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Full Length Research Paper

Tumor formation in hybrids between *Solanum lycopersicum* and *S. habrochaites*

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Genetic tumors are neoplastic growths that arise spontaneously in particular genotypes in plants. In the present study, we observed the occurrence of tumors in interspecific hybrids between *Solanum lycopersicum* L. and *Solanum habrochaites* S. Knapp and D. M. Spooner. The hybridity of these plants was confirmed based on morphological characteristics, flow cytometry and random amplified polymorphic DNA analysis. Hybrids formed tumors when the plants were grown in a growth chamber. These tumors were formed most frequently on leaves, but also on cotyledons, stems and petioles. When hybrid plants bearing tumors were transferred to a greenhouse, the tumors disappeared and necrotic brown spots were visible in the positions where tumors existed previously. Further analyses indicated that tumors were never formed in the greenhouse. These results suggest that environmental conditions influenced tumorigenesis in hybrids raised from *S. lycopersicum* × *S. habrochaites* crosses. In addition, when stem segments of hybrids were cultured *in vitro* on plant growth regulator-free medium, tumors and shoot formation were induced. Thus, hybrids between *S. lycopersicum* × *S. habrochaites* provide an excellent experimental system to study plant tumorigenesis and to understand cell division and differentiation.

Key words: Environmental condition, growth abnormality, interspecific cross, tomato, tissue culture, tumor.

INTRODUCTION

Interspecific hybrids obtained from crosses between normal and healthy plant species often show growth abnormalities. For instance, hybrid lethality, a phenomenon that causes death of hybrids (Song et al., 2009; Tezuka, 2012; Tezuka and Marubashi, 2012), and hybrid sterility (Nikova et al., 1999; Trojak-Goluch and Berbeć, 2003; Yamagata et al., 2010) are reported in some genera, such as *Nicotiana*, *Oryza* and *Gossypium*. In addition to these abnormalities, the formation of so-called genetic tumors (spontaneous neoplastic growths that develop independent of a causal agent) is sometimes observed in interspecific hybrids of certain genera, such as *Nicotiana* (Kostoff, 1935), *Solanum* (Martin, 1966), *Gossypium* (Phillips and Merritt, 1972), and *Triticum* (Joshi, 1972). Tumors may cause a reduction in quality of crop products. Therefore, development of methods to suppress tumorigenesis is required. In addition, investigation of tumorigenesis is important to understand cell division and differentiation.

Tumors may be caused also by infection with viruses, bacteria, fungi, nematodes and insects (Matveeva et al., 2001; Dodueva et al., 2007). Among these causal agents, crown gall tumors induced by the bacterium *Agrobacterium tumefaciens* are the best studied. The *A. tumefaciens*-induced crown gall is mediated by production of auxin and cytokinin by the enzymes encoded in T-DNA of the Ti plasmid (Matveeva et al., 2001; Dodueva et al., 2007). In contrast, genetic tumors are caused by the genetic constitution of the individual and distinguished from other types of tumors induced by pathogens or other

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Abbreviations: ***Frs***, *Frosty spot*; **RAPD**, random amplified polymorphic DNA.

causal agents.

Genetic tumors have been studied extensively in interspecific hybrids between *Nicotiana glauca* and *Nicotiana langsdorffii*. Genetic tumors are formed at various stages of development, most frequently at the end of flowering or a later stage, and in a variety of organs, including roots, stems and leaves (Kostoff, 1935). These tumors often differentiate into teratomas with rudimentary buds and shoots (Ahuja, 1965; Ichikawa and Syono, 1988; Fujita et al., 1994; Dodueva et al., 2007). In addition, genetic tumors are induced or enhanced by wounding of hybrids (Takenaka and Yoneda, 1965; Ichikawa and Syono, 1988). In contrast to normal tissues, genetic tumors can be cultured on plant growth regulator (PGR)-free medium, and shoots can be regenerated from the tumors (Ichikawa and Syono, 1988; Ichikawa et al., 1989). Many researchers have sought to elucidate the mechanism of genetic tumor formation and have demonstrated that changes in the levels of auxin and cytokinin and/or sensitivity to these phytohormones are involved (Ichikawa et al., 1989; 1990; Feng et al., 1990; Fujita et al., 1991; Ichikawa and Syōno, 1991). Several genes associated with genetic tumors have been identified (Ichikawa et al., 1990; Fujita et al., 1994; Wang and Rhee, 2000; Jin et al., 2008; Yang et al., 2009). However, causal genes for genetic tumors have yet to be identified.

In tomato plants, genetic tumors are reported to develop in progeny of the cross *Solanum lycopersicum* × *Solanum chilense*. The genetic tumors are caused by the dominant allele of the *Frosty spot* (*Frs*) gene from *S. chilense* in the background of *S. lycopersicum* (Martin, 1966). From observations using tumorous and non-tumorous plants obtained by six generations of successive backcrossing to *S. lycopersicum* of F_1 hybrids from the cross *S. lycopersicum* × *S. chilense*, the genetic tumors appear occasionally on the cotyledons and first and second leaves, but generally appear on the lower surface of the third leaf and leaves that form subsequently (Doering and Ahuja, 1967). These tumors do not differentiate into teratomas. Therefore, the timing of tumor formation during plant growth and the characteristics of tumors caused by the *Frs* gene in tomato differ from those observed in *Nicotiana* hybrids (Doering and Ahuja, 1967). Although, the formation of genetic tumors is greatly affected by environmental conditions (Doering and Ahuja, 1967), the specific environmental factors that influence tumorigenesis have yet to be identified. Ahuja and Doering (1967) reported that treatment with gibberellic acid suppressed the formation of genetic tumors caused by the *Frs* gene.

In the present study, we report the spontaneous occurrence of genetic tumors in interspecific hybrids between *S. lycopersicum* and *S. habrochaites*. Hybridity of the plants raised from two cross combinations were confirmed by morphological characteristics, flow cytometry and random amplified polymorphic DNA (RAPD) analysis. We cultivated hybrid seedlings in a greenhouse and a growth chamber, and discovered that frequencies of tumor formation differed markedly between the two environments. In addition, we investigated whether tumors were induced from stem segments cultured on PGR-free medium.

MATERIALS AND METHODS

Plant materials

S. lycopersicum (2*n* = 24) was used for crosses with *S. habrochaites* (2*n* = 24). *S. lycopersicum* was used as the female parent, because the cross *S. habrochaites* × *S. lycopersicum* is incongruous due to inhibition of *S. lycopersicum* pollen tube growth in the pistil of *S. habrochaites*, whereas the reciprocal cross is successful (Lewis and Crowe, 1958; Martin, 1967; Bernacchi and Tanksley, 1997). Cultivars or lines of each species used in the present study are listed in Table 1. Seeds of genotypes with a LA accession number were obtained from the C.M. Rick Tomato Genetics Resource Center, University of California, Davis, CA, and those with a PI accession number were obtained from the Northeast Regional Plant Introduction Station, Geneva, NY. Seeds were placed on moist filter paper in Petri dishes and kept in darkness at 25°C for several days. The germinated seeds were sown in a 72-cell tray (50 ml $cell^{-1}$) filled with a 1:1 (v/v) mixture of peatmoss (Super Cell-Top V; Sakata Seed Co., Kanagawa, Japan) and vermiculite (Nittai Co., Osaka, Japan) and were grown in a greenhouse under natural light conditions. Seedlings were fertigated at each watering with a nutrient solution containing 4.6 mM N, 1.3 mM P, 2.2 mM K, 1.1 mM Ca and 0.4 mM Mg.

Production and cultivation of hybrid seedlings

Flowers of *S. lycopersicum* plants used as female parents were emasculated 1 day before anthesis and pollinated with pollen from *S. habrochaites* plants. F_1 seeds were sown using an identical method to that used for the parental seeds mentioned above. Hybrid seedlings were grown in a growth chamber (25°C, 60 to 85% relative humidity) with a 16 h light (white fluorescent light, approximately 60 µmol m^{-2} s^{-1}) and 8 h dark photoperiod for 4 weeks after germination. Subsequently, hybrid seedlings were transplanted to 1.2 l pots filled with a 1:1 (v/v) mixture of peatmoss and vermiculite, and were grown in a greenhouse under natural light conditions. Seedlings were fertigated at each watering with a nutrient solution containing 4.6 mM N, 1.3 mM P, 2.2 mM K, 1.1 mM Ca and 0.4 mM Mg.

Hybrid plants from the crosses *S. lycopersicum* 'Heinz 1706-BG' × *S. habrochaites* PI 365934 and *S. lycopersicum* 'Micro-Tom' × *S. habrochaites* PI 365904 were propagated by cuttings. The propagated plants were planted in 72-cell trays (50 ml $cell^{-1}$) filled with a 1:1 (v/v) mixture of peatmoss and vermiculite, and were grown in a growth chamber (28°C, 75% relative humidity) with a 16 h light (white fluorescent light, approximately 80 µmol m^{-2} s^{-1}) and 8 h dark photoperiod or in a greenhouse under natural light conditions. Plants were fertigated at each watering with a nutrient solution containing 4.6 mM N, 1.3 mM P, 2.2 mM K, 1.1 mM Ca and 0.4 mM Mg.

Flow cytometry

Flow cytometry was conducted in accordance with the manufacturer's instructions (Partec GmbH, Münster, Germany). The leaves were chopped with a sharp razor blade in extraction buffer (Solution A in the CyStain UV Precise P Kit; Partec) and the extract was filtered through a 30 µm nylon mesh. Subsequently, 4',6-diamino-2-phenylindole dihydrochloride staining buffer (Solution B in the kit) was added. For each sample, the DNA content of at least 10,000 nuclei was analyzed using a flow cytometer (CyFlow Space; Partec).

Table 1. Tomato accessions used in the present study.

Specie	Accession number	Cultivar or line name
S. lycopersicum	LA2091	
	LA3911	'Micro-Tom'
	LA4345	'Heinz 1706-BG'
	PI 212416	'Pearson'
	PI 281554	'Hikari'
	PI 281555	'Kiyosu No. 2'
	PI 330336	'Kurihara'
	PI 355122	'Sekaiichi'
	PI 355126	'World Best'
	PI 639219	'Hillbilly Potato Leaf'
S. lycopersicum var. *cerasiforme*	PI 390504	W-C 1033
	PI 451973	'Sub-Arctic Cherry'
	PI 647522	'California Red Cherry'
S. habrochaites	LA1559	
	LA1695	
	LA1731	
	PI 199381	
	PI 365903	Cimbalo
	PI 365904	Monte Gallinazo
	PI 365905	SAL 335
	PI 365907	SAL 346
	PI 365934	
	PI 390516	W-C 1046

RAPD analysis

Total DNA was extracted from leaves of each plant using a cetyltrimethylammonium bromide-based method (Murray and Thompson, 1980). RAPD analysis was carried out as described by Williams et al. (1990) with some modifications. 20 random 10-mer oligonucleotide primers (Kit A) were obtained from Operon Technologies, Inc., Alameda, CA, USA. Reaction mixtures consisted of 1× ThermoPol reaction buffer (New England Biolabs, Tokyo, Japan), 0.2 mM each dNTP, 0.5 µM primer, 20 ng template DNA, and 1.0 U of *Taq* DNA polymerase (New England Biolabs) in a total volume of 20 µl. PCR amplification was performed using a PC-818A Program Temp Control System (Astec, Fukuoka, Japan) programmed for 3 min at 94°C for initial denaturation, followed by 45 cycles of 30 s at 94°C, 30 s at 36°C, and 2 min at 72°C, with a final extension at 72°C for 5 min. PCR products were separated by electrophoresis in 1.5% agarose gels with TBE buffer and visualized by staining with ethidium bromide. Intense and clear bands were scored.

Cytological observation of pollen grains

Three anthers for each hybrid plant were collected from still-closed flowers 1 day before anthesis. Pollen grains were released on glass slides by squashing anthers with tweezers in a drop of 1% acetocarmine [1 g acetocarmine dissolved in 100 ml of 45% (v/v) acetic acid]. Anther debris was removed and staining solution containing pollen grains was sealed with a glass cover. At least 300 pollen grains for each anther were observed under a light microscope (BX50; Olympus, Tokyo, Japan). Pollen grains stained with acetocarmine were assumed to be fertile.

Tissue culture

F_1 seeds were sterilized with 70% ethanol for 30 s followed by 5% sodium hypochlorite solution for 15 min. The sterilized seeds were sown on PGR-free MS medium (Murashige and Skoog, 1962) supplemented with 1% sucrose and 0.2% Gelrite (pH 5.7), and then incubated at 25°C with a 16 h light (approximately 80 µmol m^{-2} s^{-1}) and 8 h dark photoperiod. The stems of hybrid seedlings obtained from the seeds were sectioned at approximately 5-mm intervals, and those without axillary buds were placed vertically on PGR-free MS medium and cultured at 25°C with a 16 h light (approximately 80 µmol m^{-2} s^{-1}) and 8 h dark photoperiod.

RESULTS AND DISCUSSION

Tumorigenesis in interspecific hybrids

Hand-pollinations representing 91 cross-combinations between *S. lycopersicum* and *S. habrochaites* were performed. Fruit set was observed in 57 cross-combinations. Fruits from 10 cross-combinations contained only immature seeds, thus hybrid seeds were obtained from 47 cross-combinations (Table 2). After sowing, hybrid seedlings were raised from 42 cross-combinations (Table 3). All hybrid seedlings were grown in a growth chamber for 4

Table 2. Fruit and seed set in crosses between *S. lycopersicum* and *S. habrochaites*.

S. lycopersicum (♀)	*S. habrochaites* (♂)									
	LA1559	**LA1695**	**LA1731**	**PI 199381**	**PI 365903**	**PI 365904**	**PI 365905**	**PI 365907**	**PI 365934**	**PI 390516**
'Heinz 1706-BG'	2 / 2 / 2[a]	1 / 1 / 1	0 / 1 / 1	– / 0 / 2	1 / 1 / 1		1 / 1 / 1	2 / 2 / 2	2 / 2 / 2	1 / 1 / 1
'Hikari'		1 / 1 / 1		0 / 1 / 1					1 / 1 / 1	
'Hillbilly Potato Leaf'		– / 0 / 2		– / 0 / 3					– / 0 / 1	
'Kiyosu No. 2'	1 / 1 / 2	1 / 1 / 1	– / 0 / 2	0 / 1 / 1	– / 0 / 1	1 / 1 / 1			1 / 1 / 1	
'Kurihara'	– / 0 / 1	– / 0 / 1	– / 0 / 1	– / 0 / 2	– / 0 / 1	– / 0 / 1	– / 0 / 1		1 / 1 / 2	– / 0 / 1
LA2091	1 / 1 / 1	– / 0 / 1	1 / 1 / 1	0 / 1 / 2	– / 0 / 1				2 / 2 / 2	1 / 1 / 1
'Micro-Tom'	2 / 2 / 2		0 / 1 / 1	– / 0 / 1	1 / 1 / 1	1 / 1 / 1		– / 0 / 1		
'Pearson'	0 / 1 / 3	– / 0 / 1	0 / 1 / 1	0 / 1 / 1	1 / 1 / 1	– / 0 / 1	1 / 1 / 1	0 / 1 / 1	1 / 1 / 2	1 / 1 / 1
'Sekaiichi'		1 / 1 / 1	1 / 1 / 1	0 / 1 / 1	1 / 1 / 1				2 / 2 / 2	
'World Best'				– / 0 / 1	– / 0 / 1	– / 0 / 1	– / 0 / 1	– / 0 / 1	1 / 1 / 1	
'California Red Cherry'	1 / 1 / 1		2 / 2 / 3	– / 0 / 1	1 / 1 / 1	2 / 2 / 2		– / 0 / 1	1 / 1 / 1	
'Sub-Arctic Cherry'	1 / 1 / 1	– / 0 / 1	1 / 1 / 1	0 / 1 / 2	1 / 1 / 1	1 / 1 / 1	1 / 1 / 1	1 / 1 / 2	2 / 2 / 2	– / 0 / 2
W-C 1033	– / 0 / 1	1 / 1 / 1		– / 0 / 2	1 / 1 / 1	1 / 1 / 2	– / 0 / 1	– / 0 / 1	1 / 2 / 2	– / 0 / 1

[a] Number of capsules that contained mature seeds / number of capsules obtained / number of flowers pollinated.

Table 3. Tumor formation in hybrid seedlings between *S. lycopersicum* and *S. habrochaites* cultivated in a growth chamber.

S. lycopersicum (♀)	*S. habrochaites* (♂)									
	LA1559	**LA1695**	**LA1731**	**PI 199381**	**PI 365903**	**PI 365904**	**PI 365905**	**PI 365907**	**PI 365934**	**PI 390516**
'Heinz 1706-BG'	4 / 4 / 5 [a]	0 / 0 / 5			5 / 5 / 5		1 / 1 / 2	5 / 5 / 5	4 / 4 / 5	5 / 5 / 5
'Hikari'		1 / 1/ 1							4 / 4 / 5	
'Hillbilly Potato Leaf'										
'Kiyosu No.2'	5 / 5 / 5	0 / 0 / 5				5 / 5 / 5			1 / 1 / 5	
'Kurihara'									5 / 5 / 5	
LA 2091	3 / 3 / 5		5 / 5 / 5						4 / 4 / 5	5 / 5 / 5
'Micro-Tom'	0 / 1 / 5				5 / 5 / 5	4 / 4 / 5				
'Pearson'					4 / 4 / 5		1 / 1 / 1		4 / 4 / 5	5 / 5 / 5
'Sekaiichi'		0 / 0 / 5	1 / 1 / 2		4 / 4 / 5				3 / 3 / 5	
'World Best'									4 / 4 / 5	
'California Red Cherry'	3 / 3 / 5		1 / 1 / 5		4 / 4 / 5	5 / 5 / 5			2 / 2 / 5	
'Sub-Arctic Cherry'	5 / 5 / 5		3 / 3 / 5		5 / 5 / 5	3 / 3 / 4	5 / 5 / 5	4 / 4 / 5	4 / 4 / 5	
W-C 1033		0 / 0 / 5	5 / 5 / 5		4 / 4 / 5	5 / 5 / 5			5 / 5 / 5	

[a] Number of hybrids that formed tumors / number of hybrids obtained / number of seeds sown.

weeks after germination. During cultivation, hybrid seedlings from 41 cross-combinations developed soft callus-like structures (Table 3). These tumors were formed most frequently on leaves, but also on cotyledons, stems and petioles (Figure 1A to D). The tumors did not differentiate into teratomas, unlike interspecific *Nicotiana* hybrids (Ahuja, 1965; Dodueva et al., 2007). No differences in tumorigenesis were observed among hybrid seedlings from different crosses. Although a hybrid seedling from the cross 'Micro-Tom' × LA1559 did not develop a tumor, only one hybrid was raised (Table 3).

When hybrid seedlings that showed tumors were transferred to a greenhouse at four weeks after germination, the tumors disappeared and necrotic brown spots were visible at the positions where the tumors existed previously (Figure 1E). All hybrids from the 41 cross-combinations grew to maturity and flowered without forming new tumors. These results suggest that tumorigenesis was affected by environmental conditions and we reinvestigated this phenomenon in an additional experiment (see below).

Confirmation of hybridity

The analyses were conducted using hybrid plants from two crosses, 'Heinz 1706-BG' × PI 365934 and 'Micro - Tom' × PI 365904. The morphological characteristics of these

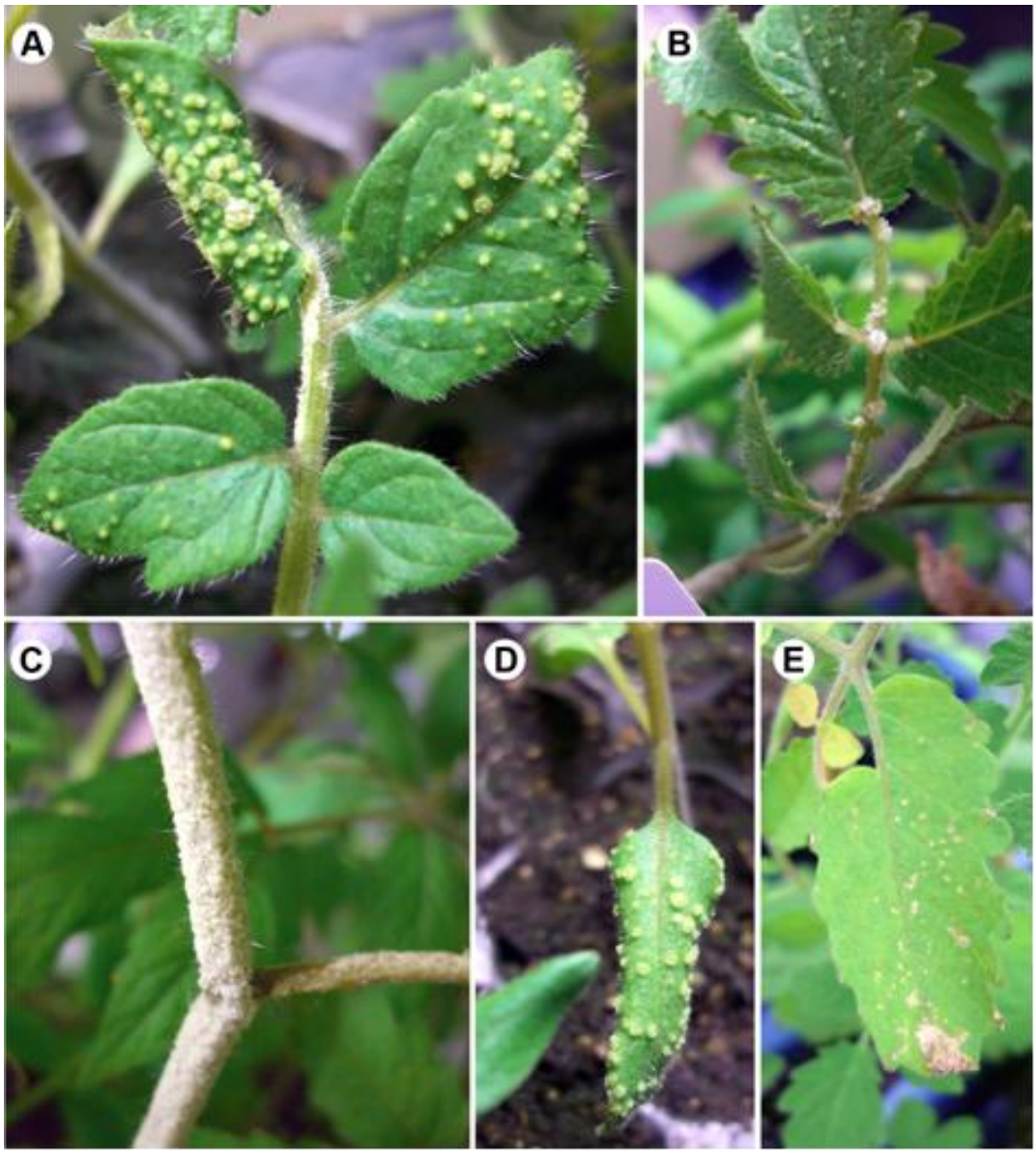

Figure 1. Tumor formation in *S. lycopersicum* × *S. habrochaites* hybrids grown in a growth chamber. (A) Tumors on the leaves of a hybrid from the cross 'Micro-Tom' × PI 365904. (B) Tumors on the petiole of a hybrid from the cross 'Heinz 1706-BG' × PI 365934. (C) Tumors on the stem surface of a hybrid from the cross 'Micro-Tom' × PI 365904. (D) Tumors on the cotyledon of a hybrid from the cross 'World Best' × PI 365934. (E) Necrotic brown spots on leaves of a hybrid from the cross 'Heinz 1706-BG' × PI 365934. When the tumorous hybrids were transferred from the growth chamber to the greenhouse, the tumors formed on the leaves disappeared and was replaced by necrotic brown spots.

hybrid plants were uniform within each cross. The leaf shape and flower shape of the hybrid plants were intermediate in appearance between those of the parents (Figure 2). Flow cytometric analysis was conducted on the hybrid plants and their parents. If the DNA contents of the parents differ significantly, those of true hybrids are expected to be intermediate between the parents. For analyses of hybrid plants, leaves lacking a tumor were used. The positions of G1 peaks differed between 'Heinz 1706-BG' and PI 365934, and the four hybrid plants analyzed showed DNA contents intermediate between those of the parents (Figure 3). Because the nuclear DNA contents of 'Micro - Tom' and PI 365904 were similar, verification of hybridity by flow cytometry was impossible for the cross 'Micro-Tom' × PI 365904.

RAPD analysis was conducted with 20 random primers on hybrid plants from the crosses 'Heinz 1706-BG' × PI 365934 and 'Micro-Tom' × PI 365904 to confirm if these plants were true hybrids (Figure 4). For analyses of hybrid plants, leaves lacking a tumor were used. In the cross 'Heinz

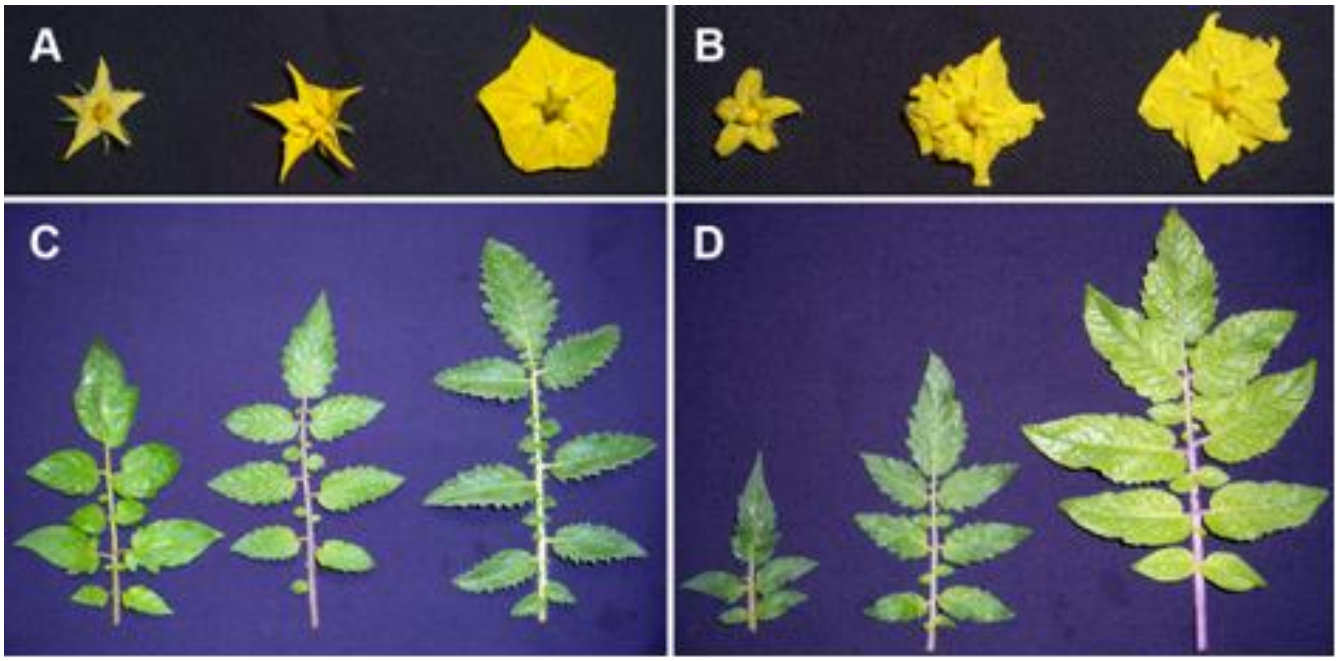

Figure 2. Morphological characteristics of flowers (A, B) and leaves (C, D) of hybrids raised from *S. lycopersicum* × *S. habrochaites* crosses. (A, C) 'Heinz 1706-BG', a hybrid obtained from the cross 'Heinz 1706-BG' × PI 365934, and PI 365934 (left to right). (B, D) 'Micro-Tom', a hybrid obtained from the cross 'Micro-Tom' × PI 365904, and PI 365904 (left to right).

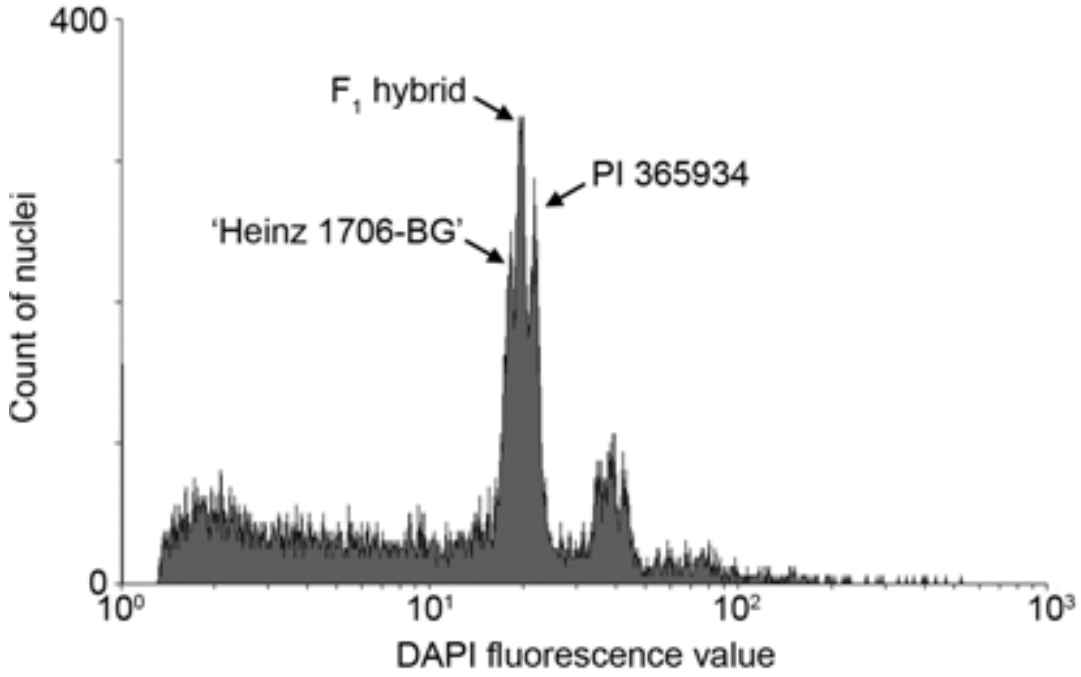

Figure 3. Histogram obtained by flow cytometric analysis of nuclei from leaves of a mixed sample of a hybrid from the cross 'Heinz 1706-BG' × PI 365934 and the parents.

1706-BG' × PI 365934, 18 primers gave RAPD patterns that showed clear polymorphisms between the parents; 26 bands were detected only in 'Heinz 1706-BG' and 31 bands were detected only in PI 365934. Four hybrid plants analyzed had all 57 bands characteristic of both parents. In the cross 'Micro-Tom' × PI 365904, 18 primers have

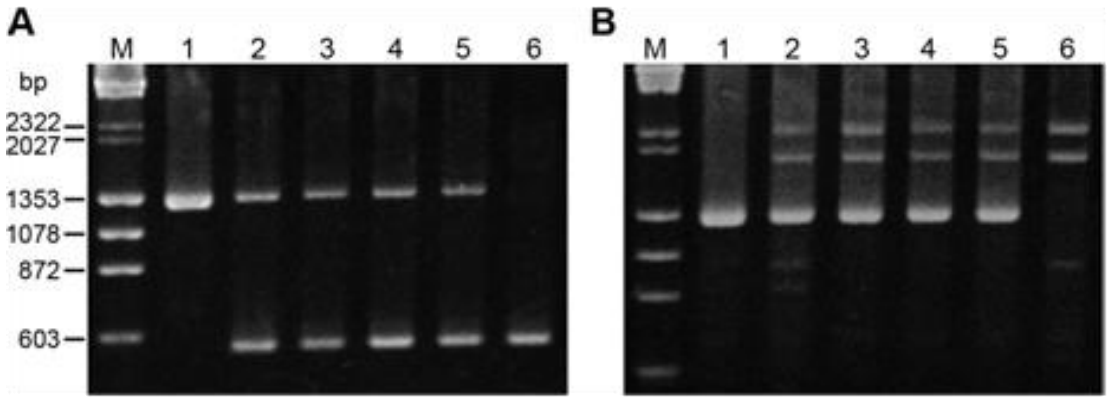

Figure 4. Confirmation of hybridity between *S. lycopersicum* and *S. habrochaites* by RAPD analysis with the primer OPA-08. **(A)** RAPD analysis of hybrid plants raised from the cross 'Heinz 1706-BG' × PI 365934. **(B)** RAPD analysis of hybrid plants raised from the cross 'Micro-Tom' × PI 365904. M, size markers (λ/*Hin*d III and φX174/*Hae* III). Lane 1, *S. lycopersicum*; lanes 2–5; hybrid plants; lane 6, *S. habrochaites*.

Table 4. Tumor formation in hybrid seedlings between *S. lycopersicum* and *S. habrochaites* cultivated in a growth chamber or greenhouse.

Cross-combination	Environment	Number of hybrid		Percentage of hybrids that developed tumors
		Cultivated	Tumors developed	
'Heinz 1706-BG' × PI 365934	Growth chamber	39	14	36
	Greenhouse	15	0	0
'Micro-Tom' × PI 365904	Growth chamber	39	6	15
	Greenhouse	15	0	0

gave RAPD patterns that showed clear polymorphisms between the parents; 29 bands were detected only in 'Micro-Tom' and 23 bands were detected only in PI 365904. Four hybrid plants analyzed had all 52 bands characteristic of both parents. Thus, the plants raised from the two crosses were confirmed to be true hybrids.

Self-pollination of hybrid plants from the two crosses was conducted. Seven of the 10 pollinated flowers produced seeds in the cross 'Micro-Tom' × PI 365904, whereas in the cross 'Heinz 1706-BG' × PI 365934 the 10 pollinated flowers abscised without enlargement of the ovary and no seeds were obtained. This result might be because of hybrid sterility or the fact that *S. habrochaites* is self - incompatible. Acetocarmine staining suggested that 86 and 68% of pollen grains were fertile in hybrid plants from the crosses 'Heinz 1706-BG' × PI 365934 and 'Micro-Tom' × PI 365904, respectively.

Tumorigenesis affected by environmental conditions

For each of the crosses 'Heinz 1706-BG' × PI 365934 and 'Micro-Tom' × PI 365904, 54 hybrid plants were propagated by cuttings and used to determine whether environmental conditions influence tumorigenesis. For both crosses, 39 and 15 hybrid plants were grown for 60 days in a growth chamber and in a greenhouse, respectively (Table 4). When grown in a growth chamber, 36% of 'Heinz 1706-BG' × PI 365934 hybrids and 15% of 'Micro-Tom' × PI 365904 hybrids formed tumors. Conversely, tumors were never formed in hybrid plants grown in a greenhouse.

The formation of genetic tumors caused by the *Frs* gene in the cross *S. lycopersicum* × *S. chilense* is affected by environmental conditions (Doering and Ahuja, 1967). The tumors are formed only in leaves when the plants carrying *Frs* are cultivated in greenhouse conditions. However, the tumors are formed over the entire surface of stems, flower-bearing branches within the inflorescence and leaves when the plants are cultivated in a growth chamber in which the humidity is considerably higher than that in the greenhouse. When the plants are transferred to the greenhouse, the stem tumors dry up, leaving necrotic brown spots. These characteristics are similar to the tumorigenesis responses observed in hybrids raised from *S. lycopersicum* × *S. habrochaites* crosses in the present study. The environmental conditions in the greenhouse and growth chamber differed in several regards, including temperature, humidity, and the quality, quantity and intensity of light. The specific environmental factors that influence tumorigenesis require determination in a future study.

Table 5. Production of callus-like structures and shoots from stem explants in hybrid seedlings between *S. lycopersicum* and *S. habrochaites*.

Cross-combination	Number of explants			Percentage of explants that produced shoot
	Cultured	Callus-like structures produced	Shoot produced	
'Heinz 1706-BG' × PI 365934	83	83	20	24
'Micro-Tom' × PI 365904	85	85	4	5

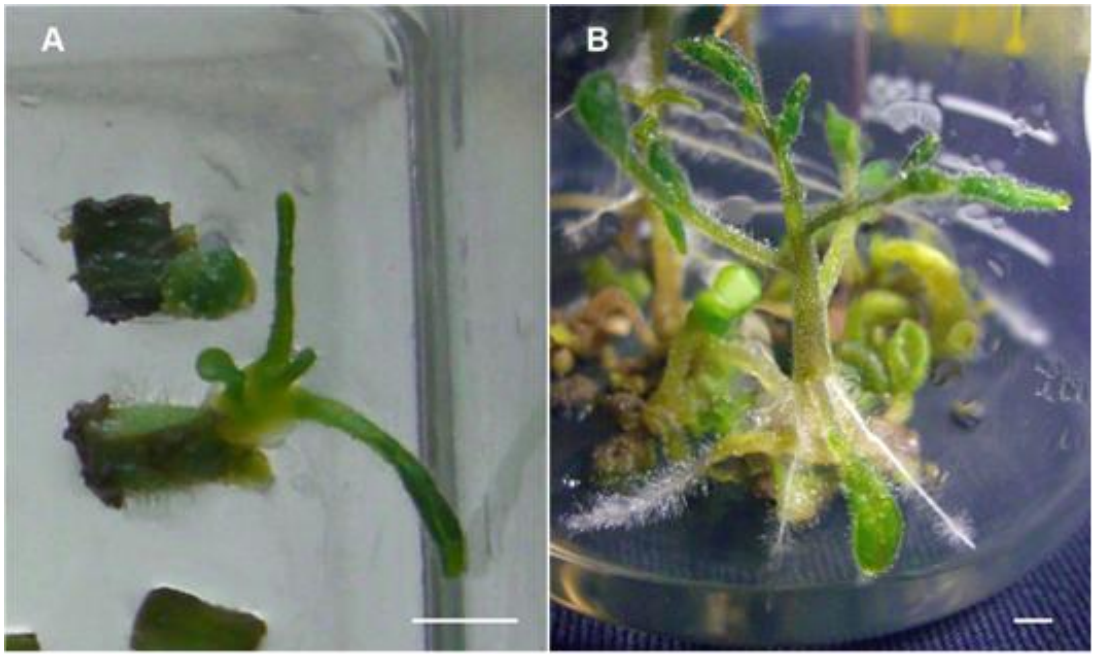

Figure 5. Shoot regeneration from cultured stem segments of hybrids between *S. lycopersicum* 'Heinz 1706-BG' and *S. habrochaites* PI 365904. **(A)** Formation of callus like-structures and shoots. **(B)** Rooted plantlet. Bars = 5 mm.

Formation of callus-like structures and shoots from stem explants

Attempts to culture tumors produced by hybrids obtained from *S. lycopersicum* × *S. habrochaites* crosses were unsuccessful. Next, we cultured stem segments of hybrids raised from crosses 'Heinz 1706-BG' × PI 365934 and 'Micro-Tom' × PI 365904 on PGR-free medium. Callus-like structures or tumors were observed in all explants after about one week of culture. After culture for 8 weeks, 24 and 5% of explants from the respective crosses produced adventitious shoots from the tumors (Table 5, Figure 5). When internodal segments of hybrid seedlings raised from the cross *N. glauca* × *N. langsdorffii* are cultured *in vitro* on PGR-free MS medium, tumorous tissues are induced from the segments and subsequently shoots can be regenerated from the tumors (Ichikawa and Syono, 1988; Ichikawa et al., 1989). Changes in the levels of auxin and cytokinin, or in the sensitivity to these phytohormones, are considered to be related to tumorigenesis in this cross (Ichikawa et al., 1989, 1990; Feng et al., 1990; Fujita et al., 1991; Ichikawa and Syōno, 1991). Because tumors and shoots were induced in *S. lycopersicum* × *S. habrochaites* hybrids through *in vitro* culture of stem segments (Table 5, Figure 5), auxin and cytokinin might also be related to tumorigenesis in this cross.

In conclusion, we revealed that hybrid plants obtained from crosses between *S. lycopersicum* and *S. habrochaites* produced tumors on their leaves, cotyledons, stems and petioles. Tumor formation was indicated to be influenced by environmental conditions and was induced by *in vitro* culture of stem segments of the hybrids. Thus, hybrids between *S. lycopersicum* × *S. habrochaites* provide an excellent experimental system to study plant tumorigenesis and to understand cell division and differentiation.

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