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ORIGINAL PAPER

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Hybrid lethality in interspecific hybrids between *Nicotiana tabacum* and *N. suaveolens*: evidence that the Q chromosome causes hybrid lethality based on Q-chromosome-specific DNA markers

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Abstract Hybrid seedlings from the cross *Nicotiana tabacum* × *N. suaveolens* express lethality at 28°C. We carried out a cross between monosomic lines of *N. tabacum* lacking the Q chromosome and *N. suaveolens* by test-tube pollination and ovule culture at 28°C. To suppress hybrid lethality, hybrid seedlings obtained were transferred to 36°C immediately after germination and cultured. We determined whether Q-chromosome-specific DNA markers were detected among hybrid seedlings. When hybrid seedlings cultured at 36°C were transferred to 28°C, hybrid seedlings in which Q-chromosome-specific DNA markers were detected expressed hybrid lethality, while hybrid seedlings in which Q-chromosome-specific DNA markers were not detected did not express hybrid lethality. From these results, we concluded that the presence of the Q chromosome of *N. tabacum* is related to hybrid lethality observed in crosses between *N. tabacum* and *N. suaveolens*. This is the first report that clearly demonstrates the relationship between a certain chromosome and hybrid lethality in the genus *Nicotiana* using chromosome-specific DNA markers. Additionally, we confirmed that the Q chromosome belongs to the S subgenome because Q-chromosome-specific DNA markers were detected only in *N. sylvestris*.

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Introduction

Reproductive isolation is a mechanism that separates species. This mechanism can be an obstacle when desirable genes are to be introduced into cultivated species by wide hybridization. Reproductive isolation is classified into two groups, prezygotic and postzygotic (Stebbins 1966). Hybrid lethality or hybrid weakness belongs to the postzygotic mechanisms of reproductive isolation and often causes death of hybrids. These phenomena have been observed in several intergeneric, interspecific and intraspecific hybrids (Hollingshead 1930; Sawant 1956; Oka 1957; Hermsen 1963; Takahashi et al. 1970; Shii et al. 1980; Lee 1981).

In the genus *Nicotiana*, several interspecific hybrids express lethal symptoms and eventually die (Christoff 1928; Kostoff 1930). These hybrids often involve cultivated tobacco, *N. tabacum*, as one of the parents. *N. tabacum* is a natural amphidiploid ($2n = 48$, SSTT), which presumably originated by interspecific hybridization of *N. sylvestris* ($2n = 24$, SS) with *N. tomentosiformis* ($2n = 24$, TT) and subsequent chromosome doubling (Sheen 1972; Gray et al. 1974; Lim et al. 2000; Murad et al. 2002). Each chromosome of *N. tabacum* is lettered alphabetically (A–Z, excluding X and Y); chromosomes A–L belong to the T subgenome and M–Z to the S subgenome. A complete set of 24 monosomic lines of *N. tabacum* (Haplo-A–Z) has been established on a genetic background of the tobacco cultivar Red Russian (var. Purpurea) and they are classified based on morphological characteristics (Clausen and Cameron 1944; Cameron 1959). These monosomic lines are useful for locating genes on specific chromosomes (Clausen and Cameron 1944).

Genetic studies of hybrid lethality observed in the genus *Nicotiana* have previously been conducted in three interspecific crosses, *N. tabacum* × *N. africana* (Gerstel et al. 1979), *N. repanda* × *N. tabacum* (Kobori and Marubashi 2004) and *N. tabacum* × *N. suaveolens* (Inoue

et al. 1996; Marubashi and Onosato 2002). Gerstel et al. (1979) carried out crosses between the entire series of 24 monosomic lines of *N. tabacum* and *N. africana*, and reported that many viable hybrids were obtained when Haplo-H was pollinated by *N. africana*. As the surviving hybrids lacked one chromosome, presumably the H chromosome, the authors inferred that the H chromosome is the causes of hybrid lethality. In another study of hybrid lethality involving *N. repanda* × *N. tabacum*, a test cross of *N. repanda* with *N. sylvestris* grew normally while another with *N. tomentosiformis* expressed lethality, demonstrating that the T subgenome in *N. tabacum* is responsible for lethality (Kobori and Marubashi 2004).

Reciprocal hybrids from crosses between *N. tabacum* and *N. suaveolens* ($2n = 32$) express hybrid lethality accompanied by programmed cell death (PCD) at 28°C, but not at 36°C (Yamada et al. 2000; Tezuka and Marubashi 2004). Thus, it is considered that hybrid lethality is due to the interaction of coexisting heterogeneous genomes, and not to a cytoplasmic effect. From test crosses involving two progenitors of *N. tabacum*, *N. sylvestris* and *N. tomentosiformis*, with *N. suaveolens*, Inoue et al. (1996) demonstrated that the S subgenome in *N. tabacum* is responsible for lethality. Later, Marubashi and Onosato (2002) carried out a series of crosses between *N. tabacum* monosomic lines of the S subgenome (Haplo-M–Z, except for Haplo-P and Haplo-V) and *N. suaveolens*. In Haplo-Q, they obtained 4 viable hybrids possessing 38 or 39 chromosomes, suggesting that these hybrids lacked the Q chromosome. However, no direct experimental data demonstrates that the Q chromosome of *N. tabacum* is the causes of hybrid lethality involving *N. tabacum* × *N. suaveolens*.

In the present study, we report obtaining viable hybrids at 36°C from crosses between Q-chromosome monosomic lines of *N. tabacum* and *N. suaveolens*. Some of the hybrids expressed hybrid lethality after transfer to 28°C. The analysis of hybrids with Q-chromosome-specific DNA markers provides clear evidence that the presence of the Q chromosome of *N. tabacum* is related to hybrid lethality observed in crosses between *N. tabacum* and *N. suaveolens*.

Materials and methods

Plant materials

For crosses, *N. tabacum* monosomic lines Haplo-Q ($2n = 47$) and F_1 progeny ($2n = 47$; Tezuka et al. 2004) derived from the cross Haplo-Q × *N. tabacum* cv. Samsun NN were used as female parent and *N. suaveolens* ($2n = 32$) was used as male parent. For the conversion of DNA markers to a sequence tagged site (STS), *N. tabacum* cv. Red Russian ($2n = 48$), *N. suaveolens* and two hybrid lines were used. One hybrid line ($2n = 40$) was obtained from the cross Red Russian × *N. suaveolens* by cultivation at 36°C (Tezuka and Marubashi 2004) and the other hybrid line ($2n = 39$) was obtained from the cross Haplo-Q × *N. suaveolens* by cultivation at 28°C (Marubashi and Onosato 2002). The latter line consisted of two hybrid plants designated Q-1 and Q-2, which appear to lack the Q chromosome of *N. tabacum* (Marubashi and Onosato 2002). Q-1 and Q-2 have been maintained by cuttings. We also used *N. sylvestris* ($2n = 24$, SS), *N. tomentosiformis* ($2n = 24$, TT) and *N. otophora* ($2n = 24$, TT) for confirmation of Q chromosome origin. All plants were cultivated under greenhouse conditions except for a hybrid line with 40 chromosomes cultivated in an incubator at 36°C. Haplo-Q was supplied by Dr. T. Kubo, a former director of the Iwata tobacco experiment station of Japan Tobacco Inc. Seeds of Red Russian and wild species were supplied by Japan Tobacco Inc. (Iwata, Japan).

Test-tube pollination and ovule culture were carried out as previously described (Tezuka and Marubashi 2004). Hybrid seedlings obtained by test-tube pollination and ovule culture at 28°C were transferred to fresh 1/2 MS medium supplemented with 1% sucrose and 0.2% Gelrite (pH 5.8) immediately after germination and cultured at 36°C under continuous illumination. After analysis using Q-chromosome-specific DNA markers, the hybrid seedlings were transferred to 28°C under continuous illumination. Hybrid seedlings that did not express lethal symptoms more than 30 days after transfer to 28°C were potted and cultivated under greenhouse conditions.

DNA extraction

Total DNA was extracted from leaves of each plant using a cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980) with minor modifications.

RAPD and ISSR analysis

RAPD and ISSR analysis were carried out as previously described (Tezuka et al. 2004). For RAPD analysis, random primers OPA-06, OPB-07 and OPB-13 (Operon Technologies, Inc., Alameda, CA, USA) were used. For ISSR analysis, unanchored SSR primers SP8-1 5'-$(TGTC)_4$ and SP16-1 5'-$(CGAA)_4$ (Yang et al. 2001) were used. PCR products were separated by electrophoresis in a 1.5% agarose gel in TAE buffer, stained with ethidium bromide and photographed under UV light.

Cloning and sequencing of DNA markers

Cloning and sequencing of DNA markers were carried out as previously described (Tezuka et al. 2004). Briefly, bands specific to the Q chromosome were excised from the gel, reamplified and cloned using the pGEM-T Easy

Vector System (Promega). The correct clone was confirmed by Southern blotting of ISSR fragments amplified from two hybrid lines using the insert as a hybridization probe. The selected clones were sequenced from both ends using T7 and SP6 promoter primers using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Primer design and PCR analysis

Based on the determined sequences, PCR primers were designed using Primer3 software (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Two pairs of primers designed in a previous study (Tezuka et al. 2004) were also used. The primer sequences are shown in Table 1.

For PCR analysis, reaction mixtures contained 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, 20 ng of template DNA, 0.5 U of AmpliTaq DNA polymerase (Applied Biosystems) in a total volume of 20 μl. PCR amplification was performed in the GeneAmp PCR System 2400 (Applied Biosystems) under the following conditions: 3 min at 94°C for initial denaturation, followed by 35 cycles of 30 s at 94°C, 1 min at 60°C, 1 min at 72°C, and a final extension of 5 min at 72°C. For the marker QCS2, the extension time was 2 min. PCR products were separated by electrophoresis in a 1.5% agarose gel in TAE buffer, stained with ethidium bromide and photographed under UV light.

Chromosome analysis

To determine chromosome numbers, root tips were pretreated with distilled water for 24 h at 4°C and with 2 mM 8-hydroxyquinoline for 4 h at 18°C, and fixed in ethanol/acetic acid (3:1) overnight. Then, the root tips were hydrolyzed in 1 N HCl for 8 min at 60°C, stained with Schiff's reagent and squashed in 45% acetic acid. The number of chromosomes in at least 5 root tip cells for each plant was counted under a light microscope (ECLIPSE E600; Nikon, Japan).

Results and discussion

Q-chromosome-specific STS markers

Two Q-chromosome-specific STS markers, designated QCS3 and QCS4 (Table 1), were obtained after cloning and sequencing of $SP8\text{-}1_{1400}$ and $SP16\text{-}1_{500}$ developed in a previous study (Tezuka et al. 2004). PCR analysis with the selected specific primer pairs showed expected amplifications from DNA isolated from *N. tabacum* cv. Red Russian and from a hybrid line with 40 chromosomes, but not from a hybrid line with 39 chromosomes (Fig. 1). This demonstrated that the generated STS markers are specific to the Q chromosome of *N. tabacum* and could be used for its identification together with the QCS1 and QCS2 markers developed in a previous study (Tezuka et al. 2004).

Test-tube pollination and ovule culture

Since pollen of Haplo-Q aborts at a high frequency (Cameron 1959), and the transmission of the monosomic condition through pollen is very low (Olmo 1935), two monosomic lines were used as maternal parents. In the cross combination of *N. tabacum* × *N. suaveolens*, test-tube pollination and ovule culture are necessary to obtain hybrid seedlings because pollen tubes do not reach the base of the style and fertilization does not occur in conventional crossing (Kostoff 1930; Marubashi and Onosato 2002). After test-tube pollination and ovule culture, a total of 11 hybrid seedlings were obtained from the cross Haplo-Q × *N. suaveolens*, and 13 from the cross (Haplo-Q × Samsun NN) × *N. suaveolens* (Table 2). All hybrid seedlings were cultured at 36°C, which suppresses hybrid lethality (Tezuka and Marubashi 2004). Three of the obtained seedlings had abnormal morphology, but the rest had normal morphology similar to that of Q-1 and Q-2 observed earlier (Marubashi and Onosato 2002).

Detection of Q-chromosome-specific DNA markers in hybrids

To investigate whether hybrid seedlings possess the Q chromosome, total DNA was extracted from leaves of

Table 1 Nucleotide sequences of primers for an STS

STS marker	Primer	Sequence	Expected product size (bp)
QCS1[a]	QC11-1	AAAGCTGCGGGGCCCTCAATAA	922
	QC11-2	AAAGCTGCGGCAAATCGACTTA	
QCS2[a]	QG04-1	AGCGTGTCTGGTCAACTGTTTT	1,700
	QG04-2	AGCGTGTCTGCATAGAACAATC	
QCS3	QSP8-1-3	CCACACCCGAGTTTAGGAAA	613
	QSP8-1-4	GTGATTCTGGGCACCAATTT	
QCS4	QSP16-1-3	AAGACGACGCAGCAGTTACA	443
	QSP16-1-4	TTGTCTAGCTGCCATGAACG	

[a]QCS1 and QCS2 were respectively $OPC\text{-}11_{922}$ and $OPG\text{-}04_{1700}$ developed in a previous study (Tezuka et al. 2004).

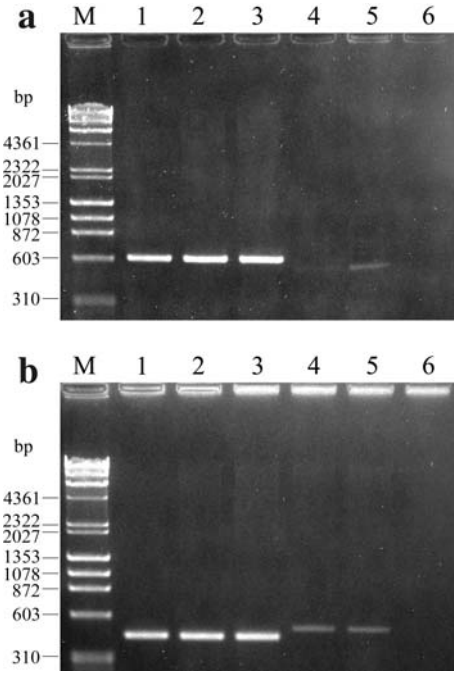

Fig. 1 PCR amplification with QSP8-1-3 and QSP8-1-4 primers (**a**) and QSP16-1-3 and QSP16-1-4 primers (**b**). *M*, DNA markers (λ/*Hin*d III and ϕX174/*Hae* III). *Lane 1*, *N. tabacum* cv. Red Russian; *lanes 2 and 3*, *N. tabacum* cv. Red Russian × *N. suaveolens* cultivated at 36°C; *lane 4*, Q-1; *lane 5*, Q-2; *lane 6*, *N. suaveolens*

hybrid seedlings cultured at 36°C, and PCR was carried out. Two RAPD markers (OPB-07_{870} and OPB-13_{1400}), two ISSR markers (SP8-1_{1400} and SP16-1_{500}) and four STS markers (QCS1, QCS2, QCS3 and QCS4) developed in the present and a previous study (Tezuka et al. 2004) were investigated. For hybrid seedlings from the cross (Haplo-Q × Samsun NN) × *N. suaveolens*, QCS1 was not used because this marker was not detected in monosomic plants from the cross Haplo-Q × Samsun NN (Tezuka et al. 2004). A comparison of the data obtained showed that the Q-chromosome-specific DNA markers analyzed were present or absent in the same manner in the analyzed hybrids. The results from analyses with QCS2 are shown in Fig. 2. As a result of investigation of the Q-chromosome-specific DNA markers, hybrid seedlings could be divided into two groups, 7 hybrids possessing the Q chromosome and 14 hybrids lacking the Q chromosome (Table 3).

We were unable to isolate good-quality DNA from three seedlings with abnormal morphology, which died after transfer from 36°C to 28°C (data not shown).

Table 2 Test-tube pollination and ovule culture

Cross combination	No. of placentas pollinated	No. of ovules cultured	No. of hybrids obtained
Haplo-Q × *N. suaveolens*	104	622	11
(Haplo-Q × Samsun NN) × *N. suaveolens*	80	681	13

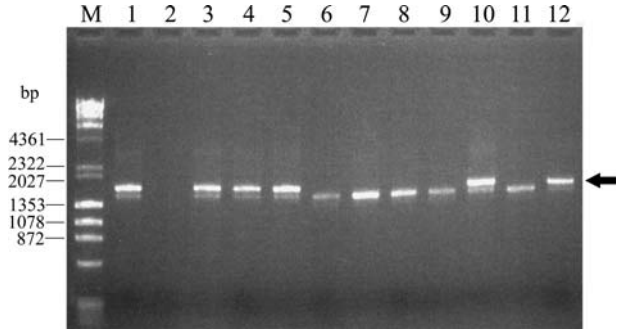

Fig. 2 Detection of the marker QCS2 in hybrid seedlings from the cross Haplo-Q × *N. suaveolens*. The marker QCS2 is indicated by an *arrow*. *M* DNA markers (λ/*Hin*d III and ϕX174/*Hae* III). *Lane 1*, Haplo-Q; *lane 2*, *N. suaveolens*; *lanes 3–12*, hybrid seedlings from the cross Haplo-Q × *N. suaveolens*

The relationship between the Q chromosome and hybrid lethality

To investigate the relationship between possession of the Q chromosome and hybrid lethality, hybrid seedlings cultured at 36°C were transferred to 28°C. As a result, all 7 hybrid seedlings possessing the Q chromosome expressed hybrid lethality and died 20 days after transfer (Table 3, Fig. 3a). The other 14 hybrid seedlings lacking the Q chromosome did not express hybrid lethality after transfer (Table 3, Fig. 3b). In this study, the number of hybrids possessing the Q chromosome and those lacking the Q chromosome deviated from the expected 1:1 ratio, even if the three hybrid seedlings with abnormal morphology had the Q chromosome. Some kind of barrier, such as hybrid lethality expressed in hybrid embryos (Inoue et al. 2000), may exist during ovule development.

When hybrid seedlings lacking the Q chromosome were potted and cultivated under greenhouse conditions, all of them grew to maturity and flowered (Fig. 4a). Based on morphological characteristics, we confirmed that all viable hybrids were true hybrids, although the

Table 3 Relationship between the Q chromosome and hybrid lethality observed in the cross between monosomic lines of *N. tabacum* lacking one Q chromosome and *N. suaveolens*

Cross combination	DNA markers[a]	No. of hybrids		
		Total	Viable	Lethal
Haplo-Q × *N. suaveolens*	+	5	0	5
	–	5	5	0
(Haplo-Q × Samsun NN) × *N. suaveolens*	+	2	0	2
	–	9	9	0
Total	+	7	0	7
	–	14	14	0

[a]The number of DNA markers used was 8 for Haplo-Q × *N. suaveolens* and 7 for (Haplo-Q × Samsun NN) × *N. suaveolens*. "+" indicates that DNA markers were detected and "–" indicates that DNA markers were not detected

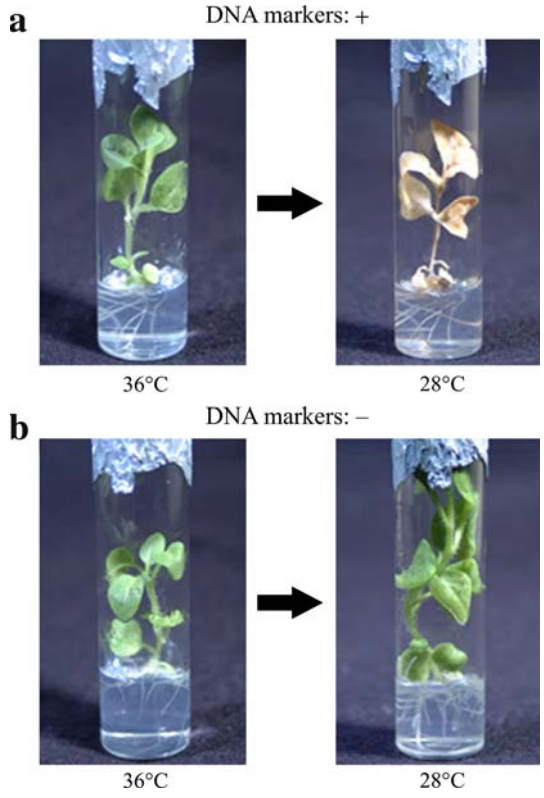

Fig. 3 Appearance of hybrid seedlings from the cross Haplo-Q × *N. suaveolens* after transfer from 36°C to 28°C. Hybrid seedlings were cultured at 36°C for 30 DAG and then transferred to 28°C. When a hybrid seedling in which DNA markers were detected was transferred to 28°C, the seedling expressed hybrid lethality and had died 20 days after transfer from 36°C to 28°C (**a**). When a hybrid seedling in which DNA markers were not detected was transferred to 28°C, the seedling did not express hybrid lethality and had survived 20 days after transfer (**b**)

flower color of hybrids from the cross (Haplo-Q × Samsun NN) × *N. suaveolens* was pink or deep pink, possibly due to the heterozygosity of monosomic plants used as the parent. Furthermore, all viable hybrids had 39 chromosomes, suggesting that viable hybrids had haploid chromosomes of *N. tabacum*, except for the Q chromosome, and haploid chromosomes of *N. suaveolens* (Fig. 4b–d). Therefore, we concluded that presence of the Q chromosome causes hybrid lethality.

The results of the present study raise two possibilities. First, the Q chromosome is the only factor in *N. tabacum* responsible for hybrid lethality and hybrid lethality is caused by the interaction between the Q chromosome and the genome of *N. suaveolens*. The second possibility is that hybrid lethality is caused by the coexistence of the Q chromosome and one or more other chromosomes belonging to the S subgenome of *N. tabacum* and the genome of *N. suaveolens*. We cannot determine which hypothesis is correct since the number of genes that cause hybrid lethality is still unclear. Previously, Marubashi and Onosato (2002) reported that only Haplo-Q among *N. tabacum* monosomic lines of the S subgenome produced viable hybrids from a cross with *N. suaveolens*. Furthermore, in other plants, there are many examples of hybrid lethality caused by two complementary genes of nuclear genomes (Hollingshead 1930; Sawant 1956; Oka 1957; Hermsen 1963; Takahashi et al. 1970; Shii et al. 1980; Lee 1981). Therefore, we speculate that hybrid lethality observed in the cross between *N. tabacum* and *N. suaveolens* is more likely to be caused by the interaction between the Q chromosome and the genome of *N. suaveolens*. In this regard, further study will be needed to determine whether hybrid lethality of this cross combination is caused by two complementary factors.

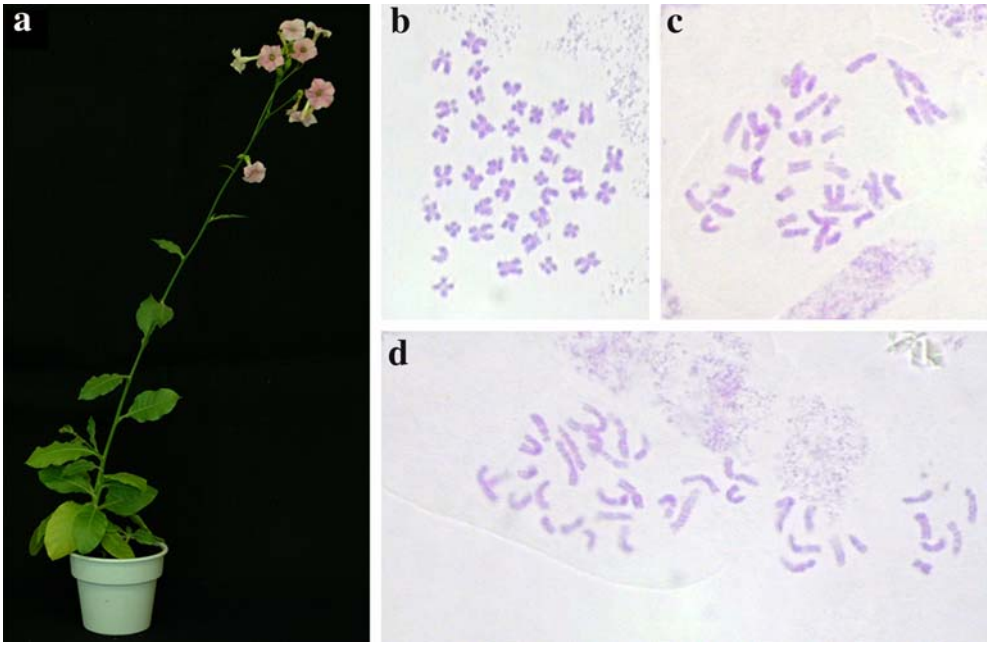

Fig. 4 Cytology of hybrids that did not express hybrid lethality. Hybrid seedlings lacking the Q chromosome grew to maturity and flowered (**a**). All of them had 39 chromosomes (**b**–**d**). **a**, **b** Hybrid plant from the cross Haplo-Q × *N. suaveolens*. **c** Hybrid plant from the cross (Haplo-Q × Samsun NN) × *N. suaveolens* with deep pink flowers. **d** Hybrid plant from the cross (Haplo-Q × Samsun NN) × *N. suaveolens* with pink flowers

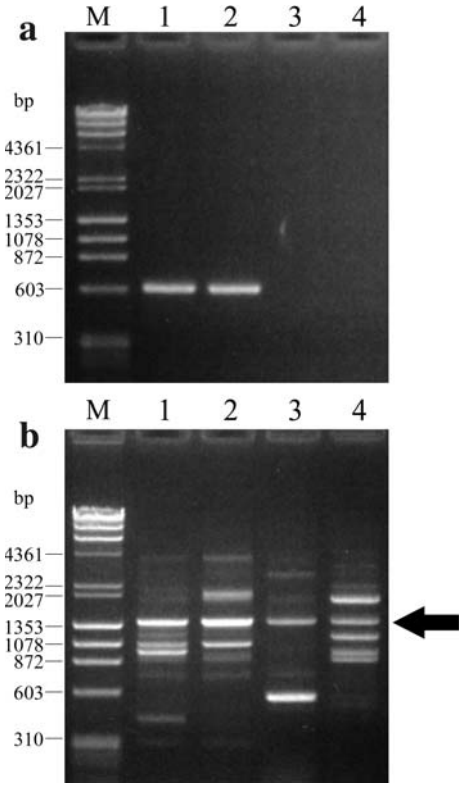

Fig. 5 Detection of markers QCS3 (**a**) and OPB-13_{1400} (**b**) in progenitors of *N. tabacum*. The marker OPB-13_{1400} is indicated by an *arrow*. *M*, DNA markers (λ/*Hin*d III and ϕX174/*Hae* III). *Lane 1*, *N. tabacum* cv. Red Russian; *lane 2*, *N. sylvestris*; *lane 3*, *N. tomentosiformis*; *lane 4*, *N. otophora*

Confirmation that the Q chromosome belongs to the S subgenome of *N. tabacum*

The Q chromosome of *N. tabacum* was assigned to the S subgenome by observation of chromosome pairing in hybrids (2*n*-1) from the cross Haplo-Q × *N. sylvestris* (Clausen and Cameron 1944). To confirm that the Q chromosome belongs to the S subgenome, we determined whether Q-chromosome-specific DNA markers could be detected in *N. sylvestris*, *N. tomentosiformis* and *N. otophora*. When eight Q-chromosome-specific DNA markers (OPB-07_{870}, OPB-13_{1400}, SP8-1_{1400}, SP16-1_{500}, QCS1, QCS2, QCS3 and QCS4) were tested, all of them except OPB-13_{1400} were detected in *N. sylvestris* and not in *N. tomentosiformis* or *N. otophora*. Results obtained with the marker QCS3 are shown in Fig. 5a as an example. For OPB-13_{1400}, bands that were about the same size as OPB-13_{1400} were detected in *N. tomentosiformis* and *N. otophora* (Fig. 5b). This result puzzled us since OPB-13_{1400} was not detected in Q-1 and Q-2, which had the T subgenome (Tezuka et al. 2004). Although it is not clear whether the sequences of the bands detected in *N. tomentosiformis* and *N. otophora* are identical with those in *N. tabacum* and *N. sylvestris*, this result suggests that similar sequences are present in both the S and T genomes.

Results of the present study using progenitors of *N. tabacum* and Q-chromosome-specific DNA markers support the hypothesis that the Q chromosome belongs to the S subgenome of *N. tabacum*. For further confirmation, characterization and identification of the Q chromosome would be needed.

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References

Cameron DR (1959) The monosomics of *Nicotiana tabacum*. Tob Sci 3:164-166

Christoff M (1928) Cytological studies in the genus *Nicotiana*. Genetics 13:233–277

Clausen RE, Cameron DR (1944) Inheritance in *Nicotiana tabacum*. XVIII. Monosomic analysis. Genetics 29:447–477

Gerstel DU, Burns JA, Burk LG (1979) Interspecific hybridizations with an African tobacco, *Nicotiana africana* Merxm. J Hered 70:342–344

Gray JC, Kung SD, Wildman SG, Sheen SJ (1974) Origin of *Nicotiana tabacum* L. detected by polypeptide composition of Fraction I protein. Nature 252:226–227

Hermsen JGTh (1963) Hybrid necrosis as a problem for the wheat breeder. Euphytica 12:1–16

Hollingshead L (1930) A lethal factor in *Crepis* effective only in an interspecific hybrid. Genetics 15:114–140

Inoue E, Marubashi W, Niwa M (1996) Genomic factors controlling the lethality exhibited in the hybrid between *Nicotiana suaveolens* Lehm. and *N. tabacum* L. Theor Appl Genet 93:341–347

Inoue E, Marubashi W, Niwa M (2000) Characterization and overcoming of temperature-dependent lethality exhibited by hybrid embryos from the cross *Nicotiana suaveolens* × *N. sylvestris*. Breed Sci 50:297–302

Kobori S, Marubashi W (2004) Programmed cell death detected in interspecific hybrids of *Nicotiana repanda* × *N. tomentosiformis* expressing hybrid lethality. Breed Sci 54:347–350

Kostoff D (1930) Ontogeny, genetics, and cytology of *Nicotiana* hybrids. Genetica 12:33–139

Lee JA (1981) Genetics of D_3 complementary lethality in *Gossypium hirsutum* and *G. barbadense*. J Hered 72:299–300

Lim KY, Matyášek R, Lichtenstein CP, Leitch AR (2000) Molecular cytogenetic analyses and phylogenetic studies in the *Nicotiana* section Tomentosae. Chromosoma 109:245–258

Marubashi W, Onosato K (2002) Q chromosome controls the lethality of interspecific hybrids between *Nicotiana tabacum* and *N. suaveolens*. Breed Sci 52:137–142

Murad L, Lim KY, Christopodulou V, Matyasek R, Lichtenstein CP, Kovarik A, Leitch AR (2002) The origin of tobacco's T genome is traced to a particular lineage within *Nicotiana tomentosiformis* (Solanaceae). Am J Bot 89:921–928

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Oka H (1957) Phylogenetic differentiation of cultivated rice. XV. Complementary lethal genes in rice. Jpn J Genet 32:83–87

Olmo HP (1935) Genetical studies of monosomic types of *Nicotiana tabacum*. Genetics 20:286–300

Sawant AC (1956) Semilethal complementary factors in a tomato species hybrid. Evolution 10:93–96

Sheen SJ (1972) Isozymic evidence bearing on the origin of *Nicotiana tabacum* L. Evolution 26:143–154

Shii CT, Mok MC, Temple SR, Mok DWS (1980) Expression of developmental abnormalities in hybrids of *Phaseolus vulgaris* L. Interaction between temperature and allelic dosage. J Hered 71:218–222

Stebbins GL (1966) Reproductive isolation and the origin of species. In: Processes of organic evolution. Prentice-Hall, New Jersey, pp 85–112

Takahashi R, Hayashi J, Moriya I (1970) Studies on lethal seedlings of barley by complementary genes. I. Mode of inheritance and the geographical distribution of the lethal genes. Nogaku Kenkyu 53:197–204

Tezuka T, Marubashi W (2004) Apoptotic cell death observed during the expression of hybrid lethality in interspecific hybrids between *Nicotiana tabacum* and *N. suaveolens*. Breed Sci 54:59–66

Tezuka T, Onosato K, Hijishita S, Marubashi W (2004) Development of Q-chromosome-specific DNA markers in tobacco and their use for identification of a tobacco monosomic line. Plant Cell Physiol 45:1863–1869

Yamada T, Marubashi W, Niwa M (2000) Apoptotic cell death induces temperature-sensitive lethality in hybrid seedlings and calli derived from the cross of *Nicotiana suaveolens* × *N. tabacum*. Planta 211:614–622

Yang D, Naito Y, Ishikawa M, Kojima T, Tazuke A, Niwa M (2001) Abundant DNA markers detected by PCR using simple sequence repeat primers without anchor sequences in soybean. Breed Sci 51:207–210