Selection of Nuclear Genotypes Associated with the Thermo-inducibility of Owen-type Cytoplasmic Male Sterility in Sugar Beet (Beta vulgaris L.)

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Research Article

Keywords: Cytoplasmic male sterility, environmental sensitivity, hybrid breeding, restorer of fertility, sugar beet

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Selection of nuclear genotypes associated with the thermo-inducibility of Owen-type cytoplasmic male sterility in sugar beet (*Beta vulgaris* L.)

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Abstract

The stability of cytoplasmic male sterility expression in several genetic backgrounds was investigated in sugar beet (Beta vulgaris L.). Nine genetically heterogenous plants from old cultivars were crossed with a cytoplasmic male-sterile line to obtain 266 F₁ plants. Based on marker analysis using a multiallelic DNA marker linked to restorer-of-fertility 1 (Rf1), we divided the F₁ plants into 15 genotypes. We evaluated the phenotypes of the F₁ plants under two environmental conditions: greenhouse rooms with or without daytime heating during the flowering season. Three phenotypic groups appeared: those consistently expressing male sterility (MS), those consistently having restored pollen fertility, and those expressing MS in a thermo-sensitive manner. All plants in the consistently male-sterile group inherited a specific Rf1 marker type named p4. We tested the potential for thermo-induced male-sterile plants to serve as seed parents for hybrid seed production, and three genotypes were selected. Open pollination by a pollen parental line with a dominant trait of red-pigmented hypocotyls and leaf veins resulted in seed setting on thermo-induced male-sterile plants, indicating that their female organs were functional. More than 99.9% of the progeny expressed the red pigmentation trait; hence, highly pure hybrids were obtained. We determined the nucleotide sequences of Rf1 from the three genotypes: one had a novel allele and two had known alleles, of which one was reported to have been selected previously as a nonrestoring allele at a single US breeding station but not at other stations in the US, or in Europe or Japan, suggesting environmental sensitivity.

Keywords

Cytoplasmic male sterility, environmental sensitivity, hybrid breeding, restorer of fertility, sugar beet

Key message

Cytoplasmic male sterility in sugar beet becomes thermo-inducible when combined with specific genotypes, suggesting a means to environmentally control pollination by this trait.

Introduction

Hybrid seed production of some crops relies on heritable male sterility (MS) because it converts a hermaphroditic plant into a seed parent that never self-pollinates (Budar et al. 2006). On the other hand, using male-sterile plants for hybrid seed production at a commercial scale is costly because the system is complicated, in part, due to problems with how seed parents are propagated. MS can be caused by a nuclear gene (genic MS) or by the combination of nuclear and mitochondrial genes (gene-cytoplasmic...
MS or cytoplasmic male sterility, CMS) (Budar et al. 2006). In genic MS, the gene responsible for MS cannot be fixed, and male-sterile plants should be selected from a population in which male fertile plants co-occur. The male-fertile plants should be removed from the field before the flowers open (Budar et al. 2006). In the case of CMS, cytoplasmic segregation does not occur; however, in order to propagate the seed parent, pollen must be obtained from a maintainer line with the identical genotype as the cytoplasmic male-sterile line but be male fertile (i.e., have a non-sterility-inducing cytoplasm) (Budar et al. 2006). In both cases, such complexity can be reduced if the gene or cytoplasm expresses MS in response to an environmental cue such as temperature or photoperiod but otherwise permits pollen production (Chen and Liu 2014). Environmentally inducible MS has been reported in rice, wheat, rapeseed, and Chinese cabbage, but only the rice and wheat male-sterile plants have been practically applied to hybrid seed production (Li et al. 2007; Murai et al. 2008; Sha et al. 2019). The use of environmentally inducible MS for the breeding of other crops is rare at present.

Almost all commercial varieties of sugar beet (Beta vulgaris L.) are hybrids using CMS (McGrath and Panella 2019). Since the discovery of CMS by Owen (1945), breeders have selected maintainer lines with the aim to achieve stable MS expression (Kaul 1988; Bosemark 2006). A prerequisite to the maintainer genotype is that two loci, X and Z, are homozygous recessive (Owen 1945). Whereas little is known about Z (Honma et al. 2014), X was identified as restorer-of-fertility 1 (Rf1), and its nucleotide sequence was determined (Matsuhira et al. 2012). Rf1 is a complex locus consisting of a gene cluster whose constituents have sequence similarity with Oma1, a gene involved in mitochondrial quality control (Arakawa et al. 2020b; Migdal et al. 2017). The clustered gene copies (hereafter, each copy is referred to as Rf1-Oma1) were divided into two classes based on a single property of their encoded protein; namely, those with or without the ability to bind to a CMS-associated sugar beet mitochondrial protein (Arakawa et al. 2020b). This binding activity is crucial for altering the higher-order structure of the CMS-associated mitochondrial protein to restore male fertility (Kitazaki et al. 2015).

The Rf1 locus is highly polymorphic in the number of clustered Rf1-Oma1 copies and their nucleotide sequences (Arakawa et al. 2020a). The molecular diversity of the Rf1 locus is associated with the variety of its alleles: dominant, semi-dominant, hypomorphic and recessive alleles with very different types of gene organization are known. Thus, Rf1 is considered a multiallelic locus (Arakawa et al. 2018, 2019); however, the entire suite of Rf1 alleles in the sugar beet gene pool is unknown.

In our previous study, we genetically analyzed maintainer lines that were developed outside of Japan (Ohgami et al. 2016). These lines were crossed with a cytoplasmic male-sterile line to obtain F1 plants that should express MS if the pollen parent had the maintainer genotype (this experimental procedure is called a test cross). We found a major recessive rf1 allele that predominates among many
maintainer lines used by the world’s breeders (Ohgami et al. 2016). In addition, several minor \( rf_1 \) alleles that could condition the male-sterile phenotype but varied in their gene organization were identified. It was puzzling that these minor \( rf_1 \) alleles were favored by a specific breeding station (Ohgami et al. 2016). We also observed that some lines recorded as ‘maintainers’ in the Gene Bank gave rise to fertility-restored \( F_1 \) plants, indicating that these lines were not maintainer genotypes in our experimental growing conditions (Ohgami et al. 2016). \( Rf_1 \) alleles of these apparently non-maintainer lines were organizationally different from the genetically-proven recessive \( rf_1 \) alleles at the DNA level (Ohgami et al. 2016). Such \( Rf_1 \) alleles seemed to have escaped maintainer selection, but another possibility remained.

Theurer and Ryser (1969) reported that the male fertility of beets with Owen cytoplasm fluctuated depending on such environmental conditions as moisture stress and temperature. We also observed that the minor \( rf_1 \) allele identified in Ohgami et al. (2016) was not selected in another of our studies examining Japanese sugar beets (Moritani et al. 2013). These two studies differed in how male fertility was evaluated, e.g., the \( F_1 \) plants were grown in different fields and seasons. Here, we investigated whether environmental conditions during maintainer selection affected the selected \( Rf_1 \) allele. As a model experiment, we exposed \( F_1 \) plants to normal and high temperatures during flowering. The resultant phenotypes were classified into three categories: consistently male fertile, consistently male sterile, and male fertile at normal temperature but male sterile at high temperature. Our results indicated that sugar beet CMS becomes environmentally sensitive in specific genotypes.

**Materials and methods**

**Plant materials**

Sugar beet (\( Beta vulgaris \) L.) open-pollinated cultivars used in this study were TA-30 (developed in Poland), TA-36 (Germany), and TA-37 (Sweden), all of which were introduced into Japan before the 1960s (Taguchi et al. 2014). Sugar beet line NK-305 was a selection of unknown origin (Arakawa et al. 2019). TA-33BB-CMS is a cytoplasmic male-sterile line with Owen cytoplasm, and TA-33BB-O is its maintainer (i.e., the nuclear genotype is the same but with a non-sterility-inducing cytoplasm) (Arakawa et al. 2018). These three lines were developed by the Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Japan. TA-8 is a US inbred line with a non-sterility-inducing cytoplasm and has deep red pigmentation in the hypocotyls and leaf veins. Inflorescences of seed parents and pollen parents were enclosed in paper bags before anthesis. After anthesis, the paper bags were exchanged for crossing. Open pollination was allowed in a closed room of a greenhouse.
Growth conditions

Seeds were sown in paper pots filled with soil, and seedlings were grown until four to five leaves developed. Plantlets were transplanted to beds in a greenhouse in Memuro, Hokkaido, Japan, in early May. A closed room of this greenhouse was equipped with a kerosene heater; the room was heated from 10:00 to 16:00 daily, from late June to early September, and the temperature did not exceed 40˚C. Temperatures were recorded once every hour by an Ondotori RTR507B data logger (T&D Corporation, Matsumoto, Japan) in each room (Fig. 1).

Pollen fertility evaluation

Pollen fertility phenotypes were visually inspected and classified into five categories (N, P, S, G, and W, from fertile to completely sterile) according to the method reported by Moritani et al. (2013). Briefly, N is fully fertile with well-rounded and yellow anthers that can dehisce; P has orange anthers and is less fertile than N; S is semi-sterile and has dull yellow and partially shrunken anthers that cannot dehisce; G is semi-sterile with light green and shrunken anthers; and W is completely sterile with white or brown shrunken anthers. Phenotypes were evaluated twice during a two-week interval.

Genotyping

Total cellular DNAs were isolated from fresh green leaves according to the procedure of Doyle and Doyle (1990) with minor modification in which the isolation buffer contained 0.2 mg/ml of RNase A (Nacalai Tesque, Kyoto, Japan). DNA markers s17 and 20L-int are detailed in Taguchi et al. (2014). DNA fragments were electrophoresed in 2% or 2.5% agarose gels.

Nucleotide sequence analysis

Rfl1-Oma1 copies were PCR amplified with primers 5'-CGGTACCGGGGATCATGGCGTGGTACAGAAATTCAAGGTTTG-3' and 5'-CGACTCTAGAGATCTTACTTACTGAAGACCTTGAATTGCACGTCC-3' using KOD FX polymerase (Toyobo, Osaka, Japan). PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Venlo, The Netherlands) and were cloned into plasmid vector pUC19 (In-Fusion HD Cloning Kit; Takara Bio, Kusatsu, Japan). Colonies with the objective DNA fragment were selected by colony PCR and cultured in LB medium (Sambrook et al. 1989). Plasmid DNAs were isolated using a QIAprep Spin Miniprep Kit (Qiagen). Sequencing was accomplished with the primer walking technique using a BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were assembled by Sequencher software (Gene Codes,
Ann Arbor, MI, USA). Sequences were compared at a website implementing ClustalW (https://clustalw.ddbj.nig.ac.jp/). Nucleotide sequences determined in this study were deposited in DDBJ/EMBL/GenBank under the accession numbers of LC628460, LC628461, LC628462 and LC628463.

**Results**

*Pollen parent selection from open-pollinated cultivars*

Pollen parents were selected from three old cultivars that are genetically heterogeneous. We selected pollen parental genotypes that contain a variety of *Rf1* alleles. The DNA marker s17 is a cleaved amplified sequence marker closely linked to *Rf1* (Taguchi et al. 2014). DNA band patterns of s17 have been linked to the *Rf1* alleles (Arakawa et al. 2018, 2019, 2020b). A total of 169 plants were genotyped using s17 (Table 1), and we observed that these plants had band patterns 1 (hereafter abbreviated as p1), p3, p4, and p5. We selected seven plants composed of the four s17 band patterns (Table 2). Because p2 was missing from our selections, we added two plants from NK-305, a sugar beet line that was previously shown to contain p2 (Arakawa et al. 2019) (Table 2). The nine plants were crossed with the cytoplasmic male-sterile line TA-33BB-CMS, whose s17 marker type was homozygous at p4 (hereafter p4p4) to obtain F1 seeds. These F1 selections were grouped into 15 genotypes according to their parentage and s17 band patterns (Table 2).

*Fertility restoration without supplemental heating*

The F1 plantlets were divided into two groups to grow in two different environmental conditions. One group was planted in a greenhouse room that had no supplemental heat. The pollen fertility of the F1 plants is summarized in Table 2. Of the 92 plants, eleven were completely male sterile (phenotype class W). The s17 marker type of all eleven plants was p4p4; considering that the s17 marker type of the seed parental line TA-33BB-CMS is p4p4, all eleven plants inherited the p4 allele from their pollen parent. The remaining 81 plants had restored pollen fertility (Table 2). Their phenotypes were either N (nine plants) or P (71 plants), both of which are phenotype classes capable of shedding pollen grains. According to their s17 marker types, these fertility-restored plants inherited p1, p2, p3 or p5 from their pollen parent (Table 2).

*Fertility restoration with supplemental heating*
The other F₁ group was planted in a greenhouse room with a heater that operated only during the daytime. The heating protocol was started just before stalk elongation and ceased at the end of flowering (Fig. 1 and see the Materials and methods section). In the same greenhouse room, plants of TA-33BB-CMS and its maintainer line TA-33BB-O were grown as controls; the phenotypes of twelve TA-33BB-CMS were W, whereas those of TA-33BB-O were N (four plants) and P (seven plants).

Of these F₁ plants, the phenotypes of all p4p4 plants were W (Table 2). Unlike the phenotypes of plants receiving no supplemental heat, the other s17 marker types were associated with W. The phenotypes of all six F₁ plants that inherited p3 from plant #14 of TA-30 (hereafter, this s17 allele is referred to as TA-30-14 p3) were W, and the phenotypes of all twenty-four F₁ plants that inherited TA-37-19 p3 were W (Table 2). W plants occasionally occurred in the F₁ population that had the TA-30-14 p1, NK-305-3 p5, TA-36-1 p3 and TA-36-3 p5 alleles (Table 2). Some other F₁ plants with these four s17 alleles exhibited an indeterminant phenotype in which a plant had flowers with different levels of pollen fertility (denoted as an admixture in Table 2). For example, each of three plants with the TA-30-14 p1 allele had flowers of P, S, and W phenotypes. The admixture phenotype was also observed in plants with other s17 alleles. We identified the admixture phenotype in eight out of the fifteen F₁ populations (Table 2). Note that the admixture phenotype was not present in plants receiving no supplemental heat (Table 2). On the other hand, plants with the TA-30-32 p1 and TA-36-32 p3 alleles had restored pollen fertility when grown in the heated room, as was also seen in plants not receiving supplemental heat, indicating that they were phenotypically stable.

Purity of hybrid seed from thermo-induced male-sterile plants

We tested whether heat-induced male-sterile plants had the potential to be seed parents for hybrid seed production. We chose three F₁ genotypes with the TA-37-19-p3, TA30-37-p5 and TA-36-3-p5 alleles as seed parents because these genotypes produced flowers of the W phenotype at high frequency (Table 2). Seedlings with these alleles were selected from F₁ populations by screening with the s17 marker and planted in the greenhouse room with supplemental heat.

Pollen fertility phenotypes are summarized in Table 3. All 24 plants with the TA-37-19-p3 allele exhibited the W phenotype, whereas plants with either the TA30-37-p5 or TA-36-3-p5 alleles exhibited W and an admixture that consisted of S, G and W flowers (Table 3). Note that anthers did not dehisce in the S and G flowers. We planted TA-8 as a pollen parent; this sugar beet line has a non-sterility-inducing cytoplasm. TA-8 plants shed pollen grains in the same greenhouse room, allowing for open pollination.
Plants with the TA-37-19-p3, TA30-37-p5 and TA-36-3-p5 alleles set seeds that were harvested to test whether they were hybrids with TA-8. Deep red pigmentation in the hypocotyls and leaf veins was a unique characteristic of TA-8 and this phenotype is governed by a dominant gene, as is also documented in garden beet (Hatlestad et al. 2012). We sowed the seeds to determine the ratio of seedlings with deep red pigmentation. The emerged seedlings exhibited deep red pigmentation that was clearly distinguishable from sugar beet plants with pink hypocotyls and unpigmented veins, phenotypes seen in some sugar beet cultivars (Fig. 2). Of 1111 seedlings derived from the seed parents with the TA-37-19-p3 allele, 1110 seedlings were intensely pigmented. All 371 seedlings from seed parents with the TA30-37-p5 allele and 2316 seedlings from seed parents with the TA-36-3-p5 allele were highly pigmented.

Molecular identity of the $Rf1$ alleles in plants expressing heat-induced male sterility

We investigated whether the TA-37-19-p3, TA30-37-p5 and TA-36-3-p5 alleles were identical to any of the previously identified $Rf1$ alleles and sought to determine the nucleotide sequences of $Rf1$-$Oma1$ copies in these $Rf1$ alleles. $Rf1$-$Oma1$ coding regions were PCR amplified from the three $F_1$ plants that expressed heat-induced MS. Because the PCR products were mixtures of the objective DNA fragments and non-objective DNA fragments from the TA-33BB-CMS allele (see Table 3), the PCR products were cloned into plasmid vectors to sort the DNA fragments. To eliminate clones with copies of TA-33BB-CMS $Rf1$-$Oma1$, we focused on the length of the first intron; TA-30-37, TA-36-3 and TA-37-19 had exclusively short introns, whereas TA-33BB-CMS had a long intron as shown using 20L-int, a DNA marker targeting the intron (Fig. 3). Therefore, we selected and sequenced plasmids with short first introns and compared the nucleotide sequences with those of known $Rf1$ alleles.

$Rf1$-$Oma1$ derived from TA-37-19-p3 was identical to that found from PI 615522, a US maintainer line (Fig. S1). The s17 marker type of PI 615522 was also reported as p3 (Ohgami et al. 2016). As such, we concluded that the TA-37-19-p3 allele was the same as PI 615522 $rf1$.

The $Rf1$-$Oma1$ nucleotide sequence of the TA-30-37-p5 allele was identical to that of the TA-37-19-p3 allele (Fig. S1), but the s17 type of the TA-30-37-p5 allele differed from that of PI 615522 $rf1$. Another US maintainer line, PI 590689, had an $Rf1$-$Oma1$ copy identical to PI 615522 $rf1$, but its s17 type was p5 (Ohgami et al. 2016). Therefore, the TA-30-37-p5 allele was likely the same as that of PI 590689 $rf1$.

We obtained two $Rf1$-$Oma1$ sequences from the TA-36-3-p5 allele. This result was consistent with the band pattern of the 20L-int marker in which two short S bands appeared from TA-33BB-CMS x TA-36-3 (Fig. 3). No known $Rf1$-$Oma1$ matched either of the obtained sequences.
Discussion

Sugar beet breeders have preferred stable MS in their quest to establish ideal seed parents, and maintainers have been selected to achieve stable MS. Unstable MS is problematic in breeding if it is uncontrollable, and such genotypes have been discarded in the past without further characterization. In our current study, we observed a novel phenotype of Owen-type CMS in which MS is expressed only when the plant experienced overheating, offering a novel means to control MS expression. This phenotype can also be interpreted as fertility restoration when not heated but non-restoring when heated. We propose the existence of two phenotypes for the Owen-type CMS: consistent sterility and thermo-sensitive sterility. These phenotypes were conditioned by their nuclear genotypes, indicating that nuclear-cytoplasmic interaction was involved in these phenotypes. The manifestation of nuclear-cytoplasmic interactions is known to fluctuate depending on environmental conditions, e.g., *Arabidopsis* nuclear

substitution lines exhibit differential adaptation to different environmental conditions (Roux et al. 2016).

*Rf1* is a principal genetic factor for Owen-type CMS expression, with several different alleles recently reported (Arakawa et al. 2020b). Our previous study identified a molecular variant linked to s17 marker type p4 as the most prevalent form in maintainer lines (Ohgami et al. 2016). Marker-assisted selection of p4 can be used for maintainer line selection (Moritani et al. 2013). The current study showed that plants with p4 expressed MS irrespective of heat treatment (Table 2). This result suggests that breeders have preferred p4 because its ability to condition MS is least affected by environmental conditions. This observation, in turn, implies that the other *Rf1* alleles are restoring alleles or unstable MS conditioners; however, because not all *Rf1* alleles have been genetically characterized yet, we may expect novel stable MS conditioning alleles to be found in the future.

Although the involvement of *Rf1* alleles in thermo-inducible MS remains unclear, it may be possible. We selected three thermo-inducible male-sterile genotypes whose *Rf1* loci were heterozygous for p4 and either of the three *Rf1* alleles. TA-36-3-p5 is, as yet, an uncharacterized *Rf1* allele with two novel *Rf1-Oma1* copies; further genetic and molecular characterization is necessary. The TA-37-19-p3 and TA-30-37-p5 alleles were likely PI 615522 rf1 and PI 59068 rf1, respectively, whose *Rf1-Oma1* coding regions were identical but s17 types differed from each other (Ohgami et al. 2016). Arakawa et al. (2020b) investigated the molecular function of PI 615522 rf1 and concluded that the ability to bind to the CMS-associated sugar beet protein was missing. Therefore, we infer that other genes such as *Z* are associated with thermo-inducible MS. Whereas the major *rf1* allele linked to p4 was selected in many breeding stations of the world, including in the US, Europe and Japan, PI 615522 rf1 was preferentially
selected by a breeding station in California (Ohgami et al. 2016). Therefore, we cannot exclude the possibility that PI 615522 rf1 (and PI 59068 rf1) are unstable MS conditioners that allow MS expression in an environment-specific manner. Detailed genetic analysis with special care for environmental factors will be required.

Female fertility of our thermo-inducible male sterile plants was normal in those receiving the supplemental daytime heat treatment. The purity of hybrid seeds from induced male-sterile plants was over 99.9%, indicating that male sterile plants can be seed parents for hybrid seed production. Our supplemental heating protocol increased the daytime temperature only and did not alter the nighttime temperature. Therefore, we favor the notion that daytime temperature is critical for MS induction.

Temperature conditions similar to that in our heated room can be seen in the southern part of Japan, i.e., >35°C during the daytime and 15-20°C at night can occur during the summer season. Investigations of MS expression at the field level should be conducted where the environmental conditions are similar to those in this greenhouse study.

Many commercial sugar beet seeds are the products of three-way crosses in which male-sterile F₁ plants are crossed with a pollinator line (Bosemark 2006). In this breeding scheme, the F₁ plants are derived from the cross between a CMS line and an unrelated maintainer line to ensure the male-sterile phenotype (Bosemark 2006). Because the nuclear genotype of a CMS line is equivalent to that of the cognate maintainer line (i.e., a CMS line is produced by recurrent backcrosses using a maintainer line as the pollen parent), developing various maintainer lines is a prerequisite to conducting a sugar beet hybrid breeding program. To obtain the desired stability of MS, sugar beet breeding must rely on the rf1 allele linked to the p4, an infrequent occurrence in the sugar beet gene pool (Taguchi et al. 2014). The dependency of p4 in sugar beet hybrid breeding could be alleviated by introducing thermo-inducible MS because the male-sterile phenotype can be expressed in the F₁ of a CMS x non-maintainer genotype. Moreover, it is interesting to consider whether thermo-inducible male-sterile lines can be developed, as is the case for rice (Li et al. 2007). A practical test of this possibility is ongoing.

Declarations

Funding

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Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Author contribution statement

H. Matsuhira, K. Kitazaki, and T. Kubo conceptualized and designed the study. H. Matsuhira, K. Kitazaki, K. Matsui, K. Kubota, and Y. Kuroda prepared materials and collected and analyzed the data. The draft was written by H. Matsuhira and K. Kitazaki and finalized by T. Kubo. All authors read and approved the final manuscript.

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Table 1 Number of plants with the DNA marker type in three open-pollinated cultivars

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<th>p1p5</th>
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<th>p5p5</th>
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Table 2  Pollen parental plants and the results of test crosses

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* Pollen fertility phenotypes found in the admixtures are shown in parentheses.
Table 3  Phenotype of F₁ plants used as seed parents in supplemental heat treatment

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<th>F₁ population</th>
<th>Selected  s17 type</th>
<th>Phenotype</th>
<th>Admixtures*</th>
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</table>

* Pollen fertility phenotypes found in the admixtures are shown in parentheses.
Figure legends

Fig. 1  Temperature fluctuations in closed greenhouse rooms where sugar beets were grown. Vertical and horizontal axes depict the temperature in degrees Celsius and the date, respectively. Red and blue lines denote greenhouse room temperatures with and without supplemental heating, respectively.

Fig. 2  Phenotypes of sugar beet seedlings. a. Hybrid seedlings emerged from the seeds harvested from heat-induced male-sterile plants (TA-33BB-CMS x TA37-19). The pollen parent was TA-8. b. Seedlings of a sugar beet cultivar that segregate for the pink hypocotyl phenotype.

Fig. 3  Gel electrophoresis of PCR fragments targeting the first intron of Rf1-Oma1. Plant IDs and cross combinations are shown above. Size markers are shown on the left (bp). Positions of the short intron (S) and long intron (L) are shown on the right.
TA-30-37
TA-36-3
TA-37-19
TA-33BB-CMS
TA-33BB-CMS x TA-30-37
TA-33BB-CMS x TA-36-3
TA-33BB-CMS x TA-37-19
No template DNA
**Figure 1**

Temperature fluctuations in closed greenhouse rooms where sugar beets were grown. Vertical and horizontal axes depict the temperature in degrees Celsius and the date, respectively. Red and blue lines denote greenhouse room temperatures with and without supplemental heating, respectively.
Figure 2

Phenotypes of sugar beet seedlings. a. Hybrid seedlings emerged from the seeds harvested from heat-induced male-sterile plants (TA-33BB-CMS x TA37-19). The pollen parent was TA-8. b. Seedlings of a sugar beet cultivar that segregate for the pink hypocotyl phenotype
Figure 3

Gel electrophoresis of PCR fragments targeting the first intron of Rf1-Oma1. Plant IDs and cross combinations are shown above. Size markers are shown on the left (bp). Positions of the short intron (S) and long intron (L) are shown on the right.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigS1.pdf