrand 1999, Hammer 2001, Ford-Lloyd 2005).

The historical development of beet cultivars has been documented by several authors (Ford-Lloyd 1986, De Bock 1986, Fischer 1989, Van Geyt *et al*. 1990, Frese 1991, Winner 1993). Domestication probably began in the eastern Mediterranean area between 2000 and 1500 BCE, where the plants were mainly used as leaf vegetables (Winner 1993). According to De Bock (1986), the first reliable documentation of beet plants with swollen roots came from the 12th century. There is no indication that beet roots were used for fodder before around 1700, but from 1800 fodder beets had become an established part of mixed farming systems in Europe (De Bock 1986). Fodder beet most likely originated from garden beet, which was initially domesticated as a leaf vegetable and, by the Middle Ages, was used as a root vegetable. White fodder beet from Silesia (Fischer 1989) provided the germplasm from which the earliest sugar beet crop was bred. Each domestication event may have imposed a genetic bottleneck on cultivated beet, so that genetic diversity has been reduced during the domestication process.

Polymorphisms in organellar DNA are invaluable sources of information for inferring the course of plant evolution (*e.g*. Skuza *et al*. 2010, Ni *et al*. 2011). We

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Abbreviations: CTAB - cetyltrimethylammonium bromide; PCR - polymerase chain reaction; SDS - sodiumdodecyl sulphate.
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recently investigated cytoplasmic genetic diversity in leaf and garden beets and their wild relatives using mitochondrial minisatellites (Nishizawa *et al*. 2007, Cheng *et al*. 2011). The same minisatellites were used to characterize cytoplasmic diversity in Chinese sugar beet breeding lines (Cheng *et al*. 2009). However, there has been only fragmented information available on cytoplasmic genetic variation in fodder beets (Ecke and Michaelis 1990, Senda *et al*. 1998). The present study was aimed at filling in this gap, and was based on an evaluation of 131 plants representing 29 fodder beet cultivars (27 were obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 1 was from the United States Department of Agriculture Research Service, and 1 was purchased from Snow Brand Seed, Japan) and 86 plants representing 8 sugar beet cultivars (2 from IPK; 4 from the National Agricultural Research Center for the Hokkaido Region of Japan (NARCH); 1 from the *Syngenta* company of Sweden, and 1 from the *KWS SAAT* company of Germany) using mitochondrial minisatellite polymorphisms. We previously used 4 mitochondrial minisatellite loci (TR1, TR2, TR3, and TR4; Nishizawa *et al*. 2007) to analyze 748 individual plants from the leaf and garden beet groups or their close relatives. Among these we were able to distinguish 21 haplotypes, named min01 to min21 (Nishizawa *et al*. 2007, Cheng *et al*. 2011).

Plants used in the present study were grown in a greenhouse. Total cellular DNA was isolated using the cetyltrimethylammonium bromide (CTAB)-based method described by Cheng *et al*. (2009). Individual plants were genotyped using the mitochondrial minisatellite loci TR1, TR2, TR3, and TR4 and some plants (see below) were also analyzed using the male-sterility-associated locus *orf129* (Cheng *et al*. 2009). The polymerase chain reaction (PCR) primers and procedures for amplification were as described by Cheng *et al*. (2009, 2011). Polymorphisms were revealed using 2 % agarose gel or 4 % polyacrylamide gel electrophoresis, and visualized after ethidium bromide staining with known individuals as internal size standards. Any ambiguities in the numbers of repeat units in the minisatellite loci were resolved by determining the nucleotide sequences of the amplicons.

Five haplotypes (min04, min06, min09, min15, and min18) were detected among the 131 fodder beet plants (Table 1 and data not shown; a complete dataset is available upon request). The haplotype min18 predominated and occurred in 61 individuals from 18 accessions. The second-most frequent haplotype was min09, which was found in 34 individuals from 9 accessions. These two haplotypes (min18 and min09) correspond to the non-sterile normal-1 and normal-2 cytoplasms, respectively (Cheng *et al*. 2009). Two of the three less frequent haplotypes, min04 and min06, are associated with the Owen male-sterile and I-12CMS(3) cytoplasms, respectively (Nishizawa *et al*. 2007, Cheng *et al*. 2009).

In sugar beet plants carrying the Owen cytoplasm, cytoplasmic male sterility (CMS) is correlated with an open reading frame (designated *preSatp6*) of unknown origin and its translation product of 39 kDa (Yamamoto *et al*. 2005). Immunoblots of total proteins from two fodder beet plants with the min04 haplotype (the Russian cultivar BETA 53 and the Polish cultivar BETA 1599, both obtained from IPK) were probed using antiserum raised against an oligopeptide corresponding to the carboxyl-terminal 17 residues of preSATP6 (αpreS-C; Yamamoto *et al.* 2005). The sugar beet lines TK81-MS (with the male-sterile Owen cytoplasm) and TK81-O (with a normal fertile cytoplasm, used as the maintainer of TK81-MS) were included as controls. These control lines were developed at HARC. Total proteins were isolated from fresh green leaves according to Cheng *et al.* (2009). A 15 % SDS polyacrylamide gel was used for electrophoresis. Fractionated proteins were electronically blotted onto *Hybond P* (*GE Healthcare*, Amersham, UK). The secondary antibody was HRP-conjugated goat anti-rabbit IgG (*GE Healthcare*). After washing three times

Table 1. Distributions of mitochondrial minisatellite haplotypes among the fodder and sugar beet plants used in this study. Each haplotype is based on the combination of alleles from the TR1, TR2, TR3, and TR4 loci. The five haplotypes min04, min06, min09, min15, and min18 are the same as those listed in Table 3 of Nishizawa *et al*. (2007). The haplotypes were based on the numbers of repeat units obtained with the four TR loci as follows: 4 (TR1)/ 3 (TR2)/ 2 (TR3)/ 4 (TR4) for min04, 5/ 3/ 2/ 3 for min06, 6/ 3/ 3/ 3 for min09, 9/ 3/ 3/ 3 for min15, and 13/ 3/ 3/ 3 for min18.

	min04	min06/-orf129	min09	min15	min18
Fodder beet	8	14	34	14	61
Sugar beet	11	0	5	0	70

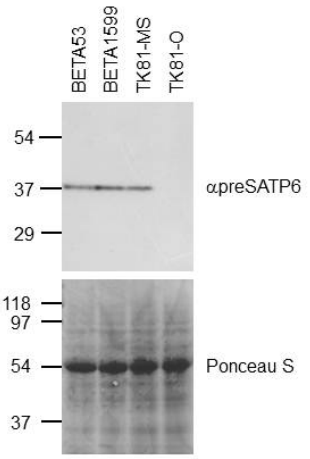

Fig. 1. Western blot analysis of proteins extracted from fresh green leaves of 2 fodder beet plants, BETA53 and BETA1599. The blot was probed with the anti-preSATP6 antiserum. The sugar beet lines TK81-MS and TK81-O were used as positive and negative controls, respectively. Size markers are shown *on the left* [kDa]. After blotting, the membrane was stained with Ponceau S as a loading control.

with *TBS-T*, the membrane was soaked in *ECL-plus* (*GE Healthcare*), then exposed to X-ray film. A single protein of 39 kDa was detected in both plants, confirming that the Owen cytoplasm is present in the fodder beet gene pool (Fig. 1). Cheng *et al*. (2009) found that the haplotype min06 could be further divided into two subtypes (min06/+orf129 and min06/-orf129) based on the presence or absence of *orf129*. In this study, the 14 fodder beet plants with min06 were subjected to a PCR assay to detect *orf129*, but the sequence was not detected in any of the plants (data not shown). It is also worth mentioning that 21 of the 29 fodder beet accessions examined appeared to be monomorphic, while 8 accessions exhibited two or three haplotypes (data not shown).

Three haplotypes (min04, min09, and min18) were detected among the 86 plants representing 6 old and 2 modern sugar beet cultivars (Table 1 and data not shown). This result was expected because we previously found that only these three haplotypes were present in a total of 437 individuals representing 42 Chinese sugar beet breeding lines (Cheng *et al*. 2009).

The distributions of mitochondrial minisatellite haplotypes detected in the fodder and sugar beet gene pools are shown in Fig. 2, along with the distributions in the leaf and garden beet gene pools reported previously (Cheng *et al*. 2009, 2011). Only 3 haplotypes (min04, min09, and min18) were found among the 50 sugar beet cultivars that we have examined (Cheng *et al*. 2009; this study). Interestingly, Fenart *et al*. (2008) found that a mitochondrial minisatellite haplotype unique to the Owen CMS cytoplasm predominated in so-called weed beet populations sampled from France and Belgium, and that two Nvulg-unique haplotypes were the next most abundant in these populations. They considered that the majority of weed beets would share cytoplasms with sugar beet cultivars, since weed beets generally result from the accidental pollination of sugar beet seed parents by ruderal beets. Judging from their raw data [see Fig. 2 of Fenart *et al*. (2008)], the Owen CMS-unique haplotype seems to correspond to min04, while the Nvulg haplotypes correspond to min09 and min18. It thus appears that the results of Fenart *et al*. (2008) are consistent with our results.

The haplotypes min04, min09, and min18 are a subset of the five haplotypes present in fodder beets. In turn, all but one (min15) of the fodder beet haplotypes constitute a subset of the 12 haplotypes distributed in the leaf beet germplasm (Cheng *et al*. 2011). Furthermore, all six of the haplotypes detected in garden beets are present in the leaf beet germplasm (Cheng *et al*. 2011). These decreases in cytoplasmic diversity must result from genetic bottlenecks associated with domestication and the ensuing breeding processes. Such bottlenecks have been documented in several crop species (*e.g*. Neale *et al*. 1988, Provan *et al*. 1999, Tesfaye *et al*. 2007, Fenart *et al*. 2008).

Another noteworthy finding is the occurrence of the Owen cytoplasm in the fodder beet gene pool. The male sterility caused by this cytoplasm was first discovered in the open-pollinated, curly top-resistant sugar beet cultivar US1, which was released in 1931 (Owen 1945, Doney 1995). Owen (1945) found that approximately 2 % of US1 individuals were male sterile. Forty years later, Powling and Ellis (1983) demonstrated that 6 out of 8 US1 plants contained mitochondrial DNA typical of plants with normal cytoplasms, and the remaining 2 contained mitochondrial DNA typical of Owen CMS plants. However, the donor of the Owen cytoplasm has remained uncertain.

Bonavent *et al*. (1989) found that the old garden beet cultivar Crapaudine exhibited the same chloroplast DNA restriction profile as the Owen CMS sugar beet plants. This led them to hypothesize that a cross occurred

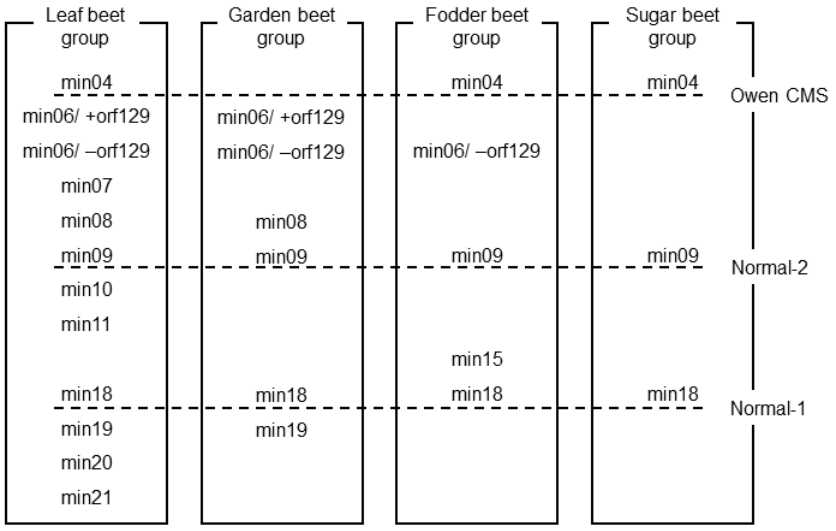

Fig. 2. Mitochondrial minisatellite haplotypes found in four cultivar groups of *B. vulgaris* ssp. *vulgaris*. The haplotypes corresponding to normal-1, normal-2, and Owen CMS are underlined with *dashed lines*. Data are from Cheng *et al*. (2009), Cheng *et al*. (2011), and this study.

between a sugar beet plant and cv. Crapaudine carrying the Owen cytoplasm, and that some individuals of the progeny were accidentally collected by Owen. The origin of sugar beet breeding has been attributed to Franz Carl Achard (1753-1821), who was the first to grow beets for sugar production (Biancardi 2005). He reportedly noticed that conical, white beet roots that were deeply set in the soil were the highest in sugar content (Winner 1993). Such traits undoubtedly became the basis for sugar beet breeding, while the plants with unwanted garden beet traits (such as colored, exposed roots) must have been carefully eliminated. This weakens the afore-mentioned hypothesis regarding the origin of the Owen cytoplasm in sugar beets. On the other hand, our results favor the idea of fodder beet as the donor of the Owen cytoplasm. We speculate that both the Owen cytoplasm and the normal cytoplasm were present in the fodder beet germplasm from which early sugar beet cultivars were derived. This assumption is in keeping with the view of Knapp (1958), who suggested that the first beet that Achard worked with was probably a fodder beet known as Runkelrübe, which was highly heterogeneous for root morphology and color.

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