Note

A maternally inherited DNA marker, descended from *Solanum demissum* (2*n* = 6*x* = 72) to *S. tuberosum* (2*n* = 4*x* = 48)

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A Mexican hexaploid wild potato species, *Solanum demissum* (*dms*), was only used as a female in previous breeding programs. The resulting clones with *dms* cytoplasm produced abundant, but non-functional pollen. A 170 bp DNA fragment, named Band 1, was originally detected in the F$_1$ hybrid between *dms* and *S. tuberosum*. In this study, the sequenced region was extended to 1,032 bp; nevertheless, it did not show any homology to known sequences. This extended region harboring Band 1 was, without introns, all transcribed to mRNA and was maternally inherited from *dms* to *S. tuberosum* through backcrosses. Three *dms* accessions, 168 accessions of 38 cultivated and closely related wild species, and 158 varieties and breeding lines were surveyed, which demonstrated that Band 1 was specific to *dms* and varieties and breeding lines with *dms* cytoplasm. Thus, Band 1 is a useful marker to distinguish *dms* cytoplasm, which enables us to design efficient mating combinations in breeding programs.

Key Words: backcrossing, cytoplasmic male sterility, DNA marker, *Solanum demissum*, transcribed region, unknown sequence, W/α cytoplasm.

Introduction

Solanum demissum Lindl. (2*n* = 6*x* = 72) is a hexaploid wild potato species distributed in Mexico (Hawkes 1990); this species could be one of the oldest wild species used in the history of modern breeding. As early as 1906, Salaman recognized resistance to late blight in this species (Salaman 1941). Since then, *S. demissum* has been extensively used as a resistance source to late blight (Plaisted and Hoopes 1989, Ross 1986, Rudorf 1950). So far, 11 hypersensitive-type resistance genes have been identified and incorporated into cultivars (Ross 1986). *S. demissum* is highly self-fertile, yet it shows unilateral incompatibility with the common potato (*S. tuberosum* L., 2*n* = 4*x* = 48). *S. demissum* can be easily crossed with the pollen of *S. tuberosum* and produces pentaploid hybrids which, as well as backcross progenies, are only crossable as female parents (Black 1943, Dionne 1961, Irikura 1968). Thus, the *S. demissum* cytoplasm was preferentially transmitted to the bred varieties.

Such unilateral incompatibility, or cytoplasmic male sterility, is common in potato. The common potato shares at least seven different cytoplasmic sterility factors ([*ASF^s*],

[*Fm^s*], [*In^s*], [*SM^s*], [*Sp^s*], [*TA^s*] and [*VSA^s*]) that condition sterility in the presence of dominant chromosomal genes (*ASF*, *Fm*, *In*, *SM*, *Sp*, *TA* and *VSA*) (Grun *et al.* 1977). The cytoplasmic genome of potato is characterized by possessing T-type chloroplast DNA (Hosaka 1986) and β-type mitochondrial DNA (Lössl *et al.* 1999). Although the cytoplasmic sterility factors likely reside on mitochondrial DNA (Hosaka *et al.* 1988, Lössl *et al.* 2000), β-type mitochondrial DNA so far shows complete association with T-type chloroplast DNA (hereinafter, T/β cytoplasm) (Lössl *et al.* 2000). The T/β cytoplasm is predominant in the common potato (Bryan *et al.* 1999, Hosaka and Hanneman 1988, Lössl *et al.* 2000, Powell *et al.* 1993, Provan *et al.* 1999, Waugh *et al.* 1990), so sterility problems are unavoidable when T/β cytoplasm is present. In addition to such intrinsic sterility, specific male sterility associated with cytoplasmic genome is known. Varieties carrying *Ry$_{sto}$* (a resistance gene to *Potato virus Y*), released mainly in Germany (Ross 1986), show male sterility caused by the association with the characteristic mitochondrial DNA derived from *S. stoloniferum* Schlechtd. et Bché. (W/γ cytoplasm) (Lössl *et al.* 2000). In these cases of T/β and W/γ cytoplasm, sterility is always characterized by visible abnormalities, such as no pollen, no or poor pollen-shedding, or various deformities of anthers (Grun 1979). In contrast, F$_1$ and backcrossed progenies carrying *S. demissum* cytoplasm (W/α) produce abundant and

normal-looking pollen, but this pollen is non-functional on *S. tuberosum* (Dionne 1961). According to Lössl *et al.* (2000), the W/α cytoplasm occupied 40% of German varieties.

We reconfirmed the unilateral incompatibility between *S. demissum* and *S. tuberosum*; 395 berries were obtained from 488 pollinations in *S. demissum* (♀) × *S. tuberosum* (♂), while in the reciprocal cross, 45 berries from 232 pollinations (Sanetomo *et al.* 2011). Further, we found that the hybrid from a cross *S. tuberosum* (♀) × *S. demissum* (♂) showed higher crossability than the reciprocal hybrid: for example, the former hybrid when crossed as a male to *S. demissum* resulted in a significantly higher berry setting rate (64.9%) than the latter hybrid (24.2%) (Sanetomo *et al.* 2011). To elucidate this differential crossability, pollen DNA samples from reciprocal hybrids were compared using methylation-sensitive amplified polymorphism (MSAP) analysis. Six distinct DNA sequences were found to be different between the reciprocal hybrids (Sanetomo and Hosaka 2011).

We noticed that one of the six bands, named Band 1 in Sanetomo and Hosaka (2011), was very interesting and worthy of further investigation. We report here that the extended DNA sequence harboring Band 1 was strictly specific to *S. demissum* and maternally inherited to *S. tuberosum*, so it was a useful indicator of *S. demissum* cytoplasm. Although this sequence was transcribed to mRNA without an intron, it did not show any significant homology with known sequences, and the intra-cellular origin remains unknown.

Materials and Methods

Plant materials

Saikai 35 (a *S. tuberosum* breeding line) and 5H109-5 (*S. demissum* PI 186551) (referred to as T and D, respectively) were reciprocally crossed, deriving DT (D as female) and TD (T as female) F$_1$ populations (6H37 and 6H38 families, respectively). One TD plant (6H38-19) and one DT plant (6H37-6) were backcrossed with the pollen of Saikai 35, deriving (TD)T and (DT)T BC$_1$ populations (7H1 and 7H2 families), respectively. Since *S. demissum* is highly self-pollinated in nature and homozygous, we assumed that all seedlings derived by selfing were genetically identical to each other and to the parental clone 5H109-5, and were used as D. Saikai 35 has S/ε cytoplasm, while 5H109-5 has W/α cytoplasm.

The presence/absence of Band 1 was examined for three *S. demissum* accessions and 168 accessions of 38 species (Table 1), which covered all 164 different cytoplasms previously distinguished among cultivated species and wild species closely related to cultivated species, except distantly related *S. pinnatisectum* Dun. and *S. stoloniferum* (Hosaka and Sanetomo 2010). A further survey was conducted for 158 varieties and breeding lines.

Extending Band 1 sequence

The sequenced region was extended from both ends of Band 1 using an LA PCR™ *in vitro* Cloning Kit (Takara Bio Inc., Japan) by the manufacturer's protocol briefly described below. Total DNA of D was extracted from fresh leaves by the method of Hosaka and Hanneman (1998), digested separately with various restriction enzymes, and ligated with appropriate adapters to the end of restriction digests. Using a pair of primers (one assigned to internal sequence of Band 1 and the other to the adapter sequence), polymerase chain reaction (PCR) was performed. If a single band was obtained, it was directly sequenced.

PCR detection of Band 1

The extended Band 1 sequence was divided into three overlapped regions, Region 1, 2 and 3, and the three primer sets were designed to amplify these regions (Table 2). PCR reaction was performed using a volume of 5 µl consisting of 1 µl template DNA (approximately 5 ng/µl), 2.5 µl Ampdirect Plus (Shimadzu Co., Japan), 0.125 units of *Taq* DNA polymerase (BIOTAQ HS DNA Polymerase; Bioline Ltd., UK) and 0.3 µM primers (Table 2). Thermal cycling was performed using a Veriti 96-well thermal cycler (Applied Biosystems) (one cycle of 10 min at 95°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 60°C and 1 min at 72°C, and then terminated with one cycle of 5 min at 72°C). PCR products were separated by electrophoresis on a 1.4% agarose gel in 1× TAE buffer (40 mM Tris-acetate and 1 mM EDTA pH 8.0), stained in 2.5 µl Midori Green DNA Stain (Nippon Genetics Europe GmbH, Germany) per 100 ml of 1× TAE buffer for 30 min with gentle shaking, followed by de-staining using 1× TAE buffer for 30 min with gentle shaking. Photographic images were captured using a UV lamp.

For a wide survey of wild and cultivated potatoes, Region 2 of the extended Band 1 was amplified by the same protocol described above except that the granule-bound starch synthase I gene (GBSS) marker (Table 2) was included at 0.3 µM concentration in the reaction as a positive control to check whether the PCR was performed correctly.

Southern hybridization

Approximately 15 µg total DNA of T and D were digested with a restriction enzyme *Msp*I, *Hin*dIII or *Eco*RI, and Southern-hybridized with a PCR product of Band 1 (primers shown in Table 2) as probe DNA by the method described by Hosaka and Hanneman (1998).

Transcription analysis

Fresh leaves were sampled from each of T, D, TD and DT plants, quick-frozen in liquid nitrogen and ground to powder with a mortar and a pestle. Total RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN, ME, USA). To eliminate possibly contaminated genomic DNA, all RNA samples were treated for 20 min at 37°C with DNase (TURBO DNA-*free*; Ambion, TX, USA). RNA concentration was measured by a fluorometer (QuantiFluor-P; Promega, WI, USA) using the Quanti-iT RiboGreen RNA Assay Kit (Invitrogen).

Table 1. Wild and Andean cultivated potatoes surveyed by Band 1

Taxonomic series and species	Accession[a]
Series *Pinnatisecta* (Rydb.) Hawkes	
S. pinnatisectum Dun.	PI 184764, PI 275230
Series *Yungasensa* Corr.	
S. chacoense Bitt.	PI 537025, chc 525-3
S. tarijense Hawkes	PI 498399*
Series *Megistacroloba* Cárd. et Hawkes	
S. boliviense Dun.	PI 498215, PI 545964*
S. megistacrolobum Bitt.	PI 265874, PI 473356, PI 473361, PI 545999
S. raphaniforium Cárd. et Hawkes	PI 473371
S. sogarandinum Ochoa	PI 230510
Series *Conicibaccata* Bitt.	
S. chomatophilum Bitt.	PI 266387, PI 365327
S. irosinum Ochoa	PI 568985
Series *Piurana* Hawkes	
S. acroglossum Juz.	PI 498204
S. blanco-galdosii Ochoa	PI 442701
Series *Tuberosa* (Rydb.) Hawkes	
(Wild species)	
S. acroscopicum Ochoa	PI 365314, 365315
S. brevicaule Bitt.	PI 498110*, PI 498111*, PI 498112*, PI 498113*, PI 498114*, PI 498115*, PI 498218, PI 545967, PI 545968, PI 545970*
S. bukasovii Juz.	PI 210042, PI 210051, PI 265876, PI 275271, PI 283074, PI 310937, PI 365304, PI 365318, PI 365321, PI 365349, PI 365350, PI 365355, PI 414155, PI 442698, PI 458379, PI 473447, PI 473450, PI 473453, PI 473491, PI 473492, PI 498219, PI 498220, PI 568932, PI 568933, PI 568939, PI 568944, PI 568949, PI 568954
S. canasense Hawkes	PI 246533, PI 283080, PI 310938, PI 310956, PI 473346, PI 473347, PI 473348
S. candolleanum Berth.	PI 498227, PI 545972, PI 568969
S. coelestipetalum Vargas	PI 473354, PI 590904
S. dolichocremastrum Bitt.	PI 498234
S. immite Dun.	PI 365330, PI 498245
S. leptophyes Bitt.	PI 283090*, PI 320340*, PI 458378, PI 473342*, PI 473343*, PI 473344*, PI 473445, PI 473451, PI 473495*, PI 545895, PI 545896, PI 545985*, PI 545986*, PI 545987, PI 545988*, PI 545990, PI 545991*, PI 545992*, PI 545993*, PI 545995
S. marinasense Vargas	PI 210040, PI 310946
S. medians Bitt.	PI 210045, PI 442703, PI 473496
S. multidissectum Hawkes	PI 210043, PI 210044, PI 210052, PI 210055, PI 473349, PI 473353, PI 498304
S. multiinterruptum Bitt.	PI 275272, PI 498267*
S. oplocense Hawkes	PI 435079*, PI 442693*, PI 458390*, PI 498067, PI 545876, PI 545908*, PI 545910*
S. pampasense Hawkes	PI 275274, PI 442697
S. sparsipilum (Bitt.) Juz. et Buk.	PI 498136*, PI 498138*, PI 498139*, PI 498140*, PI 498305*
S. × sucrense Hawkes	PI 473506
S. vernei Bitt. et Wittm.	PI 458373, PI 458374, PI 473306, PI 473311, PI 500067, PI 545884*, PI 558148, PI 558151
(Cultivated species)	
S. ajanhuiri Juz. et Buk.	CIP 702677
S. curtilobum Juz. et Buk.	CIP 700273, CIP 702455
S. juzepczukii Buk.	CIP 700895
S. phureja Juz. et Buk.	CIP 703275
S. stenotomum Juz. et Buk.	CIP 701165, CIP 701985, CIP 702583, CIP 703088, CIP 703710, CIP 703808, CIP 703933, CIP 707297
S. tuberosum L. ssp. *andigena* Hawkes	PI 243363, PI 246497, PI 255508, PI 265882*, PI 281080, PI 281105, PI 292089, PI 365345, PI 473285*, PI 473391*, PI 473393*, PI 498076, PI 498294, PI 498310, PI 546017*, PI 546023, CIP 700790, CIP 703268
S. tuberosum L. ssp. *tuberosum*	CIP 703252
Series *Acaulia* Juz.	
S. acaule Bitt.	PI 210030, CIP 761143
S. albicans (Ochoa) Ochoa	PI 266381, PI 365306
Series *Longipedicellata* Buk.	
S. stoloniferum Schlechtd. et Bché.	PI 186544*, PI 195167*
Series *Demissa* Buk.	
S. demissum Lindl.	PI 175411*, PI 186551*, PI 498012*

[a] Accessions having W/α cytoplasm are shown by asterisks.

Reverse transcription was performed using 1 μg total RNA primed with an oligo (dT)$_{21}$ primer using SuperScript III First-Strand Synthesis SuperMix (Invitrogen). The synthesized cDNA was diluted to 1/10 and subjected to PCR amplification for the three regions. PCR reactions were as described above. The following thermal profile was used: 10 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 60°C and 1 min at 72°C, followed by a final extension step of 5 min at 72°C. To confirm the absence of genomic DNA contaminations in cDNA samples, PCR was carried out under the same condition with primers Tubf and Tubr (Table 2), which amplified a 525 bp β-tubulin gene fragment from cDNA instead of an approximately 1,600 bp fragment from genomic DNA containing an intron in potato (Turra *et al.* 2009).

Determination of chloroplast and mitochondrial DNA types

Chloroplast DNA types were determined by restriction fragment length polymorphism (RFLP) analysis of chloroplast DNA as described earlier (Sukhotu *et al.* 2004). Mitochondrial DNA type was determined by PCR using primers ALM_4 and ALM_5, which amplified a 2.4 kb, 1.6 kb or no band from α-, β- or γ-type mitochondrial DNA, respectively (Lössl *et al.* 2000); however, if a 1.6 kb band was associated with S or A-type chloroplast DNA, the mitochondrial DNA type was deduced to be ε type, based on circumstantial information from Lössl *et al.* (1999).

Results

Extended Band 1 sequence

Band 1 has been detected as a 170 bp *Eco*RI and *Msp*I (or *Hpa*II) double-digested DNA fragment (NCBI Genbank Accession No. HR505437), specifically found in D and DT

Table 2. Primers used in this study

| Target	Primer (5′-3′ sequence)[a]	Size (bp)
Band 1	Band 1-F (GCCTATGGCTCTCATCTTCAA)	163
	Band 1-R (GGACCAGATCCAGAAGGTAACG)	
Region 1	Band 1-F11 (CGGGAGGTGGTGTACTTTCT)	527
	Band 1-R6 (ACGGCTGACTGTGTGTTTGA)	
Region 2	Band 1-F8 (AACTTGGAAGCGAAAGCTCA)	434
	Band 1-R9 (ATTGCCGATGTCCAAGTAGG)	
Region 3	Band 1-F9 (CCCTTTGTTTGAGCCCTTGT)	446
	Band 1-R3 (GCTCCCGTTTCCCACTATTT)	
GBSS	GBSS-01 (ATGGCAAGCATCACAG)	981
	GBSS-02 (CAAAACTTTAGGTGCCTC)	
β-tubulin	Tubf (ATGGATCTAGAGCCTGGTACTATG)	525[b]
	Tubr (CAAACAGCAAGTAACACCACTC)	

[a] Primer sequences for amplification of GBSS and β-tubulin were obtained from Takeuchi *et al.* (2009) and Turra *et al.* (2009), respectively.

[b] cDNA size (Turra *et al.* 2009).

(Sanetomo and Hosaka 2011). This sequence was extended to 1,032 bp containing the 170 bp Band 1 (Fig. 1A). Homology search was carried out for the extended Band 1 sequence using the BLASTN program; however, it did not show high homology to any known sequences, even to those of the latest potato genome database including chloroplast and mitochondrial genome sequences (The Potato Genome Sequencing Consortium 2011).

*Inheritance and specificity of Band 1*

Using three primer sets for amplification of Region 1 to 3, or a primer set that specifically amplified Band 1 (Table 2), D, T, 5 DT, 5 TD, 90 (DT)T and 38 (TD)T plants were examined for the presence/absence of Band 1. As shown in
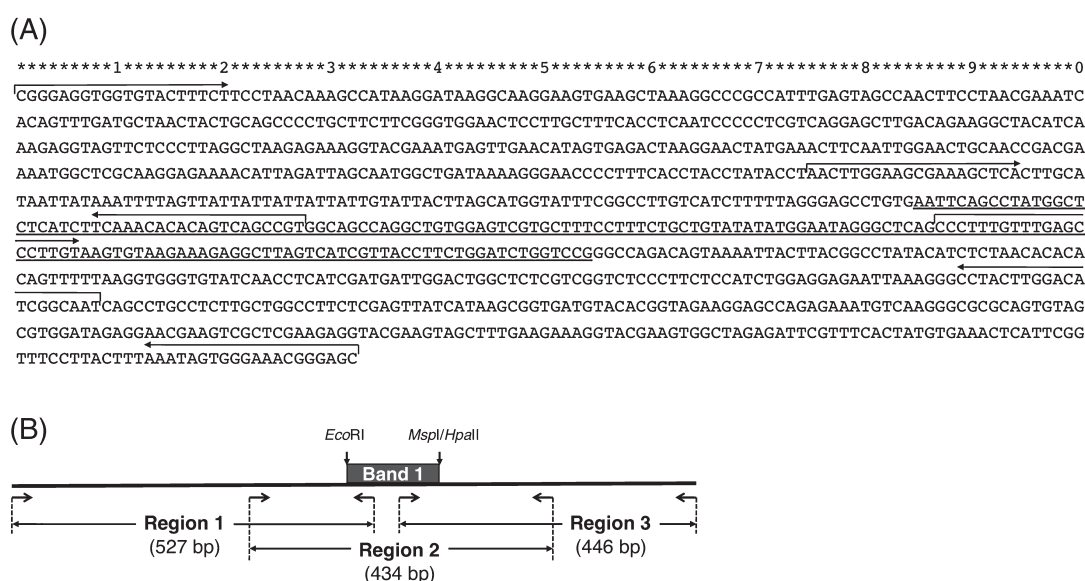
Fig. 1. A DNA fragment of 1,032 bp harboring Band 1 (underlined in A). Three pairs of primer sequences for amplification of Region 1, 2 and 3 are shown in (A) and the schematic representation in (B).
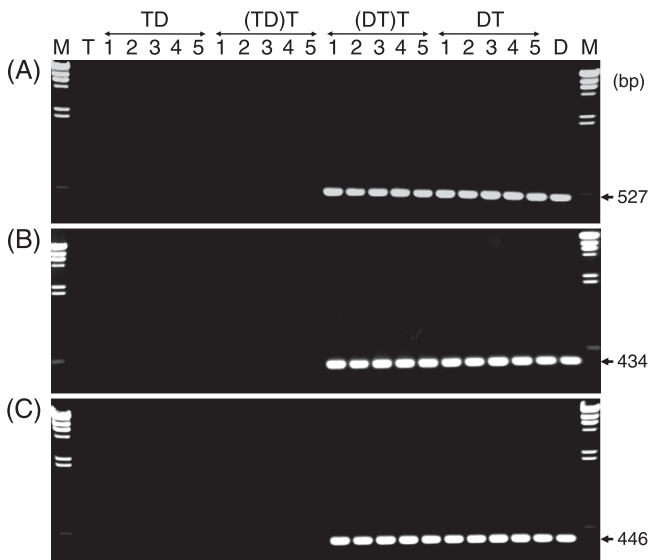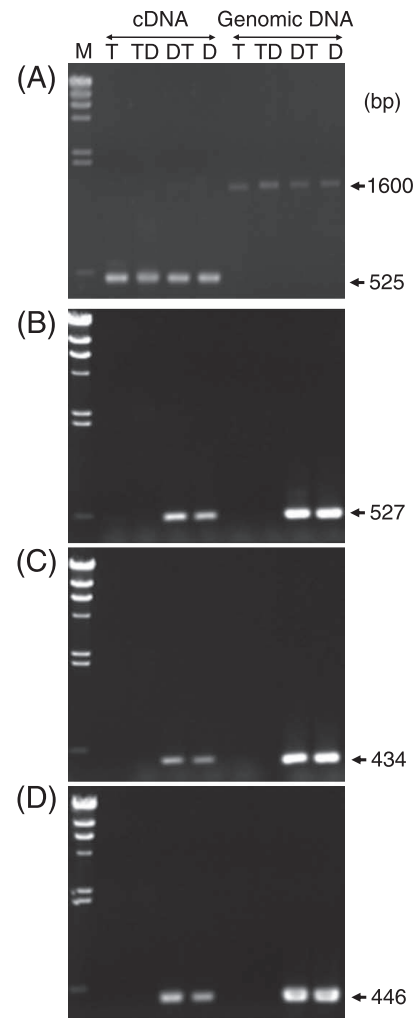
Fig. 2. Amplification of Region 1 (A), Region 2 (B) and Region 3 (C) from T, D, 5 reciprocal F_1 plants of TD and DT, and 5 BC_1 plants of (TD)T and (DT)T. M denotes λDNA *Hin*dIII digests.
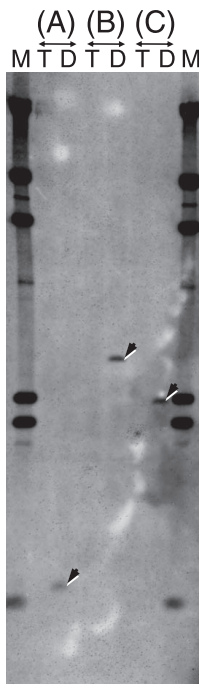
Fig. 3. Southern hybridization of *Msp*I (A), *Hin*dIII (B) and *Eco*RI (C) digests of T and D probed with Band 1. Arrows indicate hybridization signals. M denotes λDNA *Hin*dIII digests.

Fig. 4. Leaf cDNA and genomic DNA of T, TD, DT and D were amplified for Region 1 (B), Region 2 (C) and Region 3 (D). A β-tubulin gene amplified from leaf cDNA samples exhibited only 525 bp band (A), demonstrating no contamination with genomic DNA. M denotes λDNA *Hin*dIII digests.

Transcription of Band 1

Leaf mRNA was extracted and cDNA was amplified for the Band 1 region (Fig. 4). Compared with a band of β-tubulin amplified from genomic DNA, only a smaller band (525 bp) was amplified from cDNA, indicating that an intron was removed and no genomic DNA was contaminated in cDNA samples (Fig. 4A). As shown in Fig. 4B–4D, three regions were all transcribed to mRNA in leaves of D and DT plants, but not in those of T and TD plants. The sizes of amplified bands from cDNA were similar to those from genomic DNA for all three regions. Furthermore, Region 1 PCR products from cDNA were sequenced, and showed similar sequences to those of genomic DNA. This indicates that the entire sequence of extended Band 1 is transcribed to mRNA and contains no intron.

Fig. 2, DNA fragments of expected sizes were present in D, all DT and (DT)T plants, but not in T, all TD and (TD)T plants. By Southern hybridization analysis, hybridization signals were obtained as single bands from all three restriction digests of D, whereas no signal was obtained from T, indicating the lack of the homologous sequence to Band 1 in T (Fig. 3).

Screening Band 1 against various cytoplasms

Band 1 was surveyed in three *S. demissum* accessions and 168 accessions of 38 species with various cytoplasms (Table 1). Although 43 accessions had W/α cytoplasm, none except the three accessions of *S. demissum* (all had W/α cytoplasm) had Band 1.

Characterization of varieties and breeding lines

Cytoplasm types were determined for 158 varieties and breeding lines. One hundred and eight (68.4%) had T/β cytoplasm typical of *S. tuberosum* (Table 3). The W/α cytoplasm was found in 21 varieties and 12 breeding lines (20.9%) (Table 4). One of the landraces, Nemuromurasaki, and two others had A/ε cytoplasm derived possibly from *S. tuberosum* L. ssp. *andigena* Hawkes. The S/ε cytoplasm, derived from *S. phureja* Juz. et Buk., and W/γ cytoplasm, derived from *S. stoloniferum*, were found in 12 and 2 varieties or breeding lines, respectively (Table 3). As exemplified in Fig. 5, Band 1 was exclusively detected in varieties or breeding lines that had W/α cytoplasm (Table 4).

Band 1 was detected in Tunika and its three haploid clones (Table 4) and Band 1 was inherited maternally from *S. demissum* even after 10 times backcrossing (see Saikai 39 in Table 4).

Homology between Band 1 sequences of different origins

PCR products amplified from Band 1 of Rishiri (the cytoplasm originally derived from *S. demissum* T-2), Hanashibetsu (from W553-4) and Kitamurasaki (from Nemuromurasaki), and those from Region 1 of Rishiri, Tunika (from W-race) and Astarte (from MPI 19268), were sequenced, which perfectly fit the corresponding sequences of D (Fig. 1A).

Discussion

Band 1 is a useful DNA marker distinguishing the S. demissum cytoplasm

Previously, a set of PCR primers flanking a 241 bp deletion that defined T-type chloroplast DNA (Hosaka *et al.* 1988, Kawagoe and Kikuta 1991) was developed (Hosaka 2002, Lössl *et al.* 2000), and has been used frequently worldwide for various purposes (Ames and Spooner 2008, Chimote *et al.* 2008, Gavrilenko *et al.* 2007, Spooner *et al.* 2007). Such a DNA marker distinguishing *S. demissum* cytoplasm has not been available until now. Genotypes producing abundant pollen tend to be used as pollen parents. Even if a desirable genotype produces abundant pollen, it should not be used as a pollen parent when it has *S. demissum*-derived cytoplasm to avoid the difficulty of obtaining hybrid seeds; thus, it is very important to identify *S. demissum*-derived cytoplasm in breeding programs.

According to Lössl *et al.* (2000), W/α cytoplasm is specific to *S. demissum*-derived varieties. This was confirmed, as indicated in Table 4, because the W/α cytoplasm found in varieties and breeding lines was likely all descended from *S. demissum* alone; however, W-type chloroplast DNA was defined by RFLP analysis of chloroplast DNA and found in many wild species (Hosaka 1986, Hosaka and Sanetomo 2010, Sukhotu *et al.* 2004). According to the raw data used in Hosaka and Sanetomo (2010), among 164 different cytoplasms distinguished in Andean cultivated potatoes and closely related species, 73 cytoplasms had W-type chloroplast DNA, while 49 cytoplasms had α-type mitochondrial DNA. Consequently, 40 cytoplasms were disclosed to be W/α type, which is found in many wild species including *S. demissum* (shown by asterisks in Table 1). Thus, W/α cytoplasm is not specific to *S. demissum*. Alternatively, as discussed later, Band 1 was always associated with *S. demissum* cytoplasm, so it is a useful DNA marker for identifying *S. demissum*

Table 3. Varieties and breeding lines not possessing Band 1

Variety or breeding line[a]
1. Japanese variety
Aino-aka, Aiyutaka, Beni-akari, Benimaru, Bifukabeni, Bihoro, Chitose, Dejima, Early Starch, Eniwa, Fugenmaru, Haru-akari, Haruka, Hatsufubuki, Hinomaru 1, Hokkai-aka, Hokkaikogane, Inka Gold[S], Inka-no-hitomi[S], Inka-no-mezame[S], Inka Purple, Inka Red, Kannanjiro, Kita-akari, Kitahime, Konafubuki, Konayuki, Myojo, Neodelicious, Nishiyutaka, Norin 1, Norin 2, North Chip, Ohotsuku-chip, Oojiro, Ranran-chip, Red Moon, Sakurafubuki, Saya-akane, Sayaka, Shimabara, Snow March, Star Ruby, Star Queen, Tachibana, Tarumae, Tokachikogane, Tokachi Pirika, Touya, Toyoshiro, Unzen, Waseshiro, White Flyer, Yellow Shark, Yukijiro, Yukirasha, Yukitsubura
2. Japanese landrace
Ginzan-murasaki, Iya-imo (white), Kintoki-imo, Nagasaki-zairai B, Nemuromurasaki[A], Seinaiji-ki-imo
3. Japanese breeding line
529-1, 10H15[S], 10H16[S], 10H17[S], Chokei 129[S], Chokei 131[S], Hokkai 50, Hokkai 56[W], Hokkai 82, Hokkai 87, Hoku-iku 11, Hoku-iku 14, Kitakei 31, Kon-iku 35, KW85091-21, KW85093-33, Saikai 30, Saikai 31, Saikai 34, Saikai 35[S], Saikai 37[S], Saikai 38, Shimakei 569, T05003-1[A], T03097-19, T09073-88, WB77025-3, WB88055-8, WB902209-1[S]
4. Foreign variety or breeding line
Agasize, Alowa, Alwara[W], Andover, Atlantic, Cherie, Chipeta, Cimbal's Pheonix, Corolle, Cynthia, Daisy, Desiree, Early Rose, Irish Cobbler, Jenny, Juliette, Kanona, Kennebec, Kexin No. 1, Matilda, May Queen, ND860-2[S], Norking Russet, Pike, Prevalent, Russet Burbank, Salem, Shepody, Snowden, Sylvia, V2[A], Vermont Gold Coin, Yankee Chipper

a Cytoplasm type: [A] = A/ε, [S] = S/ε, [W] = W/γ, no symbol = T/β.

Table 4. Varieties and breeding lines possessing Band 1

Clones	Year released	Pedigree (Female × male)	Cytoplasm	Cytoplasmic origin (No. of generations)
1. Japanese variety				
Yoraku	1958	41089-8 × Norin 1	W/α	*S. demissum* T-2 (3)
Rishiri	1960	41089-8 × Norin 1	W/α	*S. demissum* T-2 (3)
Chijiwa	1962	S54042-15 × Unzen	W/α	*S. demissum* T-2 (5)
Shiretoko	1967	Hokkai 24 × Shimakei 291	W/α	HLT-4 (2)
Setoyutaka	1977	Saikai 10 × Unzen	W/α	*S. demissum* T-2 (7)
Meihou	1986	Chijiwa × Chokei 80	W/α	*S. demissum* T-2 (6)
Toyo-akari	1986	Tunika × WB61037-4	W/α	W-race (4)
Ezo-akari	1987	Tunika × Priekulsky Ranny	W/α	W-race (4)
Musamaru	1992	Tunika × Kon-iku 20	W/α	W-race (4)
Hanashibetsu	1997	W553-4 × R392-50	W/α	W553-4 (1)
Benihisashi	2001	W862207-1 × WB72209-5	W/α	W217H.2 (2)
Natsufubuki	2003	Musamaru × Shimakei 544	W/α	W-race (5)
Kitamurasaki	2004	Shimakei 571 × Shimakei 561	W/α	Nemuromurasaki (3)
Koganemaru	2006	Musamaru × Tokachikogane	W/α	W-race (5)
Northern Ruby	2006	Open-polination from Kitamurasaki	W/α	Nemuromurasaki (4)
Shadow Queen	2006	Open-polination from Kitamurasaki	W/α	Nemuromurasaki (4)
2. Japanese breeding line				
Chokei 108	–	Musamaru × Touya	W/α	W-race (5)
Hokkai 97	–	Meihou × Tokachikogane	W/α	*S. demissum* T-2 (7)
Hoku-iku 6	–	Kon-iku 27 × Hokkaikogane	W/α	HLT-4 (6)
Kitakei 29	–	Hanashibetsu × Tokachikogane	W/α	W553-4 (2)
Saikai 39	–	Aikei 125 × Saikai 35	W/α	*S. demissum* T-2 (10)
Shimakei 284	–	Open-pollination from Nemuromurasaki	W/α	Nemuromurasaki (1)
Shimakei 561	–	Shimakei 530 × ND860-2	W/α	W-race (5)
Shimakei 571	–	Shimakei 284 × 83015-47	W/α	Nemuromurasaki (2)
W553-4	–	A possible interspecific hybrid of unknown origin	W/α	–
W794215-H33	–	2x Tunika	W/α	–
W794215-H34	–	2x Tunika	W/α	–
W794215-H35	–	2x Tunika	W/α	–
3. Foreign variety				
Tunika	1967	Lü.56.186/21 N × Lü.51.183/2	W/α	W-race (3)
Astarte	1993	SVP RR 62-5-43 × VTN 62-69-5	W/α	MPI 19268 (4)
Eva	1999	Steuben × bulk pollen hybrids	W/α	B4494-3 (3)
Monticello	2003	Steuben × Kanona	W/α	B4494-3 (3)
Sassy	2004	G82TT137.1 × Promesse	W/α	?

cytoplasm. Precise identification of *S. demissum*-derived cytoplasm enables the design of efficient mating combinations in breeding programs.

*Cytoplasmic origin of varieties and breeding lines*

Varieties and breeding lines that had W/α cytoplasm had exclusively Band 1. Their cytoplasm was descended from at least eight parents: *S. demissum* T-2, W-race and MPI 19268 (both are famous backcross progenies from *S. demissum*, Ross 1986), Nemuromurasaki, HLT-4 (from USDA), W553-4, W217H.2 and B4494-3 (Table 4). The cytoplasm of W-race was incorporated into Japanese varieties via Tunika (introduced from the former East Germany in 1981), from which a potato cyst nematode resistance gene (*H1*) was introduced into several Japanese varieties (Mori *et al*. 2007).

Shimakei 571, Kitamurasaki, Northern Ruby and Shadow Queen had Band 1. According to their pedigree, they were maternally descended from Shimakei 284, which was developed from seedlings of an open-pollinated berry set on Nemuromurasaki (Table 4); however, the parent of Shimakei 284 was apparently misreported, because Nemuromurasaki is the oldest variety with A-type chloroplast DNA (Table 3), typical of *S. tuberosum* ssp. *andigena*, and was thought to be a relic of the early European potato (Hosaka 1993).

Although W553-4 was recorded as *S. tuberosum* ssp. *andigena* by Dr. Y. Irikura, who collected this clone in Colombia in 1977, now it has been recognized as an interspecific hybrid of unknown origin because of the extremely wide segregation observed in the progeny. As W553-4 shows a high level of late blight resistance, it might be a backcross progeny from *S. demissum*.

Furthermore, the pedigrees of other maternal parents that conferred W/α cytoplasm could not be traced. In conclusion, the W/α cytoplasm associated with Band 1 in varieties and
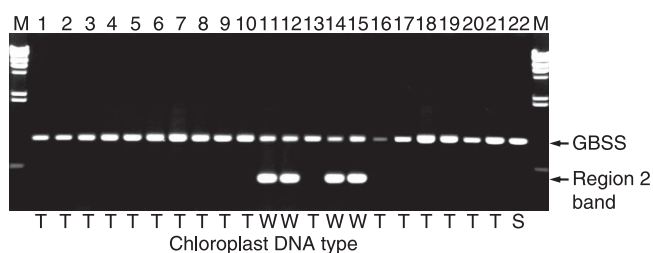
Fig. 5. Amplification of Region 2 together with GBSS as a positive control from randomly chosen varieties. Chloroplast DNA types are shown below. 1. Early Rose, 2. Irish Cobbler, 3. May Queen, 4. Desiree, 5. Russet Burbank, 6. Kennebec, 7. Atlantic, 8. Shepody, 9. Snowden, 10. Norking Russet, 11. Tunika, 12. Astarte, 13. Norin 1, 14. Rishiri, 15. Chijiwa, 16. Toyoshiro, 17. Nishiyutaka, 18. Hokkaikogane, 19. Konafubuki, 20. Kita-akari, 21. Touya, 22. Incano-mezame. M denotes λDNA *Hin*dIII digests.

breeding lines were all derived likely from *S. demissum*.

Intra-cellular origin of Band 1

We demonstrated that Band 1 is a *S. demissum*-specific DNA fragment maternally inherited from *S. demissum* to *S. tuberosum* through backcrossing; however, Band 1 did not show high homology to any known sequences, including complete sequences of potato chloroplast DNA (Chung *et al.* 2006) and mitochondrial DNA of the related genus *Nicotiana* (Sugiyama *et al.* 2005). It is generally known that plant chloroplast DNA evolves very slowly and is highly conservative in size, structure, gene content, and the linear order of genes (Palmer 1992, Palmer *et al.* 1988). Band 1 or Region 1 sequences, maternally inherited through many generations from different source accessions of *S. demissum*, were all similar to those of *S. demissum* used in this study, demonstrating the highly conservative nature of Band 1; however, *S. demissum*-specific insertion over 1 kbp was not detected in chloroplast DNA (Hosaka 1986), so that it is less likely that Band 1 is a part of chloroplast DNA.

By comparing several completely sequenced angiosperm mitochondrial DNAs, it is known that, although identified genes and introns are rather well conserved, intergenic regions are highly variable in sequence, even between two close relatives (Alverson *et al.* 2010, Handa 2003, Kubo and Mikami 2007); therefore, it is highly probable that Band 1 is part of the mitochondrial DNA of *S. demissum*.

However, mRNAs with poly(A) tails from chloroplast and mitochondrial genes are generally found only in degradation and are thus expected to occur in only a minor fraction of the steady state pool (del Campo 2009, Forner *et al.* 2007). Thus, alternative possibilities are that Band 1 is 1) located on one of the *S. demissum* chromosomes and exclusively transmitted maternally, or 2) something else, such as plasmid-like DNA, is maternally inherited. Linear or circular plasmids have been frequently reported in mitochondria in higher plants (Allen *et al.* 2007, Handa *et al.* 2002, Turpen *et al.* 1987). Further investigation of the intra-cellular origin of Band 1 is ongoing.

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