

Hybrid Lethality in the Genus *Nicotiana*

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1. Introduction

Reproductive isolation is a mechanism that separates species. It is considered to play a crucial role in the evolution of animals and plants. Reproductive isolation is divided into two types of barriers, namely prezygotic and postzygotic. In plants, a typical prezygotic barrier observed after pollination is pollen–pistil incongruity (or incompatibility). Specifically, when the pollen of one species is rejected by the pistil of another species, but the reciprocal cross is successful, the incongruity is called unilateral incongruity. Postzygotic barriers include seed abortion, and hybrid lethality and hybrid sterility in the F_1 generation. When F_1 hybrids are normal but their F_2 progeny contains lethal or sterile individuals, this phenomenon is called hybrid breakdown and is discriminated from abnormalities in the F_1 generation. Whereas these prezygotic and postzygotic barriers contribute to speciation, they are obstacles for plant breeders, especially in breeding programs involving wide hybridization. Hybrid plants from normal parents sometimes show weak growth or die before maturity. Several terms have been used to describe these phenomena, i.e., hybrid lethality, hybrid weakness, hybrid necrosis and hybrid inviability. Hybrid lethality is observed in certain cross combinations in many plant species (Bomblies & Weigel, 2007). In this chapter, I review studies of hybrid lethality in the genus *Nicotiana*.

2. The genus *Nicotiana*

The genus *Nicotiana* (Solanaceae) includes 76 species classified into 13 sections (Knapp et al., 2004). Species in most sections are distributed mainly in the Americas. The exception is section *Suaveolentes*. This section includes 25 species restricted to Australia and islands of the South Pacific, and one African species, *N. africana*, which is the only known species in Africa. These *Suaveolentes* species are geographically isolated from the majority of species in other sections. Many researchers have attempted to reveal the origin and evolution of this complex genus.

Among *Nicotiana* species, only *N. tabacum* ($2n = 48$, SSTT) and *N. rustica* ($2n = 48$, PPUU) are cultivated tobacco species, whereas the others are wild tobacco species. Of these cultivated species, *N. tabacum*, which belongs to section *Nicotiana*, is the most important for commercial purposes. In *N. tabacum* breeding programs, wild species are valuable as sources of disease resistance (Bai et al., 1995; Burk & Heggestad, 1966; Holmes, 1938; Li et al., 2006; Stavely et

al., 1973) and cytoplasmic male sterility (Nikova & Zagorska, 1990; Nikova et al., 1991, 1999). Therefore, many interspecific crosses have been conducted between *N. tabacum* and wild species. However, hybrid lethality often presents a barrier to introduction of desirable characteristics into *N. tabacum*.

3. Types of hybrid lethality in *Nicotiana*

Inviably hybrid seedlings in *Nicotiana* initially show specific phenotypes (surface symptoms) depending on cross combinations (Tezuka et al., 2009; Yamada et al., 1999). Hybrid lethality in this genus is classified into five types based on the external phenotypes as follows:

- Type I: browning of shoot apex and root tip
- Type II: browning of hypocotyl and roots
- Type III: yellowing of true leaves
- Type IV: formation of multiple shoots
- Type V: fading of shoot color.

Different physiological processes are considered to be involved in at least Types I-IV hybrid lethality, because whether three methods to rescue inviable hybrids (cultivation at elevated temperatures, cotyledon culture, and cytokinin treatment) are effective or not depends on the lethality type (Yamada et al., 1999) as described later. It is possible different causative factors control different types of hybrid lethality.

4. Hybrid lethality in crosses between *Suaveolentes* species and *N. tabacum*

In *Nicotiana*, hybrid lethality is well studied in crosses between species of section *Suaveolentes* and *N. tabacum*. To my knowledge, 22 species in section *Suaveolentes* have been crossed with *N. tabacum* and the viability of the hybrid seedlings has been reported. The results of the crosses are summarized in Table 1.

Twenty *Suaveolentes* species yield inviable hybrids in crosses with *N. tabacum*. In most cases, the hybrid lethality is of Type II (Iizuka et al., 2010; Laskowska & Berbeć, 2011; Tezuka & Marubashi, 2006b; Tezuka et al., 2006, 2007, 2010). Only hybrid seedlings between *N. occidentalis* and *N. tabacum* show Type V lethality (Tezuka et al., 2009). Types II and V lethality in these crosses is observed at or below 28°C, but is completely suppressed at elevated temperatures ranging from 34 to 36°C.

The remaining two *Suaveolentes* species, *N. benthamiana* and *N. fragrans*, yield 100% viable hybrids in crosses with *N. tabacum* (DeVerna et al., 1987; Iizuka et al., 2010, 2011; Tezuka et al., 2010). These species are exceptions in this section with respect to hybrid lethality.

4.1 Causes of hybrid lethality

Reciprocal hybrids with *N. tabacum* were produced using the above-mentioned 19 *Suaveolentes* species that yield inviable hybrids (excluding *N. wuttkei*). In all these crosses, hybrid lethality was observed regardless of cross direction, which indicates hybrid lethality is a result of the interaction of coexisting heterologous genomes, and not a cytoplasmic effect (Iizuka et al., 2010; Tezuka & Marubashi, 2004, 2006a; Tezuka et al., 2006, 2009, 2010). Subsequent genetic analyses have identified the chromosome and genes responsible for hybrid lethality.

<i>Suaveolentes</i> <i>species</i>	Haploid chromosome number	F ₁ pheno- type	Suppression at elevated temperatures	Factors responsible for hybrid lethality ^a		References ^b
				In <i>N. tabacum</i>	In <i>Suaveolentes</i> species	
<i>N. africana</i>	23	Type II lethality	Possible	Q chromosome	ND	7
<i>N. amplexicaulis</i>	18	Type II lethality	Possible	Q chromosome	ND	3
<i>N. benthamiana</i>	19	Viable	-	-	<i>hla1-2</i>	1, 6, 8
<i>N. cavicola</i>	23	Type II lethality	Possible	ND	ND	6
<i>N. debneyi</i>	24	Type II lethality	Possible	Q chromosome	<i>Hla1-1</i>	4, 8
<i>N. excelsior</i>	19	Type II lethality	Possible	Q chromosome	ND	7
<i>N. exigua</i>	16	Type II lethality	Possible	ND	ND	6
<i>N. fragrans</i>	24	Viable	-	-	<i>hla1-2</i>	7
<i>N. goodspeedii</i>	20	Type II lethality	Possible	Q chromosome	ND	7
<i>N. gossei</i>	18	Type II lethality	Possible	Q chromosome	ND	7
<i>N. hesperis</i>	21	Type II lethality	Possible	ND	ND	6
<i>N. ingulba</i>	20	Type II lethality	Possible	Q chromosome	ND	6, 10
<i>N. maritima</i>	16	Type II lethality	Possible	Q chromosome	ND	7
<i>N. megalosiphon</i>	20	Type II lethality	Possible	Q chromosome	ND	7
<i>N. occidentalis</i>	21	Type V lethality	Possible	S and T subgenomes	ND	5
<i>N. rosulata</i>	20	Type II lethality	Possible	ND	ND	6
<i>N. rotundifolia</i>	22	Type II lethality	Possible	ND	ND	6
<i>N. simulans</i>	20	Type II lethality	Possible	ND	ND	6
<i>N. suaveolens</i>	16	Type II lethality	Possible	Q chromosome	ND	2
<i>N. umbratica</i>	23	Type II lethality	Possible	ND	ND	6
<i>N. velutina</i>	16	Type II lethality	Possible	Q chromosome	ND	7
<i>N. wuttkei</i>	16	Type II lethality	ND	ND	ND	9

^a ND, not determined

^b 1, DeVerna et al. (1987); 2, Tezuka & Marubashi (2006b); 3, Tezuka et al. (2006); 4, Tezuka et al. (2007); 5, Tezuka et al. (2009); 6, Iizuka et al. (2010); 7, Tezuka et al. (2010); 8, Iizuka et al. (2011); 9, Laskowska & Berbec (2011); 10, Matsuo et al. (2011)

Table 1. Hybrid lethality observed in crosses between *Suaveolentes* species and *N. tabacum*

4.1.1 Causative genes in *N. tabacum*

Nicotiana tabacum ($2n = 48$, SSTT) is a natural allotetraploid (amphidiploid) that originated by interspecific hybridization of *N. sylvestris* ($2n = 24$, SS; section *Sylvestres*) with *N. tomentosiformis* ($2n = 24$, TT; section *Tomentosae*) and subsequent chromosome doubling (Chase et al., 2003; Clarkson et al., 2004, 2010; Gray et al., 1974; Lim et al., 2000; Murad et al., 2002; Sheen, 1972). Therefore, it is possible to determine which subgenome of *N. tabacum* is involved in hybrid lethality using these progenitors.

Nicotiana debneyi (Tezuka et al., 2007) and *N. suaveolens* (Inoue et al., 1996) were crossed with the two progenitors of *N. tabacum*. Both *N. debneyi* and *N. suaveolens* produced inviable hybrids in crosses with *N. sylvestris*, whereas the two species produced viable hybrids in crosses with *N. tomentosiformis*. These results clearly indicated that the S subgenome of *N. tabacum* is involved in hybrid lethality.

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), which lack a certain chromosome, has been established in the genetic background of 'Red Russian' (Cameron, 1959; Clausen & Cameron, 1944). These monosomic lines are useful to locate genes on specific chromosomes (Clausen & Cameron, 1944; Kubo et al., 1982).

The first application of *N. tabacum* monosomic lines to study hybrid lethality in *Nicotiana* was in a cross between *N. tabacum* and *N. africana* (Gerstel et al., 1979). When all 24 monosomic lines were crossed with *N. africana*, only Haplo-H produced a high number of viable hybrids. Based on these results, the H chromosome, which belongs to the T subgenome, is considered to be related to hybrid lethality. However, it was not clear whether the viable hybrids from the cross Haplo-H \times *N. africana* definitely lacked the H chromosome in their study.

Monosomic lines of *N. tabacum* were used next to investigate hybrid lethality in crosses between *N. tabacum* and *N. suaveolens*. Ten monosomic lines of the S subgenome (Haplo-M-Z, excluding Haplo-P and Haplo-V) were crossed with *N. suaveolens* (Marubashi & Onosato, 2002). A small number of viable hybrids were obtained only from the cross using Haplo-Q. These hybrids possessed 38 or 39 chromosomes, which indicated they lacked the Q chromosome. Therefore, it was speculated that the Q chromosome encodes one or more genes causing hybrid lethality. This was conclusively proven with analyses using Q-chromosome-specific DNA markers (Tezuka & Marubashi, 2006b; Tezuka et al., 2004).

In crosses between the other 10 *Suaveolentes* species and *N. tabacum*, causative gene(s) in *N. tabacum* of hybrid lethality were encoded on the Q chromosome (Table 1; Matsuo et al., 2011; Tezuka et al., 2006, 2007, 2010). These results suggested that many species of section *Suaveolentes* share the same gene(s) that triggers hybrid lethality by interaction with the gene(s) on the Q chromosome.

However, it seems one or more species in section *Suaveolentes* are exceptional with regard to hybrid lethality. *Nicotiana occidentalis* yields inviable hybrids showing Type V lethality in crosses with *N. tabacum* as mentioned already. Hybrid seedlings from this cross combination die despite the lack of the Q chromosome. Based on genetic analyses using the two progenitors of *N. tabacum*, both the S and T subgenomes of *N. tabacum* are apparently related to hybrid lethality in the cross with *N. occidentalis* (Table 1; Tezuka et al., 2009).

4.1.2 Causative genes in *Suaveolentes* species

A segregation analysis by classical Mendelian genetics was conducted to identify the causative genes in *Suaveolentes* species. *Nicotiana debneyi* and *N. fragrans* yield inviable and viable hybrids, respectively, in crosses with *N. tabacum* (Table 1; Tezuka et al., 2007, 2010). F₁ hybrids obtained from the cross *N. debneyi* × *N. fragrans* were crossed with *N. tabacum* (Iizuka et al., 2011). Trispecific hybrids from this cross were segregated into inviable and viable hybrids with a ratio of 1:1. Therefore, it was determined that *N. debneyi* carries a single dominant gene causing hybrid lethality in the cross with *N. tabacum*. The gene locus was designated *HYBRID LETHALITY A1 (HLA1)* and the *N. debneyi* allele, which causes hybrid lethality, was assigned as *Hla1-1* and the non-causative allele of *N. fragrans* and *N. tabacum* as *hla1-2* (Iizuka et al., 2011). In conclusion, the *Hla1-1* allele in *N. debneyi* causes hybrid lethality by interaction with the gene(s) on the Q chromosome of *N. tabacum*.

Similar to hybrid lethality in the cross using *N. debneyi*, gene(s) on the Q chromosome cause Type II lethality in other crosses involving 10 *Suaveolentes* species (Table 1; Matsuo et al., 2011; Tezuka & Marubashi, 2006b; Tezuka et al., 2006, 2010). These results infer that the *Hla1-1* allele is shared by at least 11 *Suaveolentes* species (*N. africana*, *N. amplexicaulis*, *N. debneyi*, *N. excelsior*, *N. goodspeedii*, *N. gossei*, *N. ingulba*, *N. maritima*, *N. megalosiphon*, *N. suaveolens* and *N. velutina*). This finding is consistent with the hypothesis that section *Suaveolentes* originated from a single polyploid event some 10 Mya, followed by speciation to produce the extant species (Leitch et al., 2008). This hypothesis is supported by recent studies that indicate section *Suaveolentes* is a monophyletic group based on sequence data for the internal transcribed spacer region (Chase et al., 2003), plastid genes (Clarkson et al., 2004) and nuclear-encoded chloroplast-expressed glutamine synthetase (ncpGS; Clarkson et al., 2010).

All species in section *Suaveolentes* are allotetraploids. This section contains an almost complete aneuploid series of $n = 16-24$, with only $n = 17$ unknown. According to Clarkson et al. (2004), it seems that the allotetraploid ancestor of the section occurred in South America, where its parental species are found, and subsequently the allotetraploid ancestor dispersed to Africa and Australia separately; only in Australia has an explosive radiation of taxa occurred, largely accompanied by dysploid reductions probably because of chromosomal fusions. Progenitors (parental species of the allotetraploid ancestor) of this section have been proposed by some researchers. Goodspeed (1954) considered that, based on external morphology, ancestral races related to the source of the sections *Alatae*, *Noctiflorae* and *Petunioides* were involved in the formation of section *Suaveolentes*. However, based on analyses of ncpGS sequences, Clarkson et al. (2010) recently suggested the maternal progenitor is *N. sylvestris* and the paternal progenitor is section *Trigonophyllae* ($2n = 24$). Although the evidence for the progenitors of section *Suaveolentes* is inconclusive at present, this information might shed light on the origin of the *Hla1-1* allele. *Nicotiana sylvestris* produces viable hybrids in reciprocal crosses with *N. tabacum* (Christoff, 1928; East, 1935; Kostoff, 1930; Tanaka, 1961). Therefore, the *Hla1-1* allele might be derived from section *Trigonophyllae*. Another possibility is that the allotetraploid ancestor of section *Suaveolentes* or the descendant of the allotetraploid ancestor acquired the *Hla1-1* allele after divergence of *N. benthamiana* and *N. fragrans*.

As already stated, three *Suaveolentes* species (*N. occidentalis*, *N. benthamiana* and *N. fragrans*) yielded results different from those using the above-mentioned 11 species. *Nicotiana*

benthamiana and *N. fragrans*, which yield viable hybrids in crosses with *N. tabacum*, possess the *hla1-2* allele. The causative gene(s) in *N. occidentalis* is somewhat complicated, because this species shows hybrid lethality in which the S and T subgenomes of *N. tabacum* are involved (Table 1; Tezuka et al., 2009). These results suggest genetic changes that reinforce reproductive isolation with *N. tabacum* have accumulated in the lineage leading to *N. occidentalis*. Whether *N. occidentalis* possesses the *Hla1-1* allele is an interesting issue requiring further investigation.

5. Hybrid lethality in other crosses in *Nicotiana*

In addition to crosses between *Suaveolentes* species and *N. tabacum*, hybrid lethality is reported in many interspecific crosses in *Nicotiana*. Some of these crosses involve allotetraploid species as parents, i.e., *N. tabacum* ($2n = 48$, SSTT) in section *Nicotiana*, *N. rustica* ($2n = 48$, PPUU) in section *Rusticae*, and species in sections *Polydichiae* ($2n = 48$), *Repandae* ($2n = 48$) and *Suaveolentes* ($2n = 32-48$). In addition to *N. tabacum* and section *Suaveolentes*, recent molecular phylogenetic analysis has revealed the progenitors of these species and sections (Clarkson et al., 2010). The maternal and paternal progenitors of *N. rustica* are, respectively, *N. paniculata* ($2n = 24$, PP; section *Paniculatae*) and *N. undulata* ($2n = 24$, UU; section *Undulatae*). Section *Polydichiae* is derived from section *Trigonophyllae* ($2n = 24$; the maternal progenitor) and *N. attenuata* ($2n = 24$; section *Petunioides*; the paternal progenitor). Section *Repandae* is derived from *N. sylvestris* ($2n = 24$, SS; the maternal progenitor) and section *Trigonophyllae* (the paternal progenitor). These progenitors of allotetraploid species might provide important information to determine which subgenome is responsible for hybrid lethality, as in crosses between *N. tabacum* and *Suaveolentes* species.

Tables 2–4 list interspecific crosses using *N. tabacum*, those using *N. rustica*, and other crosses, respectively. Tables 2 and 3 also include crosses using progenitors and relatives of *N. tabacum* and *N. rustica*. In addition to crosses that yield inviable hybrids, those that yield viable hybrids are also listed, because these are useful to discuss hybrid lethality. However, attention was also paid to literature that described viable hybrids. This is because it is likely some authors did not report hybrid lethality, even though they observed this phenomenon, because they placed particular emphasis on the acquisition of viable hybrids that can be used for breeding programs and in studies such as observations of chromosome pairing. For example, Christoff (1928) reported that crosses between *N. tabacum* and *N. alata* produced inviable hybrids that died at different stages of their development, and only two hybrids reached maturity. Nevertheless, Christoff determined this cross as one that produces mature hybrids. Therefore, as for crosses that yield viable hybrids, only articles that describe hybrid lethality in the same study are cited.

In several crosses in Tables 2 and 3, one author reported a certain cross to be inviable but other authors described the same cross as viable. These incongruities might be because of the above-mentioned fact that some authors have not described hybrid lethality in detail. Other explanations of the incongruities might be differences in the parental cultivars or lines used for crosses, or the environmental conditions under which hybrid seedlings were cultivated. Although verification of whether certain cross combinations listed in Tables 2–4 produce viable (or inviable) hybrids might be needed, nonetheless, these data are informative to study and discuss hybrid lethality in *Nicotiana*.

5.1 Hybrid lethality in crosses using *N. tabacum*

Nicotiana tabacum and its progenitors, including closely related species, are reported to yield inviable hybrids in crosses with species in seven *Nicotiana* sections (Table 2). In Table 2, *N. tomentosa* (2n = 24, TT) and *N. otophora* (2n = 24, TT) are included; both species belong to section *Tomentosae* and are closely related to *N. tomentosiformis* (Clarkson et al., 2010). The *N. tabacum* subgenome that encodes gene(s) for hybrid lethality can be estimated in crosses using *N. glutinosa*, which belongs to section *Undulatae*. *Nicotiana glutinosa* × *N. sylvestris* hybrids are viable (Christoff, 1928; East, 1935), but *N. glutinosa* × *N. tomentosiformis* hybrids are inviable (East, 1935; McCray, 1932). Thus, the T subgenome must encode the causative gene(s) for hybrid lethality.

Species ^a		F ₁ phenotype	References ^c
Female	Male		
Section <i>Alatae</i>			
<i>N. tabacum</i> (24)	<i>N. alata</i> (9)	Lethality	1, 2, 8
		Viable	3, 6
<i>N. alata</i> (9)	<i>N. tabacum</i> (24)	Viable	6
<i>N. tabacum</i> (24)	<i>N. langsdorffii</i> (9)	Lethality	2, 8
		Viable	6
<i>N. tabacum</i> (24)	<i>N. longiflora</i> (10)	Lethality	1, 2, 6, 8
<i>N. longiflora</i> (10)	<i>N. tabacum</i> (24)	Lethality	1, 8
<i>N. tabacum</i> (24)	<i>N. plumbaginifolia</i> (10)	Lethality	2, 6, 7
<i>N. tabacum</i> (24)	<i>N. sanderae</i> (9) ^b	Viable	2, 3, 6
<i>N. sanderae</i> (9)	<i>N. tabacum</i> (24)	Viable	6
Section <i>Noctiflorae</i>			
<i>N. tabacum</i> (24)	<i>N. glauca</i> (12)	Viable	3, 6, 8
<i>N. glauca</i> (12)	<i>N. tabacum</i> (24)	Viable	3, 6
<i>N. glauca</i> (12)	<i>N. sylvestris</i> (12)	Lethality	6
<i>N. tomentosa</i> (12)	<i>N. glauca</i> (12)	Viable	3, 6
<i>N. glauca</i> (12)	<i>N. tomentosa</i> (12)	Viable	3, 6
<i>N. tomentosiformis</i> (12)	<i>N. glauca</i> (12)	Viable	3, 6
<i>N. glauca</i> (12)	<i>N. tomentosiformis</i> (12)	Viable	3, 6
Section <i>Trigonophyllae</i>			
<i>N. tabacum</i> (24)	<i>N. trigonophylla</i> (12)	Viable	8
<i>N. trigonophylla</i> (12)	<i>N. otophora</i> (12)	Type III lethality	13
<i>N. trigonophylla</i> (12)	<i>N. tomentosa</i> (12)	Viable	4
<i>N. palmeri</i> (12)	<i>N. tomentosa</i> (12)	Viable	4
<i>N. palmeri</i> (12)	<i>N. tomentosiformis</i> (12)	Viable	4
Section <i>Undulatae</i>			
<i>N. tabacum</i> (24)	<i>N. glutinosa</i> (12)	Lethality	4, 8
		Viable	2, 4, 6
<i>N. glutinosa</i> (12)	<i>N. tabacum</i> (24)	Lethality	8
		Viable	6

Species ^a		F ₁ phenotype	References ^c
Female	Male		
Section <i>Undulatae</i>			
<i>N. glutinosa</i> (12)	<i>N. sylvestris</i> (12)	Viable	2, 6
<i>N. glutinosa</i> (12)	<i>N. tomentosa</i> (12)	Lethality	6
<i>N. glutinosa</i> (12)	<i>N. tomentosiformis</i> (12)	Lethality	4, 6
Section <i>Polydiciae</i>			
<i>N. tabacum</i> (24)	<i>N. bigelovii</i> (24)	Viable	6
<i>N. bigelovii</i> (24)	<i>N. tabacum</i> (24)	Viable	2, 6
<i>N. tabacum</i> (24)	<i>N. quadrivalvis</i> (24)	Viable	6
<i>N. quadrivalvis</i> (24)	<i>N. tabacum</i> (24)	Viable	6
<i>N. bigelovii</i> (24)	<i>N. sylvestris</i> (12)	Lethality	2
<i>N. tomentosa</i> (12)	<i>N. bigelovii</i> (24)	Lethality	6
<i>N. bigelovii</i> (24)	<i>N. tomentosa</i> (12)	Lethality	4
		Viable	6
<i>N. tomentosiformis</i> (12)	<i>N. bigelovii</i> (24)	Lethality	6
<i>N. bigelovii</i> (24)	<i>N. tomentosiformis</i> (12)	Viable	6
<i>N. tomentosa</i> (12)	<i>N. quadrivalvis</i> (24)	Lethality	6
<i>N. quadrivalvis</i> (24)	<i>N. tomentosa</i> (12)	Viable	6
<i>N. tomentosiformis</i> (12)	<i>N. quadrivalvis</i> (24)	Lethality	6
<i>N. quadrivalvis</i> (24)	<i>N. tomentosiformis</i> (12)	Viable	6
Section <i>Repandae</i>			
<i>N. nesophila</i> (24)	<i>N. tabacum</i> (24)	Viable	9
<i>N. nudicaulis</i> (24)	<i>N. tabacum</i> (24)	Type I lethality	2, 4, 5, 13
<i>N. tabacum</i> (24)	<i>N. repanda</i> (24)	Lethality	11
<i>N. repanda</i> (24)	<i>N. tabacum</i> (24)	Type III lethality	9, 10, 11, 12, 13
<i>N. stocktonii</i> (24)	<i>N. tabacum</i> (24)	Viable	9
<i>N. nudicaulis</i> (24)	<i>N. sylvestris</i> (12)	Lethality	4
<i>N. nudicaulis</i> (24)	<i>N. tomentosiformis</i> (12)	Viable	4
<i>N. repanda</i> (24)	<i>N. sylvestris</i> (12)	Viable	14
<i>N. repanda</i> (24)	<i>N. tomentosiformis</i> (12)	Lethality	14
Sections <i>Rusticae</i> and <i>Paniculatae</i>			
<i>N. tabacum</i> (24)	<i>N. rustica</i> (24)	Viable	3, 6
<i>N. rustica</i> (24)	<i>N. tabacum</i> (24)	Lethality	3
		Viable	2, 6, 8
<i>N. tabacum</i> (24)	<i>N. paniculata</i> (12)	Viable	8
<i>N. paniculata</i> (12)	<i>N. tabacum</i> (24)	Viable	3, 6, 8
<i>N. knightiana</i> (12)	<i>N. tabacum</i> (24)	Viable	8

^a Number in parentheses is the haploid chromosome number

^b Hybrid taxon, a hybrid between *N. forgetiana* (section *Alatae*) and *N. alata* (Goodspeed, 1954)

^c 1, Malloch & Malloch (1924); 2, Christoff (1928); 3, Kostoff (1930); 4, McCray (1932); 5, McCray (1933); 6, East (1935); 7, Moav & Cameron (1960); 8, Tanaka (1961); 9, Reed & Collins (1978); 10, Iwai et al. (1985); 11, DeVerna et al. (1987); 12, Shintaku et al. (1988); 13, Yamada et al. (1999); 14, Kobori & Marubashi (2004)

Table 2. Interspecific crosses using *N. tabacum* and its progenitors and closely related species

In crosses using two *Repandae* species, sufficient data are available to determine the *N. tabacum* subgenome that encodes the causative gene(s) for hybrid lethality. Hybrid lethality is observed in the crosses *N. nudicaulis* × *N. tabacum* and *N. nudicaulis* × *N. sylvestris*, but not in the cross *N. nudicaulis* × *N. tomentosiformis* (Christoff, 1928; McCray, 1932, 1933; Yamada et al., 1999). These results indicate the S subgenome encodes the causative gene(s) for hybrid lethality in the cross *N. nudicaulis* × *N. tabacum*. On the other hand, hybrid lethality is observed in the crosses *N. repanda* × *N. tabacum* and *N. repanda* × *N. tomentosiformis*, but not in the cross *N. repanda* × *N. sylvestris* (DeVerna et al., 1987; Kobori & Marubashi, 2004; Iwai et al., 1985; Reed & Collins, 1978; Shintaku et al., 1988), which indicates the T subgenome encodes the causative gene(s) for hybrid lethality in the cross *N. repanda* × *N. tabacum*. Additionally, the types of hybrid lethality differ between the crosses *N. nudicaulis* × *N. tabacum* and *N. repanda* × *N. tabacum* (Yamada et al., 1999). It is interesting that *N. nudicaulis* and *N. repanda*, which are closely related (Chase et al., 2003; Clarkson et al., 2004, 2010), yield different outcomes with regard to hybrid lethality.

Some conflicting results among crosses involving *N. tabacum* and its progenitors are apparent. In crosses with *N. glauca*, which belongs to section *Noctiflorae*, *N. sylvestris* produces inviable hybrids whereas *N. tabacum* produces viable hybrids (East, 1935; Kostoff, 1930; Tanaka, 1961). Similarly, in crosses with *N. bigelovii* and *N. quadrivalvis*, which belong to section *Polydiciae*, *N. sylvestris* and/or *N. tomentosiformis* produce inviable hybrids but *N. tabacum* produces viable hybrids (Christoff, 1928; East, 1935).

5.2 Hybrid lethality in crosses using *N. rustica*

As for *N. tabacum*, *N. rustica* has been crossed with wild tobacco species and reported to produce inviable hybrids (Table 3). In several crosses with *Alatae* species, *N. rustica* and its progenitor, *N. paniculata*, produce inviable hybrids (Christoff, 1928; East, 1935; Kostoff, 1930; Malloch & Malloch, 1924; McCray, 1932; Yamada et al., 1999). These results infer that the causative gene(s) for hybrid lethality is encoded in the P subgenome of *N. rustica*. Involvement of the U subgenome is unclear, because crossing results using another progenitor, *N. undulata*, are not available.

The causative gene(s) for hybrid lethality in the cross *N. rustica* × *N. suaveolens* would be also encoded in the P subgenome, because hybrid lethality is observed in the cross *N. paniculata* × *N. suaveolens* (Christoff, 1928; East, 1935; Yamada et al., 1999). *Nicotiana palmeri*, which belongs to section *Trigonophyllae*, produces inviable hybrids in crosses with *N. rustica* (Kostoff, 1930). Therefore, the causative gene(s) for hybrid lethality in *N. nudicaulis*, *N. suaveolens* and *N. gossei* might have been derived from section *Trigonophyllae*, although involvement of *N. sylvestris* cannot be ruled out.

5.3 Hybrid lethality in other crosses

Table 4 shows results of crosses using species other than *N. tabacum*, *N. rustica* and their relatives. Noteworthy is hybrid lethality in crosses between *Alatae* species and *Polydiciae* or *Suaveolentes* species. Sections *Polydiciae* and *Suaveolentes* share the same progenitor, i.e., section *Trigonophyllae* (Clarkson et al., 2010). *Nicotiana trigonophylla* in section *Trigonophyllae* produces inviable hybrids in crosses with *N. langsdorffii* from section *Alatae* (Christoff, 1928). Therefore, at least in the crosses *N. bigelovii* × *N. langsdorffii* and *N. suaveolens* × *N. langsdorffii*, the causative gene(s) for hybrid lethality in *N. bigelovii* and *N. suaveolens* might have been derived

from section *Trigonophyllae*. Nonetheless, gene(s) derived from other progenitors (*N. attenuata* for *N. bigelovii* and *N. sylvestris* for *N. suaveolens*) might be involved in hybrid lethality.

Species ^a		F ₁ phenotype	References ^c
Female	Male		
<i>Section Alatae</i>			
<i>N. rustica</i> (24)	<i>N. alata</i> (9)	Lethality	3
		Viable	5
<i>N. rustica</i> (24)	<i>N. langsdorffii</i> (9)	Lethality	1, 3, 4
		Viable	2, 5
<i>N. rustica</i> (24)	<i>N. sanderae</i> (9) ^b	Lethality	3
		Viable	2, 5
<i>N. paniculata</i> (12)	<i>N. alata</i> (9)	Type IV lethality	2, 6
		Viable	5
<i>N. alata</i> (9)	<i>N. paniculata</i> (12)	Lethality	5
<i>N. paniculata</i> (12)	<i>N. langsdorffii</i> (9)	Lethality	3, 5
		Viable	2
<i>N. paniculata</i> (12)	<i>N. longiflora</i> (10)	Lethality	2, 5
<i>N. paniculata</i> (12)	<i>N. plumbaginifolia</i> (10)	Lethality	2, 5
<i>N. plumbaginifolia</i> (10)	<i>N. paniculata</i> (12)	Lethality	2
<i>N. paniculata</i> (12)	<i>N. sanderae</i> (9)	Viable	2, 5
<i>N. sanderae</i> (9)	<i>N. paniculata</i> (12)	Lethality	5
<i>Section Petunioides</i>			
<i>N. rustica</i> (24)	<i>N. attenuata</i> (12)	Lethality	3
<i>Section Trigonophyllae</i>			
<i>N. rustica</i> (24)	<i>N. palmeri</i> (12)	Lethality	3
<i>Section Undulatae</i>			
<i>N. paniculata</i> (12)	<i>N. glutinosa</i> (12)	Type IV lethality	6
		Viable	2, 5
<i>N. glutinosa</i> (12)	<i>N. paniculata</i> (12)	Lethality	5
<i>Section Polydicliae</i>			
<i>N. rustica</i> (24)	<i>N. bigelovii</i> (24)	Viable	5
<i>N. rustica</i> (24)	<i>N. quadrivalvis</i> (24)	Viable	5
<i>N. bigelovii</i> (24)	<i>N. paniculata</i> (12)	Lethality	2
<i>N. paniculata</i> (12)	<i>N. bigelovii</i> (24)	Viable	5
<i>N. paniculata</i> (12)	<i>N. quadrivalvis</i> (24)	Viable	5
<i>Section Repandae</i>			
<i>N. paniculata</i> (12)	<i>N. nudicaulis</i> (24)	Type III lethality	6
<i>Section Suaveolentes</i>			
<i>N. rustica</i> (24)	<i>N. suaveolens</i> (16)	Lethality	2
<i>N. paniculata</i> (12)	<i>N. gossei</i> (18)	Type II lethality	6
<i>N. paniculata</i> (12)	<i>N. suaveolens</i> (16)	Type II lethality	2, 5, 6

^a Number in parentheses is the haploid chromosome number

^b Hybrid taxon, a hybrid between *N. forgetiana* (section *Alatae*) and *N. alata* (Goodspeed, 1954)

^c 1, Malloch & Malloch (1924); 2, Christoff (1928); 3, Kostoff (1930); 4, McCray (1932); 5, East (1935); 6, Yamada et al. (1999)

Table 3. Interspecific crosses using *N. rustica* and its progenitors

Species ^a		F ₁ phenotype	References ^c
Female	Male		
Sections <i>Alatae</i> and <i>Trigonophyllae</i>			
<i>N. trigonophylla</i> (12)	<i>N. langsdorffii</i> (9)	Lethality	2
Sections <i>Alatae</i> and <i>Undulatae</i>			
<i>N. glutinosa</i> (12)	<i>N. langsdorffii</i> (9)	Lethality	2, 4
Sections <i>Alatae</i> and <i>Polydcliae</i>			
<i>N. bigelovii</i> (24)	<i>N. langsdorffii</i> (9)	Lethality	2
<i>N. bigelovii</i> (24)	<i>N. longiflora</i> (10)	Lethality	1, 2
<i>N. bigelovii</i> (24)	<i>N. plumbaginifolia</i> (10)	Lethality	2
<i>N. bigelovii</i> (24)	<i>N. sanderae</i> (9) ^b	Lethality	1
Sections <i>Alatae</i> and <i>Suaveolentes</i>			
<i>N. alata</i> (9)	<i>N. suaveolens</i> (16)	Lethality	4
<i>N. suaveolens</i> (16)	<i>N. alata</i> (9)	Lethality	2, 4
<i>N. gossei</i> (18)	<i>N. alata</i> (9)	Type IV lethality	5
<i>N. suaveolens</i> (16)	<i>N. langsdorffii</i> (9)	Lethality	4
<i>N. megalosiphon</i> (20)	<i>N. longiflora</i> (10)	Type III lethality	5
<i>N. suaveolens</i> (16)	<i>N. longiflora</i> (10)	Viable	2, 3, 4
<i>N. megalosiphon</i> (20)	<i>N. plumbaginifolia</i> (10)	Type III lethality	5
<i>N. suaveolens</i> (16)	<i>N. plumbaginifolia</i> (10)	Viable	2, 4
<i>N. sanderae</i> (9)	<i>N. suaveolens</i> (16)	Lethality	4
<i>N. suaveolens</i> (16)	<i>N. sanderae</i> (9)	Lethality	4
Sections <i>Repandae</i> and <i>Undulatae</i>			
<i>N. glutinosa</i> (12)	<i>N. nudicaulis</i> (24)	Viable	2
<i>N. glutinosa</i> (12)	<i>N. repanda</i> (24)	Type III lethality	5
Sections <i>Repandae</i> and <i>Suaveolentes</i>			
<i>N. debneyi</i> (24)	<i>N. repanda</i> (24)	Type II lethality	5

^a Number in parentheses is the haploid chromosome number

^b Hybrid taxon, a hybrid between *N. forgetiana* (section *Alatae*) and *N. alata* (Goodspeed, 1954)

^c 1, Malloch & Malloch (1924); 2, Christoff (1928); 3, Kostoff (1930); 4, East (1935); 5, Yamada et al. (1999)

Table 4. Other interspecific crosses in *Nicotiana*

The cross between *N. debneyi* from section *Suaveolentes* and *N. repanda* from section *Repandae* produces inviable hybrids (Yamada et al., 1999). These sections are derived from shared progenitors, i.e., *N. sylvestris* and section *Trigonophyllae* (Clarkson et al., 2010). When *N. debneyi* and *N. repanda* are crossed with *N. sylvestris*, the former produces inviable hybrids (Tezuka et al., 2007), whereas the latter produces viable hybrids (Kobori & Marubashi, 2004). Therefore, the causative gene in *N. repanda* might have been derived from *N. sylvestris*.

6. Methods to overcome hybrid lethality

For plant breeders, it is important to overcome hybrid lethality, since this mechanism can be an obstacle when seeking to introduce desirable genes into cultivated species. If hybrid lethality can be overcome, a larger germplasm pool will be available in breeding programs. Several methods to overcome or suppress hybrid lethality have been developed through studies in the genus *Nicotiana*.

6.1 Cultivation at elevated temperatures

Hybrid seedlings show hybrid lethality at 28°C, a temperature suitable for the growth of tobacco plants. When hybrid seedlings are cultivated at elevated temperatures generally ranging from 32–38°C, the seedlings may grow normally without exhibiting lethal symptoms. Following the first report of temperature sensitivity in the cross *N. suaveolens* × *N. tabacum* (Manabe et al., 1989), hybrid lethality has been demonstrated to be suppressed at elevated temperatures in many *Nicotiana* crosses (Iizuka et al., 2010; Marubashi & Kobayashi, 2002; Mino et al., 2002; Tezuka et al., 2006, 2010; Watanabe & Marubashi, 2004; Yamada et al., 1999). Obtaining even hybrid seedlings that grow to maturity and flower is possible by suppressing hybrid lethality at elevated temperatures at least in some crosses (Manabe et al., 1989; Tezuka & Marubashi, 2006a; Yamada et al., 1999). This method of cultivation at elevated temperatures is very simple and convenient. However, hybrid seedlings must be cultivated continuously at elevated temperatures from germination to maturity. When hybrid seedlings are transferred from an elevated temperature to one below 28°C, they will die. In *Nicotiana*, this method is useful for Types I–III and V lethality, but might not be effective against Type IV lethality (Tezuka et al., 2009; Yamada et al., 1999). Temperature sensitivity is also observed in hybrid lethality in other plant species, including intraspecific and interspecific crosses in *Gossypium* (Phillips, 1977), *Oryza* (Saito et al., 2007), *Lactuca* (Jeuken et al., 2009) and *Arabidopsis* (Bomblies et al., 2007), and intergeneric hybrids between *Pyrus pyrifolia* and *Malus* × *domestica* (Inoue et al., 2003). However, why hybrid lethality is suppressed at elevated temperatures is still unclear.

6.2 Tissue culture

Cotyledon culture has been used to overcome hybrid lethality in *Nicotiana* (DeVerna et al., 1987; Lloyd, 1975; Ternovskii et al., 1976; Yamada et al., 1999). To raise viable hybrids with this procedure, cotyledons are excised from hybrid seedlings before showing lethal phenotypes. The cotyledons are sectioned and cultured on an appropriate medium containing auxin and cytokinin to induce callus. Adventitious shoots regenerated from the callus are normal and can be rooted. These plants grow to maturity after acclimatization. Although cotyledons are often used as explant sources, other tissues can be suitable. In the cross *N. occidentalis* × *N. tabacum*, germinated seeds were used for callus and shoot production (Ternovskii et al., 1972). Small leaves were used in the cross *N. repanda* × *N. tabacum* (Iwai et al., 1985). In *Triticum*, viable hybrids were obtained by regeneration from callus induced by culture of immature embryos (Chen et al., 1989).

In *Nicotiana*, cotyledon culture is reported to be useful to overcome Type II lethality, but not Types I, III and IV lethality (Yamada et al., 1999). Nevertheless, Type III lethality in the cross *N. repanda* × *N. tabacum* was overcome by tissue culture (Iwai et al., 1985). Tissue culture is also useful for Type V lethality in the cross *N. occidentalis* × *N. tabacum* (Ternovskii et al., 1972).

The mechanism of overcoming hybrid lethality by tissue culture may be explained by somaclonal variation that is often observed in regenerated plants (Larkin & Scowcroft, 1981). For example, the phenomenon might involve (1) deletion of a chromosome or chromosome segment, leading to deletion of the causative gene for hybrid lethality, (2) mutation in the causative gene(s), leading to loss of function in the induction of hybrid lethality, and (3) mutation in other key genes required to induce hybrid lethality.

6.3 Application of cytokinin or auxin

Application of exogenous plant growth regulators, such as auxin and cytokinin, is reported to be effective to address hybrid lethality. Hybrid seedlings from reciprocal crosses between *N. repanda* and *N. tabacum* cultivated in vermiculite supplemented with 1/4 Murashige and Skoog (MS) solution (Murashige & Skoog, 1962) and the auxin indole-3-acetic acid (IAA) grew to maturity, whereas hybrid seedlings supplied with only 1/4 MS solution died (Zhou et al., 1991). This IAA treatment needed to be continued, otherwise the hybrid seedlings died. The authors suggested the hybrid seedlings contained insufficient endogenous auxin for normal growth and that exogenous IAA compensated for this deficiency. Effectiveness of the IAA treatment in other lethal crosses is unclear.

Inoue et al. (1994) developed a method to overcome hybrid lethality in the cross *N. suaveolens* × *N. tabacum* by cytokinin treatment. This method was carried out in two steps. First, the seeds were germinated on 1/2 MS solid medium. Next, the hybrid seedlings were transplanted to 1/2 MS solid medium containing cytokinin. After this treatment, the hybrid seedlings began to die. However, adventitious buds developed from the base of the hypocotyl and the primary root of the dying seedlings, and grew into normal green shoots that were viable without exogenous cytokinin. After rooting, the plantlets grew to maturity. This two-step procedure was further improved by the same authors; hybrid seeds were cultured in 1/2 MS liquid medium containing cytokinin (Inoue et al., 1997). This simple one-step method allowed the whole surface of hybrid seedlings to absorb cytokinin. Adventitious shoots were regenerated from the root and the whole hypocotyl, and at a greater frequency compared with the two-step method. In both methods, several types of cytokinin, including kinetin, 6-benzylaminopurine, *t*-zeatin, thidiazuron and *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea, were effective to overcome hybrid lethality. However, the efficiency of shoot regeneration differed with the type and concentration of cytokinin (Inoue et al., 1994, 1997).

Although the mechanism of overcoming hybrid lethality by exogenous cytokinin application is still unclear, three hypotheses were proposed by Inoue et al. (1997): (1) cytokinin directly suppresses hybrid lethality, (2) cytokinin induces a mutation in the causative gene(s) for hybrid lethality, and (3) cytokinin enables the screening of variant cells that carry a spontaneous mutation in the causative gene(s).

In *Nicotiana*, cytokinin culture methods are effective for Types I and II lethality, but might not overcome Types III and IV lethality (Yamada et al., 1999). Effectiveness in other plant species is unclear.

6.4 Irradiation

Irradiation with γ -rays and ion beams has been used to overcome hybrid lethality. Irradiation with γ -rays was used to overcome Type III lethality in a cross between *N. repanda* and *N. tabacum*. Viable hybrids were obtained when *N. repanda* was pollinated with γ -ray-irradiated pollen of *N. tabacum*, and when γ -ray-irradiated egg cells (ovules) of *N. repanda* were fertilized by *N. tabacum* (Shintaku et al., 1988, 1989). To overcome Type II lethality in the cross *N. gossei* × *N. tabacum*, *N. tabacum* pollen irradiated with γ -rays or ion beams was used successfully (Kitamura et al., 2003).

Irradiation techniques are also effective in other plant species. In *Triticum*, chimera hybrids, which produced necrotic and normal tillers, and necrotic and normal leaves, were obtained by irradiation of hybrid seeds with γ -rays. These chimeras set seeds (Sharma, 1969). In the intergeneric cross between *Pyrus pyrifolia* and *Malus × domestica*, hybrids without lethal phenotypes were obtained by γ -ray irradiation of shoots from immature hybrid embryos (Gonai et al., 2006). Although direct evidence is not available, irradiation might allow elimination or mutation of the causative gene(s) or other key genes that contribute to hybrid lethality.

7. Conclusion

The genus *Nicotiana* is advantageous for investigation of hybrid lethality because many cross combinations show hybrid lethality. Several gene combinations probably cause hybrid lethality in *Nicotiana*. However, as yet none of the genes have been cloned and characterized. Recently, the causative gene for hybrid lethality in *Arabidopsis thaliana* was identified (Bomblies et al., 2007). In intraspecific crosses in *A. thaliana*, hybrid lethality is induced by the interaction between an allele of the *DANGEROUS MIX 1 (DM1)* locus and an allele of the *DM2* locus. *DM1* encodes a *Toll Interleukin Receptor-Nucleotide Binding-Leucine Rich Repeat (TIR-NB-LRR)* disease resistance (*R*) gene homolog. This finding strongly indicates an autoimmune-like response is the mechanism for hybrid lethality in *A. thaliana* (Bomblies et al., 2007).

In interspecific crosses of lettuce (*Lactuca saligna × L. sativa*), a specific allelic combination at two loci triggers hybrid lethality. One of the two interacting loci was *Rin4*, a homolog of *RPM1 INTERACTING PROTEIN 4 (RIN4)* of *A. thaliana* (Jeuken et al., 2009). In *A. thaliana*, *RIN4* is a target of at least three effectors (*AvrB*, *AvrRpm1* and *AvrRpt2*) from *Pseudomonas syringae*, and is guarded by two *R* proteins, *RPM1* and *RPS2* (Axtell & Staskawicz, 2003; Kim et al., 2005; Mackey et al., 2002, 2003). Interaction of *RIN4* and these *R* proteins results in the hypersensitive response to *P. syringae*. Similarly, association between hybrid lethality and disease resistance has been shown in interspecific crosses involving tomato. The *Cf-2* gene confers resistance to *Cladosporium fulvum*. When a dominant allele of *Cf-2* derived from *Solanum pimpinellifolium* is combined with a recessive allele of *RCR3* from *S. lycopersicum*, autonecrosis is observed (Day, 1958; Krüger et al., 2002; Langford, 1948; Santangelo et al., 2003). This phenomenon infers hybrid lethality, or more strictly hybrid breakdown. *Cf-2* encodes a transmembrane protein with an extracellular LRR and *RCR3* encodes a secreted papain-like cysteine endoprotease (Dixon et al., 1996; Krüger et al., 2002).

Thus, recent findings have revealed that the mechanism of hybrid lethality is related to disease resistance. However, whether all types of hybrid lethality are explained by disease resistance is uncertain. To address this question, myself and co-workers plan to elucidate the distribution of the causative genes, conduct genetic analyses, and identify and clone the causative genes in *Nicotiana*. These studies will reveal the diverse mechanisms of hybrid lethality, contribute to the development of new cultivars and also help to understand speciation mechanisms.

8. Acknowledgment

Preparation of this chapter was partly supported by a Grant-in-Aid for Young Scientists (Start-up) No. 20880024 from the Japan Society for the Promotion of Science.

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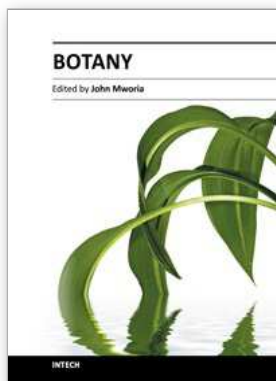
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Botany

Edited by Dr. John Mworira

ISBN 978-953-51-0355-4

Hard cover, 226 pages

Publisher InTech

Published online 16, March, 2012

Published in print edition March, 2012

This book is devoted to botany and covers topical issues in this diverse area of study. The contributions are designed for researchers, graduate students and professionals. The book also presents reviews of current issues in plant-environment interactions making it useful to environmental scientists as well. The book is organized in three sections. The first section includes contributions on responses to flood stress, tolerance to drought and desiccation, phytotoxicity to Chromium and Lead; the second has aspects of economic botany including a review of Smut disease in sugarcane and properties of plant extract used Tassaboount date juice; the last covers topical issues on morphogenesis and genetics on cotton fiber special cell, secretory glands *Asphodelus aestivus* flower ,pollen tube growth in *Leucojum aestivum* , morphological studies of *Ardisia crenata* complex, and hybrid lethality in the Genus *Nicotiana*.

How to reference

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Takahiro Tezuka (2012). Hybrid Lethality in the Genus *Nicotiana*, *Botany*, Dr. John Mworira (Ed.), ISBN: 978-953-51-0355-4, InTech, Available from: <http://www.intechopen.com/books/botany/hybrid-lethality-in-the-genus-nicotiana>

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