Plant Biotechnology 30, 179–184 (2013)
DOI: 10.5511/plantbiotechnology.13.0312a

Short Communication

Hybrid lethality with programmed cell death results from reciprocal interspecific crosses between *Nicotiana nudicaulis* Watson and *N. tabacum* L.

Hongshuo Liu, Misato Hoshio, Yusuke Masaki, Yojiro Mori, Wataru Marubashi*

Graduate School of Agriculture, Meiji University, Kawasaki, Kanagawa 214-8571, Japan
*E-mail: marubasi@isc.meiji.ac.jp Tel & Fax: +81-44-934-7811

Received November 12, 2012; accepted March 12, 2013 (Edited by M. Otani)

Abstract Hybrid seedlings from reciprocal interspecific crosses between *Nicotiana nudicaulis* and *N. tabacum* were not viable when cultured at 28°C; this an example of hybrid lethality. Characteristically, shoot apices and root tips of these hybrids began to brown immediately after germination at 28°C. However, hybrid seedlings did not exhibit any symptoms of lethality at 34°C, and their growth was normal at this higher temperature. When hybrid seedlings were cultured at 34°C and then transferred to 28°C, lethal symptoms emerged rapidly after the transfer. Therefore, hybrid lethality associated with reciprocal interspecific crosses between *N. nudicaulis* and *N. tabacum* was due to the interaction of coexisting heterologous genomes and not to a cytoplasmic effect. Furthermore, as the *N. nudicaulis*×*N. tabacum* seedlings die, features of apoptotic cell death, including chromatin condensation, nuclear fragmentation, and fragmentation of DNA, were evident. These observations indicated that hybrid lethality of *N. nudicaulis*×*N. tabacum* seedlings is accompanied by apoptotic cell death. This report is the first to demonstrate that hybrid lethality with apoptotic cell death results from *N. nudicaulis*×*N. tabacum* hybridization.

Key words: Hybrid lethality, apoptotic cell death, reciprocal interspecific crosses, *Nicotiana nudicaulis*, *N. tabacum*.

Reproductive isolation is classified into two types, prezygotic or postzygotic, based on barriers to hybridization. Hybrid lethality is a postzygotic mechanism of reproductive isolation and it is a major factor in speciation within both animal and plant lineages. In plants, hybrid lethality, which can occur in intergeneric, interspecific and intraspecific crosses, is an important obstacle in breeding programs (Stebbins 1966; Tezuka 2012).

In the genus *Nicotiana* (*Solanaceae*), several interspecific hybrids between the cultivated tobacco, *N. tabacum* and any of several wild tobacco strains exhibit hybrid lethality. The genus *Nicotiana* includes 75 species that were classified into 13 sections (Knapp et al. 2004). Most species within 12 of these 13 sections are distributed mainly in the Americas; only the species within the section *Suaveolentes* are commonly found outside the Americas. The section *Suaveolentes* includes 26 species, 20 of which produce nonviable hybrids in crosses with *N. tabacum* (Tezuka 2012).

Hybrid seedlings from reciprocal interspecific crosses between *N. suaveolens* (section *Suaveolentes*) and *N. tabacum* express lethality (Manabe et al. 1989; Tezuka and Marubashi 2004), which is accompanied by programmed cell death (PCD) (Tezuka and Marubashi 2004; Yamada et al. 2000). Furthermore, the Q chromosome belonging to the S subgenome of *N. tabacum* (SSTT) is responsible for this hybrid lethality (Inoue et al. 1996; Marubashi and Onosato 2002). Recently, the location of the gene(s) on the Q chromosome responsible for hybrid lethality was inferred by Tezuka et al. (2012).

Similarly, seedlings from reciprocal interspecific crosses of *N. debneyi* (section *Suaveolentes*)×*N. tabacum* also express lethality and PCD accompanies this lethality (Marubashi and Kobayashi 2002; Tezuka et al. 2006). This hybrid lethality was controlled by the interaction between a single dominant gene, *Hybrid Lethality A1* (*HLA1*), in *N. debneyi* and gene(s) on the Q chromosome in *N. tabacum* (Iizuka et al. 2011; Tezuka et al. 2007).

N. repanda, which is found in North America, is closely related to *N. nudicaulis* (Clarkson et al. 2004). In crosses between *N. repanda* and *N. tabacum*, hybrid lethality was observed (Reed and Collins 1978). Furthermore, Kobori and Marubashi (2004) concluded that the T subgenome encoded the causative gene(s) for hybrid lethality in this cross.

N. nudicualis×*N. tabacum* crosses also result in hybrid lethality (Yamada et al. 1999). However, an interaction of coexisting heterologous genomes leading to hybrid

This article can be found at http://www.jspcmb.jp/
Published online May 22, 2013

lethality has not been reported in the interspecific crosses of *N. nudicaulis*×*N. tabacum*. In this study, we investigated hybrid lethality in seedlings from *N. nudicaulis*×*N. tabacum* crosses and the accompanying apoptotic cell death; we also examined the hybrid lethality in seedlings from *N. tabacum*×*N. nudicaulis* crosses.

The seeds of *N. nudicaulis* Watson ($2n=48$) and *N. tabacum* ($2n=48$, SSTT) 'Red Russian' were used in this experiment and were supplied by Japan Tobacco Inc. (Oyama, Japan). Plants were grown and pollinated in the greenhouse of the School of Agriculture, Meiji University. Flower buds of *N. nudicaulis* were emasculated just before flowering and were pollinated with fresh pollen from *N. tabacum*.

F_1 seeds (*N. nudicaulis*×*N. tabacum*) were soaked in a 0.05% gibberellic acid (GA_3) solution for 30 min, sterilized with 5% sodium hypochlorite for 15 min and then rinsed three times with sterilized water. Sterilized F_1 seeds were sown on Petri dishes containing 8 ml of 1/2 MS medium (Murashige and Skoog 1962) supplemented with 1% sucrose and 0.25% Gelrite (Wako), pH 5.8; the plates were maintained at 28°C under continuous illumination (32 μmol s^{-1} m^{-2}) for seed germination.

Immediately after germination, several seedlings were transferred to flat-bottomed test tubes (25-mm diameter, 100-mm length) that contained 10 ml of 1/2 MS medium supplemented with 1% sucrose and 0.25% Gelrite, pH 5.8; these seedlings were cultured at 34°C under continuous illumination (87 μmol s^{-1} m^{-2}). Seedlings cultured at 34°C for 30–40 DAG (days after germination) were then transferred to 28°C under continuous illumination (32 μmol s^{-1} m^{-2}) to induce hybrid lethality.

Because no fertile seeds were obtained *in situ* from *N. tabacum*×*N. nudicaulis* crosses, test tube pollination and ovule culture were necessary to obtain these hybrid seedlings. Test tube pollination and ovule culture were carried out as described by Marubashi and Nakajima (1985). Anthers of *N. nudicaulis* were aseptically excised from still-closed flowers and stimulated to dehisce in an incubator (28°C). Flowers of *N. tabacum* were emasculated one day before anthesis; on the next day, the flowers were collected and their sepals, petals, and styles removed. The ovaries were surface-sterilized with 70% ethanol for 30 s and then with 5% sodium hypochlorite for 10 min. The ovary walls were peeled to expose the placenta with intact ovules and then the placentas were placed on 1/2 MS medium with 3% (w/v) sucrose. Pollen of *N. nudicaulis* was spread on the surface of the placenta. Pollinated placentas were maintained at 28°C under continuous illumination (32 μmol s^{-1} m^{-2}).

Enlarged ovules were excised 8 days after test tube pollination and cultured on 1/2 MS medium at 28°C under continuous illumination (32 μmol s^{-1} m^{-2}). The hybrid seedlings ($n=32$) were transferred to 34°C and seven hybrid seedlings were left at 28°C immediately after germination; 25 hybrid seedlings that had been cultured at 34°C for 10 days were transferred to flat-bottomed test tubes that contained 10 ml of 1/2 MS medium supplemented with 1% sucrose and 0.25% Gelrite, pH 5.8.

For detection of apoptotic changes of nuclei, protoplasts were isolated from the leaves of hybrid seedlings of *N. nudicaulis*×*N. tabacum*. Leaves were sectioned and treated with an enzyme solution containing 2% (w/v) cellulase Onozuka R-10 (Yakult), 0.2% (w/v) Macerozyme R-10 (Yakult), 0.7 M mannitol, and 10 mM $CaCl_2$, pH 5.6 for 3 h at 30°C. Protoplasts were separated from cellular debris with a 48-μm nylon mesh. After the mixture was allowed to settle for 2 h on ice, the supernatant was discarded and the protoplast pellet was fixed in a fixative solution that was a 9-to-1 mixture of 0.7 M mannitol and formaldehyde solution. Protoplasts were stained with 5 μl/ml 4′-6-diaminio-2-phenylindole dihydrochloride (DAPI), observed under a fluorescence microscope (BX51; Olympus) with U excitation (330–385 nm) and photographed using an automatic photomicrography system (DP70; Olympus).

For cytometric analysis, nuclei were isolated from leaves of *N. nudicaulis*×*N. tabacum* hybrid seedlings that had been transferred from 34 to 28°C; the leaves were chopped off and macerated in ice-cold buffer (Michaelson et al. 1991). The macerated tissue was then filtered through a 25-μm nylon mesh. The nuclei were collected from the filtrate by centrifugation (5 min at 3000 rpm and 4°C) and then suspended in ice-cold buffer supplemented with 5 μl/ml DAPI for 1 min at 4°C. The DNA content of the isolated nuclei was analyzed by flow cytometry (Cell Lab Quanta SC system, Beckman Coulter). Between 20,000 and 22,000 nuclei were counted.

For detection of DNA fragmentation, genomic DNA was extracted from the leaves of the hybrid seedlings according to a slightly modified version of the method of LoSchiavo et al. (2000). DNA was separated by electrophoresis on a 1.5% (w/v) agarose gel in TAE buffer and visualized using ethidium bromide under UV light.

Hybrid seeds from *N. nudicaulis*×*N. tabacum* crosses were sown in 1/2 MS medium and germinated at 28°C. Immediately after germination, *N. nudicaulis*×*N. tabacum* hybrid seedlings were cultured at 28°C or 34°C. Symptoms of lethality, including browning of the shoot apex and the root tip, were observed in hybrid seedlings cultured at 28°C at an early stage (Figure 1). By 2 DAG, the unfolding cotyledons had turned yellow-green (Figure 1A) and they had died by 4 DAG (Figure 1B). Yamada et al. (1999) classified hybrid lethality in the genus *Nicotiana* into four types: Type I, browning of shoot apex and root tips; Type II, hypocotyls and roots; Type III, yellowing of true leaves; and Type IV, formation

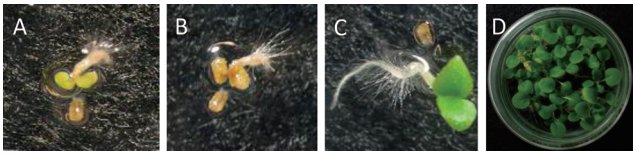

Figure 1. Hybrid seedlings from a *N. nudicaulis*×*N. tabacum* cross. (A and B) Lethal symptoms observed in hybrid seedlings cultured at 28°C for 2 DAG (A) and 4 DAG (B). (C and D) Hybrid seedlings without any lethal symptoms cultured at 34°C for 2 DAG (C) and 21 DAG (D).

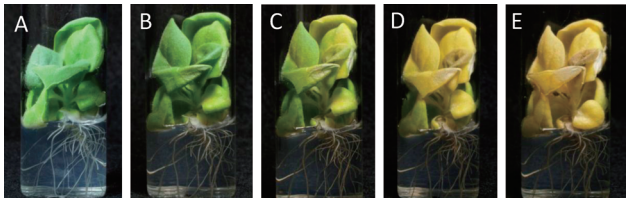

Figure 2. Morphological changes in hybrid seedlings from a *N. nudicaulis*×*N. tabacum* cross. (A) A seedling cultured at 34°C for 40 DAG grew without any lethal symptoms. (B) A seedling transferred to 28°C from 34°C and cultured for 3 days at 28°C stopped growing and the stem turned yellow. (C) A seedling cultured at 28°C for 5 days had yellow tissue in some leaves. (D) A seedling cultured at 28°C for 7 days had yellow tissue in all the leaves. (E) A seedling cultured at 28°C for 10 days dried up and died.

of multiple shoots. Based on the lethal symptoms observed in seedlings of *N. nudicaulis*×*N. tabacum*, the lethality is indicative of Type I lethality.

These symptoms were not evident in the seedlings that were cultured at 34°C (Figure 1C); in fact, seedlings grown at 34°C did not show any symptoms of lethality at 21 DAG (Figure 1D). Hybrid seedlings were cultured at 34°C for 40 DAG (Figure 2A); they were then shifted to 28°C and cultured. Subsequently, their growth stopped and lethal symptoms were also expressed. About 3 days after transfer from 34 to 28°C, the stems and roots began to turn yellow (Figure 2B). Within 5 days of transfer from 34 to 28°C, the leaves became frail and began to turn yellow (Figure 2C); all of the leaves had turned yellow by 7 days after transfer from 34 to 28°C (Figure 2D). The tip of the leaves from hybrid seedlings finally died by 10 days after transfer from 34 to 28°C (Figure 2E).

In this study, growth of hybrid seedlings was normal at 34°C; therefore, hybrid lethality was suppressed at 34°C and induced at 28°C.

Protoplasts isolated from the leaves of *N. nudicaulis*×*N. tabacum* hybrid seedling were examined and each protoplast stained with DAPI to show structural changes in the nucleus (Figure 3). Normal chromatin structure was evident in the protoplasts isolated from the leaves of hybrid seedlings cultured at 34°C (Figure 3A). In contrast, chromatin condensation was observed in the protoplasts isolated from the hybrid seedlings that had been cultured at 28°C for 3 days (Figure 3B). Nuclear fragmentation was evident in the protoplasts isolated from the leaves of hybrid seedlings that had been cultured at 28°C for 10 days (Figure 3C).

Nuclei were isolated from the leaves of *N. nudicaulis*×*N. tabacum* hybrid seedlings. Fragmented nuclei were further evaluated by flow cytometry. In

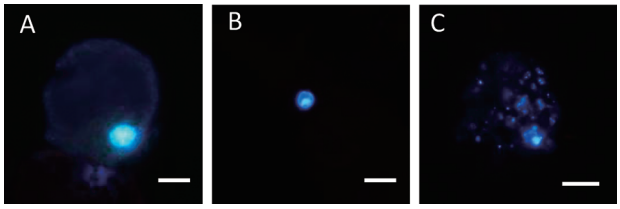

Figure 3. Progressive changes in the nuclear structure detected in protoplasts stained with DAPI. (A) Normal mesh-like structure of chromatin in green leaves of a hybrid seedling cultured at 34°C for 40 DAG. (B) Chromatin condensation in green leaves of a hybrid seedling cultured at 28°C for 3 days. (C) Nuclear fragmentation in yellow leaves of a hybrid seedling cultured at 28°C for 10 days. Scale bars are 50 μm.

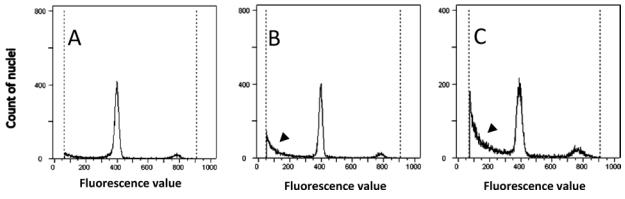

Figure 4. Histograms indicating increased fragmentation of nuclei in leaves of hybrid seedlings; the DNA content of 20000–22000 nuclei was determined by flow cytometry. (A) The two peaks were characteristic of G1 nuclei and G2 nuclei. (B) Additional peaks (arrowheads) with lower fluorescence values than that for G1 peak. (C) The areas under the additional peaks (arrowheads) increased further and the G1 peak decreased in size.

histograms of DAPI fluorescence values, the sample of leaf cells from normal hybrid seedlings (those cultured at 34°C) showed two peaks that presumably corresponded to nuclei in the G1 and the G2/M phases of the cell cycle (Figure 4A). When hybrid seedlings were transferred to and cultured at 28°C for 5 days, the G1 peak decreased slightly relative to the peak from seedlings grown at 34°C (Figure 4B). Within 10 days after transfer to 28°C,

additional peaks (arrowheads) became evident and the area of the G1 peak decreased further (Figure 4C).

Agarose gel analysis of DNA isolated from the hybrids was performed to determine whether fragmentation of DNA had occurred. DNA that was isolated from leaves of *N. nudicaulis*×*N. tabacum* that had been cultured at 34°C was separated by electrophoresis. Though these hybrid seedlings did not show morphological evidence of hybrid lethality, a distinctive ladder pattern was detected in the isolated DNA (Figure 5, lane 1). A ladder pattern was also observed in DNA extracted from the hybrid seedlings 3, 5, 7, or 10 days after transfer from 34 to 28°C (Figure 5, lanes 2–5). This ladder pattern is characteristic of internucleosomal fragmentation of DNA. The intensity of the ladder pattern was less prominent in hybrid seedlings 10 days after transfer than in seedlings 3, 5, or 7 days after transfer (Figure 5, lane 5).

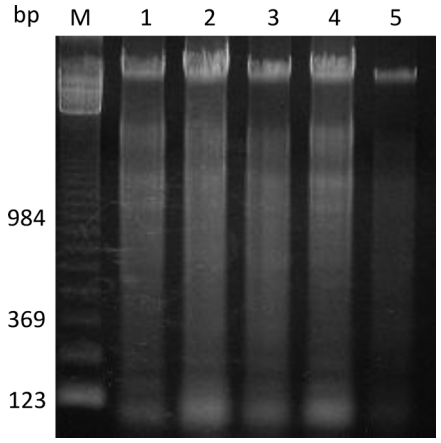

Figure 5. DNA fragmentation in the hybrid seedlings of *N. nudicaulis*×*N. tabacum*. Total DNA was extracted from the leaves of the seedlings. M: 123 bp DNA ladder maker, Lane 1: seedlings cultured at 34°C for 40 DAG, Lane 2, 3, 4, and 5: seedlings transferred to 28°C for 3, 5, 7, or 10 days, respectively.

Nuclear fragmentation and cleavage of nuclear DNA into oligonucleosomal fragments are considered the key characteristics of apoptotic cell death (Ryerson and Heath 1996; Wang et al. 1996). In the present study, these features were detected in seedlings of *N. nudicaulis*×*N. tabacum* expressing hybrid lethality. This report is the first to demonstrate that hybrid lethality resulting from *N. nudicaulis*×*N. tabacum* crosses is accompanied by apoptotic cell death.

In previous studies (Marubashi and Kobayashi 2002; Yamada et al. 2000), DNA laddering was not observed in DNA isolated from *N. suaveolens*×*N. tabacum* seedlings or *N. debneyi*×*N. tabacum* seedlings that had been cultured at 36°C and did not exhibit any lethal symptoms. In the present study, *N. nudicaulis*×*N. tabacum* hybrid seedlings were cultured at 34°C and their growth was normal; interestingly, internucleosomal fragmentation of DNA was detected in these seedlings, but chromatin condensation and nuclear fragmentation were not. These findings differ from those of *N. suaveolens*×*N. tabacum* crosses and *N. debneyi*×*N. tabacum* crosses.

When the *N. tabacum*×*N. nudicaulis* crosses were carried out via conventional cross-pollination, the flowers of *N. tabacum* dropped within 5 days; this means that there was no fertilization or embryogenesis. To obtain hybrid seedlings, test-tube pollination and ovule culture were performed. Placentas (n=15) were pollinated in 1/2 MS medium supplemented with 3%

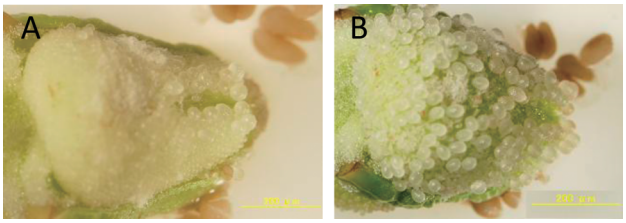

Figure 6. Fertilized ovules through the test tube pollination in the cross of *N. tabacum*×*N. nudicaulis*. (A) Fertilized ovules started to develop on the placentas 5 days after test tube pollination. (B) Fertilized ovules were enlarged 8 days after test tube pollination. Scale bars are 200 μm.

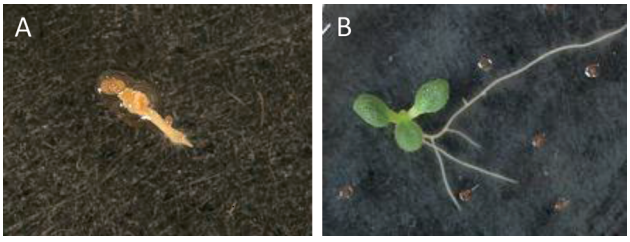

Figure 7. Hybrid seedling of *N. tabacum*×*N. nudicaulis* cultured at 28°C and 34°C. (A) A hybrid seedling of *N. tabacum*×*N. nudicaulis* was cultured at 28°C after germination. (B) A hybrid seedling of *N. tabacum*×*N. nudicaulis* was cultured at 34°C after germination.

Table 1. Viability of hybrid seedlings in the cross of *N. tabacum*×*N. nudicaulis* at 28°C.

Culture temperature	No. of placentas pollinated	No. of ovules cultured	No. of hybrids germinated	No. of hybrids cultured	No. of hybrids*		
					Viable	Non-viable	Vitrified
28°C	15	250	32	7	0	7	0
34°C	—	—	—	25	21	0	4

* Counted at 10 DAG.

Figure 8. Morphological changes in a hybrid seedling of *N. tabacum*×*N. nudicaulis* transferred to 28°C for 40 DAG. (A) The seedling was cultured at 34°C for 40 DAG. (B) By 3 days after transfer to 28°C, the stem of the seedling began to turn brown. (C) By 5 days after transfer to 28°C, the leaves began to turn yellow. (D) By 7 days after transfer to 28°C, the leaves of the seedling began to turn brown. (E) By 15 days after transfer to 28°C, the entire seedling turned brown and died.

(w/v) sucrose and 0.25% (w/v) Gelrite, pH 5.8 at 28°C. Fertilized ovules started to develop on the placentas 5 days after pollination and enlarged gradually (Figure 6A and B). Thereafter, 250 ovules were obtained 8 days after pollination, and 32 of them began to germinate during the 3 weeks after ovule culture (Table 1). To confirm the expression of hybrid lethality, seven hybrid seedlings were left at 28°C (Table 1); each of these seedlings died 2 DAG (Figure 7A). These symptoms of the dying seedlings were quite similar to those observed in seedlings from *N. nudicaulis*×*N. tabacum* crosses (Figure 7). To suppress lethality, 25 hybrid seedlings were transferred to 34°C immediately after germination. No evidence of hybrid lethality was observed in these hybrid seedlings (Figure 7B). Of the 25 hybrid seedlings, 21 showed normal growth and four hybrids showed vitrification within 10 DAG (Table 1).

To confirm that the lethality of the hybrids from the *N. tabacum*×*N. nudicaulis* crosses was temperature dependent, 21 normal hybrid seedlings were transferred at 40 DAG to flat-bottomed test tubes that contained 10 ml of 1/2 MS medium supplemented with 1% (w/v) sucrose and 0.25% (w/v) Gelrite, pH 5.8 (Figure 8A). Subsequently, these hybrid seedlings were transferred to 28°C. Lethal symptoms were expressed at 3 days after transfer to 28°C (Figure 8B). The stems and tip of leaves began to turn yellow 5 days after transfer (Figure 8C). The leaves turned yellow-green or yellow 7 days after transfer and then turned brown (Figure 8D); these hybrid seedlings finally died within 15 days after transfer (Figure 8E). In these seedlings, hybrid lethality was also expressed at 28°C and suppressed at 34°C. This lethality was the characteristic Type I that is observed in crosses of *N. nudicaulis*×*N. tabacum* immediately after germination. These results support the idea that hybrid lethality in this cross-combination is temperature sensitive and genetically controlled by nuclear genomes, not cytoplasmic factors.

Currently, we are attempting to perform the reciprocal crosses *N. nudicaulis* ($2n=48$)×*N. tomentosiformis* ($2n=24$, TT) and *N. nudicaulis* ($2n=48$)×*N. sylvestris* ($2n=24$, SS) to determine which subgenome of *N. tabacum* is responsible for this hybrid lethality.

Acknowledgments

We thank Japan Tobacco, Inc. (Oyama, Japan) for providing seeds from cultivated and wild species of the genus *Nicotiana* and Dr. N. Kadotani, former Professor of Tamagawa University, for helpful discussions. We are grateful to Dr. T. Yamada, Associate Professor at Tokyo University of Agriculture and Technology, and Dr. T. Tezuka, Assistant Professor at Osaka Prefecture University, for technical advice.

References

Clarkson JJ, Knapp S, Garcia VF, Olmstead RG, Leitch AR, Chase MW (2004) Phylogenetic relationships in Nicotiana (Solanaceae) inferred from multiple plastid DNA regions. *Mol Phylogenet Evol* 33: 75–90

Iizuka T, Kuboyama T, Marubashi W, Oda M, Tezuka T (2011) *Nicotiana debneyi* has a single dominant gene causing hybrid lethality in the cross with *N. tabacum*. *Euphytica* 186: 321–328

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

Knapp S, Chase MW, Clarkson JJ (2004) Nomenclatural changes and a new sectional classification in *Nicotiana* (Solanaceae). *Taxon* 53: 73–82

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

LoSchiavo F, Baldan B, Compagnin D, Ganz R, Mariani P, Terzi M (2000) Spontaneous and induced apoptosis in embryogenic cell cultures of carrot (*Daucus carota* L.) in different physiological states. *Eur J Cell Biol* 79: 294–298

Manabe T, Marubashi W, Onozawa Y (1989) Temperature-dependent conditional lethality in interspecific hybrids between *Nicotiana suaveolens* Lehm. and *N. tabacum* L. *Proc 6th Internatl Congr of SABRAO*: 459–462

Marubashi W, Kobayashi M (2002) Temperature-dependent apoptosis detected in hybrids between *Nicotiana debneyi* and *N. tabacum* expressing lethality. *Plant Biotechnol* 19: 267–270

Marubashi W, Nakajima T (1985) Overcoming cross-incompatibility between *Nicotiana tabacum* L. and *N. rustica* L. by test tube pollination and ovule culture. *Jpn J Breed* 35: 429–437

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

Michaelson MJ, Price HJ, Ellison JR, Johnston JS (1991) Comparison of plant DNA contents determined by Feulgen microspectrophotometry and laser flow cytometry. *Am J Bot* 78: 183–188

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

Reed SM, Collins GB (1978) Interspecific hybrids in *Nicotiana* through in vitro culture of fertilized ovules. *J Hered* 69: 311–315

Ryerson DE, Heath MC (1996) Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic treatments. *Plant Cell* 8: 393–402

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

Tezuka T (2012) Hybrid lethality in the genus *Nicotiana*. In: Mworia JK (ed) Botany. InTech, Janeza Trdine 9, 51000 Rijeka, Croatia, pp 191–210

Tezuka T, Kuboyama T, Matsuda T, Marubashi W (2007) Possible involvement of genes on the Q chromosome of *Nicotiana tabacum* in expression of hybrid lethality and programmed cell death during interspecific hybridization to *Nicotiana debneyi*. *Planta* 226: 753–764

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, Marubashi W (2006) Genomic factors lead to programmed cell death during hybrid lethality in interspecific hybrids between *Nicotiana tabacum* and *N. debneyi*. *SABRAO J of Breed Genet* 38: 69–81

Tezuka T, Matsuo C, Iizuka T, Oda M, Marubashi W (2012) Identification of *Nicotiana tabacum* linkage group corresponding to the Q chromosome gene(s) involved in hybrid lethality. *PLoS ONE* 7: e37822

Wang H, Li J, Bostock RM, Gilchrist DG (1996) Apoptosis: A functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. *Plant Cell* 8: 375–391

Yamada T, Marubashi W, Niwa M (1999) Detection of four lethality types in interspecific crosses among *Nicotiana* species through the use of three rescue methods for lethality. *Breed Sci* 49: 203–210

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