Plant Cell Rep (2002) 20:1022–1032
DOI 10.1007/s00299-001-0429-5

**F. Rizza · G. Mennella · C. Collonnier · D. Sihachakr
V. Kashyap · M. V. Rajam · M. Presterà · G. L. Rotino**

Androgenic dihaploids from somatic hybrids between *Solanum melongena* and *S. aethiopicum* group *gilo* as a source of resistance to *Fusarium oxysporum* f. sp. *melongenae*

Received: 9 September 2001 / Revised: 19 November 2001 / Accepted: 6 December 2001 / Published online: 13 February 2002

Abstract Dihaploid plants were obtained through anther culture of somatic hybrids between eggplant and *Solanum aethiopicum gilo*. The androgenic origin of the dihaploids was demonstrated by ploidy determination (flow cytometry and chloroplast counting) and isozyme and molecular (I-SSR and RAPDs) analyses. The androgenic plants showed significant morphological variability in the traits analysed. Pollen viability in the diploid androgenic plants was drastically reduced with respect to the somatic hybrids; however most of the dihaploids produced parthenocarpic fruits. *S. aethiopicum* and the somatic hybrids showed complete resistance to fungal wilt caused by *Fusarium oxysporum* f. sp. *melongenae*. Out of the 41 dihaploids inoculated, 34 were symptomless. The population of androgenic plants developed may represent a useful source for introgression of the *Fusarium* resistance trait into eggplant.

Keywords Androgenesis · Eggplant · *Solanum aethiopicum* gr. *gilo* · Somatic hybrids · *Fusarium oxysporum* f. sp. *melongenae*

Communicated by K. Glimelius

F. Rizza · M. Presterà · G.L. Rotino (✉)
Istituto Sperimentale per l'Orticoltura, Via Paullese 28,
26836 Montanaso Lombardo (LO), Italy
e-mail: pinuzzu@libero.it
Tel.: +39-0371-68171, Fax: +39-0371-68172

G. Mennella
Istituto Sperimentale per l'Orticoltura, Via Cavalleggeri 25,
84098 Pontecagnano (SA), Italy

C. Collonnier · D. Sihachakr
Bât. 360, Morphogénèse Végétale Expérimentale,
Université Paris Sud, 91405 Orsay Cedex, France

V. Kashyap · M.V. Rajam
Department of Genetics, University of Delhi – South Campus,
Benito Juarez Road, New Delhi-21, India

Present address:
F. Rizza, Istituto Sperimentale per la Cerealicoltura,
Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy

Abbreviations *BAP*: 6-Benzylaminopurine · *FDA*: Fluorescein diacetate · *Kin*: Kinetin · *IAA*: Indol-3-acetic acid · *NAA*: α-Naphthaleneacetic acid · *TDZ*: Thidiazuron · *Zeatin*: 6-(4-Hydroxyl-3-methylbot-2-enyl) aminopurine

Introduction

Progress in crop improvement is dependent on the preservation and induction of genetic variability. The cultivation of only a few improved cultivars over large areas has led to a reduction in the genetic diversity of many crops, especially in the self-pollinated ones whose genetic bases are apt to be narrower (Fulton et al. 1997). Wild and cultivated relatives are a valuable source of genes that can be exploited to increase the variability of the cultivated crops by the genetic introgression of useful agronomic traits (Daunay et al. 1991; Eshed and Zamir 1995; Chen et al. 1999). Somatic hybridisation provides an effective means for accessing allied and wild germplasm by overcoming sexual barriers between species and genera.

A main factor limiting the yield and commercial value of horticultural crops is their susceptibility to diseases. In particular, the development of cultivars resistant to soil-borne diseases is a major goal. In vegetable crops of the *Solanaceae*, resistance to soil-borne diseases has been introduced from wild into cultivated species through somatic fusion; this is particularly true in tomato (Lefrancois et al. 1993) and potato (Austin et al. 1988; Deimling et al. 1988; Brown et al. 1995; Laferriere et al. 1999; Fock et al. 2000). Likewise, somatic hybridisation has been successfully applied to obtain interspecific hybrids between eggplant (*Solanum melongena*) and its relatives resistant to soil-borne diseases caused by nematodes, *Verticillium*, *Fusarium* and *Ralstonia* (Gleddie et al. 1986; Guri and Sink 1988; Sihachakr et al. 1988; Daunay et al. 1993; Sihachakr et al. 1994; Jarl et al. 1999; Collonnier at al. 2001).

Fusarium oxysporum f. sp. *melongenae* induces vascular wilt disease in eggplant and causes heavy yield losses

in Asian countries (Kennet et al. 1970; Kishi 1974). This disease also occurs in Europe (van Steekelenburg 1976), both in greenhouse and open-field cultivations. As the symptoms are often confused with those of *Verticillium* wilt, the incidence of *Fusarium* disease is probably underestimated (Stravato et al. 1993). Since fungicides are costly, polluting and relatively ineffective, strategies for controlling *Fusarium* should be directed towards the development of resistant cultivars.

Solanum aethiopicum, a close relative species of *S. melongena*, includes accessions resistant to the soilborne pathogens *F. oxysporum* f. sp. *melongenae* and *Ralstonia solanacearum* (Hébert 1985; Daunay et al. 1991; Cappelli et al. 1995). Collonnier et al. (2001) used two accessions of *S. aethiopicum*, groups *aculeatum* and *gilo*, to produce somatic hybrids that were significantly more resistant to *Ralstonia* than the cultivated eggplant

In the investigation reported here we produced dihaploid plants through anther culture from somatic hybrids between eggplant (*S. melongena*) and *S. aethiopicum* group *gilo* in order to evaluate and exploit the potential of *Fusarium* resistance from *S. aethiopicum* for eggplant improvement. The dihaploid androgenetic plants were morphologically and molecularly characterised, evaluated for fertility and tested for resistance to *Fusarium*.

Materials and methods

Plant materials

Three somatic hybrids, D-Sa 2/1, D-Sa 2/2 and D-Sa 2/3, were obtained from protoplast electrofusion between *Solanum melongena* cv. Dourga and the wild species *S. aethiopicum* group *gilo* as described in Collonnier et al. (2001). The plants were grown both in vitro and in pots under greenhouse conditions.

Anther culture

Anther culture was carried out according to Rotino (1996) using plants grown in the greenhouse. Briefly, anthers were placed in a 60-mm petri dish containing the induction medium C3 (3 mg l^{-1} Kin + 1 mg l^{-1} IAA), C6 (5 mg l^{-1} Kin + 5 mg l^{-1} NAA), C9 (1 mg l^{-1} Zeatin + 3 mg l^{-1} NAA) or C12 (0.5 mg l^{-1} TDZ + 0.1 mg l^{-1} Zeatin + 0.5 mg l^{-1} IAA), maintained at 35°C in the dark for 8 days and then transferred to a growth chamber maintained at 25°C, a 16/8-h (day/night) photoperiod and a light intensity of 50 µE m^{-2} s^{-1}. After 4 days, the anthers were transferred to regeneration medium containing only the cytokinin (0.1 mg l^{-1}) employed in the induction medium as growth regulator. Embryos sprouting from the anthers were placed in V3 medium (Chambonnet 1985) for germination. Androgenic calli were transferred to a regeneration medium composed of MS (Murashige and Skoog l962) basal medium supplemented with Zeatin (0.5 mg l^{-1}), Kin (0.3 mg l^{-1}) and BAP (0.1 mg l^{-1}). Regenerated shoots were rooted in V3 medium.

Ploidy determination

Chloroplast counting

Small pieces (approximately 0.5 cm^2) of leaves from in vitro-grown plants were covered by one drop of FDA (50 µg ml^{-1} in acetone diluted 1:100 with distilled water) and quickly observed with a microscope under UV-filtered light. The ploidy level was indirectly detected by counting (magnification: 400×) the chloroplast number in the stomata guard cells of the lower leaf epidermis. Observations were carried out using three different leaves of in vitro-grown plants and counting the chloroplasts in ten stomata per leaf (Rotino 1996).

Flow cytometry

The protocol used for the flow cytometry analysis has been reported in Collonnier et al. (2001).

Morphological characterisation and pollen viability

Analysis was carried out using the fusion parents, the interspecific somatic hybrids and androgenic plants grown in the greenhouse. For each genotype, the number of flowers per inflorescence was recorded from seven inflorescences. Moreover, for the principal flower of each analysed inflorescence the following traits were recorded: flower width and length; number and length of anthers. The petal and stigma colour were also registered. Pollen viability was recorded with a phase-contrast microscope after staining with acetocarmine. The percentage of stained pollen grains was calculated on about 1,000 pollen grains from at least ten flowers collected at different times during the whole growing season. Colour, weight, peduncle length and polar and equatorial diameter were determined on the fruits, when the latter were produced.

Molecular characterisation

DNA was extracted from young leaves using the DNeasy Plant Minikit following the manufacturer's instructions (Qiagen, Valencia, Calif.). Inter-simple sequence repeat (I-SSR) analysis was carried out using UBC primer set no. 9 (University of British Columbia, Vancouver, B.C.). The reaction was performed in 20 µl aliquots containing 1× buffer (GibcoBRL, Gaithersburg, Md.), 2 m*M* $MgCl_2$, 0.1 m*M* dNTPs, 0.8 U platinum *Taq* Polymerase (GibcoBRL) and 1 ng $µl^{-1}$ DNA. The amplification reaction was run according to the following profile: one cycle of 3 min at 94°C; 45 cycles of 30 s at 94°C, 45 s at 55°C, 2 min at 72°C; one cycle of 5 min at 72°C.

Random amplified polymorphic DNA (RAPD) analysis was carried out using five primers (L15–L19) from Life Technologies (Gaithersburg, Md.), four primers (F4, G7, G12, D15) from Operon Technology and five primers (G1–G5) from Genmed. The reactions were performed in 25 µl aliquots, each containing 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 2 m*M* $MgCl_2$, 100 µ*M* dNTPs, 0.5 U *Taq* polymerase (MBI Fermentas), 1 µ*M* primer and 25 ng DNA. The following reaction cycle was adopted: one cycle of 94°C for 4 min, 35°C for 1 min, 72°C for 2 min; 44 cycles of 94°C for 1 min, 35°C for 1 min, 72°C for 2 min; one cycle of 72°C for 10 min.

Polymerase chain reaction (PCR) products were separated by electrophoresis on a 1.2% agarose gel containing 0.25 µg ml^{-1} ethidium bromide at 55 V/cm for 4 h in TAE buffer. The amplified DNA fragments were observed under UV light and documented using the Gel Doc 1000 (Bio-Rad, Hercules, Calif.) apparatus.

Isozyme analysis

The enzyme activity stainings of shikimate dehydrogenase (SKDH, E.C. 1.1.1.25) and glucose-6-phosphate dehydrogenase (G-6-PDH, E.C. 1.1.1.49) were analysed according to Vallejos (1983). Young leaves (200 mg) were homogenised at 4°C in two volumes (w/v) of extraction buffer with 5% insoluble polyvinylpolypyrrolidone. The extraction buffer consisted of 50 m*M* TrisHCl pH 6.8, 1% β-mercaptoethanol and 15% glycerol. The homogenates were centrifuged at 4°C for 15 min at 10,000 *g* and 8 µl of the supernatants used for electrophoresis.

Native horizontal 10–15% polyacrylamide gradient gel electrophoresis was performed using the PhastSystem (Pharmacia-LKB) according to the manufacturer's instructions. Electrophoresis separations were carried out at 4°C and 10 mA/gel.

Test for *Fusarium* resistance

Inoculation with an Italian isolate of *Fusarium oxysporum* f. sp. *melongenae* was carried out according to the root-dip method described in Cappelli et al. (1995) using ex vitro plantlets acclimatised for 3 weeks. Inoculated plants were kept in a growth chamber (25°±2°C; 50 µE m^{-2} s^{-1}), and after 4 weeks the number of healthy or dead plants was scored.

Statistical analysis

Data were analysed by a two-way analysis of variance. Seven replications, represented by the measurements on the inflorescences and the correspondent principal flowers, were employed for flower characteristics (number of flowers per inflorescence, width and length of the flower, number and length of the anther). Three replications were used for pollen viability (each replication was the mean value of 11–20 counts in a field of view at 250× magnification under a phase-contrast microscope) and fruit characteristics (weight, polar and equatorial diameter, peduncle length; each replication was the average of the data from the harvested fruits randomly pooled into three groups). The least significant difference (LSD) test was used to identify the significantly different means.

Results

Anther culture

A total of 2,760 anthers was cultured on the four induction media, giving rise to androgenic embryos and calli (Table 1; Fig. 1A). Complete plants were obtained from 83% of the embryos and 65% of the calli. In total, 159 dihaploid plants were raised. Among the media tested, C3 was ineffective in inducing androgenic embryos and calli; medium C9 gave the highest yield of embryos and calli. Medium C6 showed a higher aptitude to produce embryos than C12.

Ploidy determination

In the first experiment, the ploidy level of the fusion parents, the interspecific somatic hybrids and 26 embryo-derived androgenic plants was determined by flow cytometry and by counting the number of chloroplasts in the stomata guard cells (Table 2). There was about double the number of chloroplasts per stomata guard cell in the somatic hybrids as in the parental genotypes, as was expected for plants having a higher ploidy level. In most of the androgenic plants, the values were comparable to that of the parental species, showing a putative return to the diploid state. In three plants (D-Sa 2/1-E4, D-Sa 2/2-E15 and -E23) the number of chloroplasts was similar to that of the somatic hybrids and, consequently, these three plants were classified as tetraploids.

Data from flow cytometry analyses were consistent with the determination of the chloroplast number in the

Table 1 Number of cultured anthers and androgenic plants obtained from direct embryogenesis (*E*) or callus induction (*C*) in the somatic hybrids using four induction media. The corresponding percentages are also indicated

Induction media	Somatic hybrid															
	D-Sa 2/1					D-Sa 2/2					D-Sa 2/3					
	Number of anthers	E		C		Number of anthers	E		C		Number of anthers	E		C		
		Number	Percentage	Number	Percentage		Number	Percentage	Number	Percentage		Number	Percentage	Number	Percentage	
C3	115	0	0	0	0	331	0	0	0	0	137	0	0	0	0	
C6	157	9	5.7	1	0.6	354	9	2.5	2	1.2	206	2	0.1	0	0	
C9	166	16	9.6	1	0.6	374	29	8.2	35	9.4	214	6	2.8	7	3.3	
C12	160	3	1.9	2	1.3	349	3	0.8	10	2.9	197	5	2.5	9	4.6	
Total	298	28	5.0	4	0.7	1,408	41	2.9	47	3.3	754	13	1.7	16	2.1	

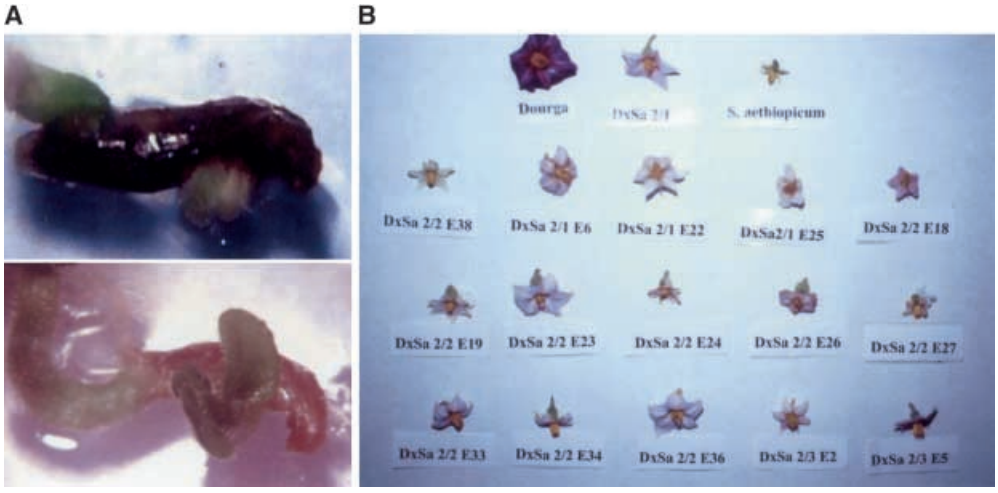

Fig. 1 **A** Androgenic embryos (*bottom*) and calli (*top*) sprouting from cultured anthers of the somatic hybrids between eggplant and *Solanum aethiopicum* group *gilo*. **B** Flowers from fusion parents, somatic hybrids and dihaploids. *Upper row* flowers of cv. Dourga (*left*), a somatic hybrid (*middle*) and *S. aethiopicum* (*left*); *lower rows* flowers of different androgenic plants**.** Note the variability in size, shape and petal colour of flowers from dihaploids

Table 2 Ploidy level, number of chloroplasts (± standard deviation) and relative amount of DNA (± standard deviation) in the fusion parents (*Solanum melongena* cv. Dourga and *S. aethiopicum* gr. *gilo*), three somatic hybrids and 26 androgenic plants obtained from anther culture of the interspecific hybrids

Genotype		Chloroplasts number	Flow cytometry	Ploidy level
S. melongena		9.1±1.2	46±6	2*x*
S. aethiopicum		12.0±1.4	47±6	2*x*
D-Sa 2/1		22.5±2.7	102±6	4*x*
D-Sa 2/2		20.9±2.1	97±4	4*x*
D-Sa 2/3		19.8±2.3	100±6	4*x*
D-Sa 2/1	E4	19.0±1.6	98±5	4*x*
D-Sa 2/2	E1	13.3±1.9	50±6	2*x*
	E2	9.2±3.5	51±6	2*x*
	E3	11.0±2.1	51±6	2*x*
	E4	11.0±2.2	50±7	2*x*
	E5	12.0±1.8	52±6	2*x*
	E6	11.8±1.3	51±7	2*x*
	E7	13.9±1.8	48±6	2*x*
	E10	13.2±1.4	51±6	2*x*
	E11	10.7±2.2	50±7	2*x*
	E13	10.0±2.4	50±6	2*x*
	E14	12.5±1.3	49±5	2*x*
	E15	28.5±4.1	103±6	4*x*
	E16	12.4±1.1	50±6	2*x*
	E17	12.1±2.0	47±5	2*x*
	E20	11.2±2.0	49±7	2*x*
	E22	9.4±1.4	49±5	2*x*
	E23	21.4±4.1	99±5	4*x*
	E27	12.5±1.7	50±5	2*x*
	E35	14.7±1.8	49±7	2*x*
D-Sa 2/3	E1	9.8±3.3	49±6	2*x*
	E3	10.9±1.4	47±6	2*x*
	E5	11.2±1.1	50±6	2*x*
	E7	15.4±3.2	51±5	2*x*
	E9	13.2±1.9	49±5	2*x*
	E11	12.0±2.2	49±6	2*x*

stomata guard cells. The analyses confirmed that the somatic hybrids contained twice the amount of DNA as the diploid parental species. Twenty-three androgenic plants were diploids, showing a DNA amount identical to that of the fusion parents (Table 2; Fig. 2); the remaining three plants, having the same DNA amount as the somatic hybrids, were confirmed to be tetraploids.

Due to the consistent agreement between the two methods, further determination of the ploidy level of the androgenic plants was carried out only by counting chloroplasts in the stomata guard cells (Table 3). The results showed that 88% of the androgenic plants obtained by direct embryogenesis and 75% of the callus-derived plants were diploids, while the remaining plants were tetraploids. In both cases, the highest percentage of tetraploids was found among the plants originating from D-Sa 2/3 (23% and 37.5%, respectively).

Morphological characterisation and pollen viability

A detailed morphological characterisation was performed on the three anther donors, the fusion parents and 42 dihaploid plants, of which 36 were obtained through direct embryogenesis and six through plant regeneration from androgenic callus. Growth habit, leaf shape (data not shown) and flower and fruit characteristics displayed a high level of variability (Table 4). Except for D-Sa 2/1-E3 and D-Sa 2/3-C2, the number of flowers per inflorescence in the dihaploids and in the somatic hybrids was significantly higher than that in the fusion parents, with the dihaploid D-Sa 2/3-E2 having the highest value. With respect to flower length, D-Sa 2/1-E11 had the highest value of all the genotypes analysed; in the other dihaploids the values of this trait were within the range of variation of the fusion parents (eggplant cv. Dourga with the maximum and *Solanum aethiopicum* with the minimum). Four dihaploids (D-Sa 2/1-E1, D-Sa 2/2-E7, -E12 and D-Sa 2/3-E7) and *S. aethiopicum* had the lowest values of flower width, while no androgenic plants reached the value of cv. Dourga. Eggplant had significantly longer anthers than *S. aethiopicum*, with some dihaploids showing values either higher (D-Sa 2/1-E4 and D-Sa 2/3-C1) than eggplant or lower (D-Sa 2/1-E8, D-Sa 2/2-E3, -E6 and -E29) than *S. aethiopicum*. Among all the plants analysed, the number of anthers per flower was higher in the fusion parents and in the dihaploids D-Sa 2/2-E1, -E2 and -E16.

The white or pale-violet colour was predominant in the petals (Fig. 1B), while the stigma colour varied from

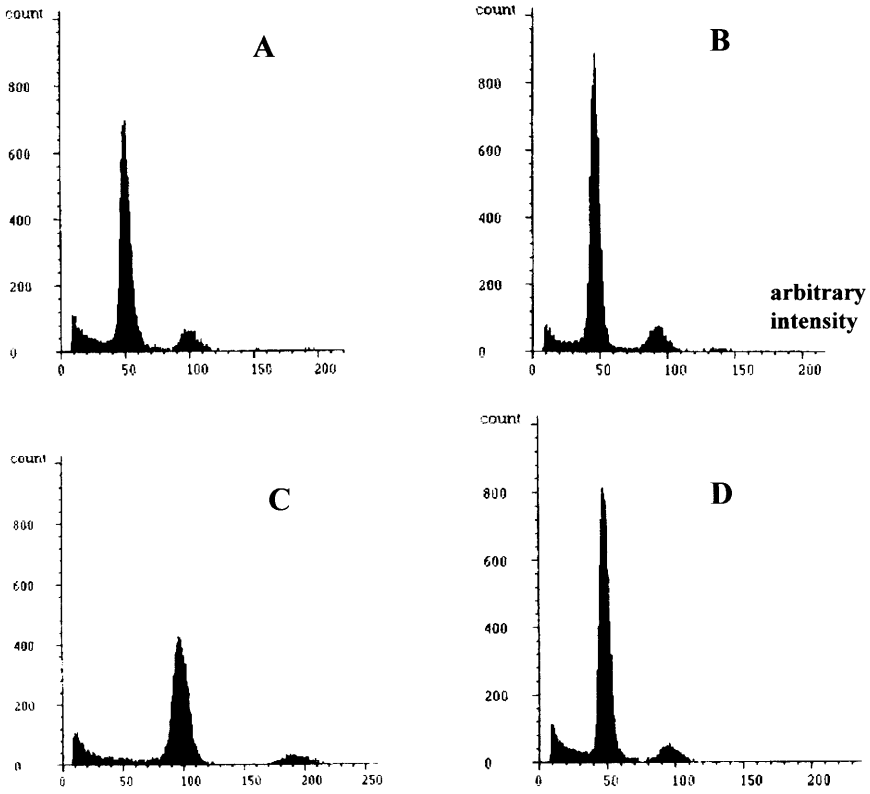

Fig. 2A–D Diagrams of relative nuclear DNA contents obtained by flow cytometric analysis: **A** *Solanum melongena* cv. Dourga, **B** *S. aethiopicum* gr. *gilo*, **C** their tetraploid somatic hybrid, **D** a diploid androgenic plant. Fluorescence intensity is proportional to nuclear DNA quantity, and the position of the dominant peak reflects the ploidy level

Table 3 Number of androgenic plants derived from embryos (*E*) or callus (*C*) and classified as diploid or tetraploid by chloroplast counting in the stomata guard cells

Anther	Number of diploids		Number of tetraploids	
Donor	E	C	E	C
D-Sa 2/1	26	3	2	1
D-Sa /2	36	37	5	10
D-Sa 2/3	10	10	3	6
Total	72	50	10	17

green to orange. The type of pistil, longi-style in the flower of the fusion parents and meso-style in the somatic hybrids, was longi-style or meso-style in the dihaploids.

Among the dihaploids analysed, three showed small spines on the leaf main vein and calyx, while the other plants were spineless (Table 4).

Pollen viability was drastically reduced in the diploid androgenic plants when compared to that of the somatic hybrids and fusion parents; two tetraploids had higher pollen viability (64% in D-Sa 2/1-E4 and 48% in D-Sa 2/2-E15). Moreover, the dihaploids D-Sa 2/2-E28 and -E35 were male-sterile as no pollen was found in their anthers.

Out of the 42 androgenic plants analysed, 30 produced parthenocarpic fruits, and some of these plants were particularly productive (Table 4; Fig. 3F–H). There was no complete agreement between the percentage of viable pollen grains and the number and size of fruit produced by the dihaploids. In fact, the dihaploids D-Sa 2/2-E15 and -E26 were not able to set any fruits at all, even though they had 48% and 25% viable pollen, respectively. A total of 770 fruits were collected from the dihaploids. All of the fruits were orange at maturity, like those of the somatic hybrids and *S. aethiopicum* (Fig. 3A). All but two of the dihaploids produced immature fruits with dark-green stripes in their upper part before physiological maturity, as observed in *S. aethiopicum* and the somatic hybrids (Fig. 3E, F, H). The stripes were absent in D-Sa 2/1-E8 and D-Sa 2/2-C7, whose fruits showed a homogeneous white skin colour as in the eggplant cv. Dourga (Table 4; Fig. 3B, C). A wide variability was found in the weight and size of the fruits (Table 4; Fig. 3B–H). The weight and size of the fruits of the cultivated eggplant were never reached by the dihaploids. The weight of the fruits produced by most of the androgenetic plants was similar to that of the anther donors and *S. aethiopicum*. However, the average weight of fruits obtained from D-Sa 2/3-C1 (Fig. 3H) and D-Sa 2/1-E4 was significantly higher than those of all the other dihaploids (Table 4). With regard to the polar and equatorial diameter of the fruits, values significantly higher or lower were evidenced in some dihaploids with respect to those of the anther donors and/or of the fusion parents. The fruit peduncle length, about 3 cm in the somatic hybrids, varied significantly among the dihaploids (mean values: 1.8–4.3 cm), which showed a minimum and maximum similar to the values of the fusion parents.

Taken globally, with respect to the morphological traits tested we found significant differences among the dihaploids.

Table 4 Morphological characteristics of flowers and fruits, pollen viability and resistance to *Fusarium oxysporum* f. sp. *melongenae* of the fusion parents, *S. melongena* cv. Dourga and *S. aethiopicum* gr. *gilo*, the somatic hybrids and androgenic plants. Means followed by a common letter are not significantly different according to LSD test (P=0.05) (*G* green, *L* long-style, *M* meso-style, *O* orange, *P* pale violet, *V* violet, *W* white, *NT* not tested)

Genotype		Flowers									Fruits					Resistance to *Fusarium*	
		Number of flowers per inflores	Flower width (mm)	Flower length (mm)	Anther length (mm)	Number of anthers per flower	Petal colour	Type of pistil	Stigma colour	Pollen viability (%)	Number of fruits	Average weight (g)	Equatorial diameter (cm)	Polar diameter (cm)	Peduncle length (cm)	Number of inoculated plants	*Fusarium* resistance (%)
Dourga[a]		1.7q	8.3a	18.6b	8.1d–j	6.3a	V	L	G	74.5b	19	225a	5.5a	19.7a	4.2a	35	0
S. aethiopicum		2.0q	4.1t	10.8r,s	6.4q	6.3a	W	L	O	91.4a	20	7.9c,d	1.8g–l	2.9c	1.5i	21	100
D-Sa 2/1		5.7c–j	6.2d,e	17.1c,d	9.3a	5.3d–f	P	M	O	72.2b,c	30	16.5c,d	2.4e–k	5.7b,c	2.7b–f	12	100
D-Sa 2/2		5.6d–k	6.7c,d	17.6c,d	8.4c–f	5.7b–d	P	M	O	67.0b,c	40	18.1c,d	2.5e–h	4.7b,c	2.8b–e	17	100
D-Sa 2/3		5.9b–i	6.8c	17.9b,c	9.1a,b	5.0f–h	P	M	O	70.8b,c	17	16.6c,d	2.5e–j	5.1b,c	3.0b–d	11	100
D-Sa 2/1	E1	4.5k–n	4.3r–t	12.9j–o	7.7h–m	5.0f–h	P	M	O	1.4m,n	53	6.3c,d	1.9f–l	3.7b,c	2.4d–h	9	0
	E2	6.0b–h	5.9 e–h	13.3 i–m	7.9 f–l	5.0 f–h	P	L	G	10.5f–k	10	12.7c,d	2.3 e–l	4.4b,c	2.0g–i	15	100
	E3	2.9p,q	5.6f–l	11.9p,q	8.1d–k	5.1e–g	P	L	O	4.1i–n	–	–	–	–	–	7	100
	E4	5.7c–j	6.9b,c	16.8d,e	9.3a	5.7b–d	P	M	O	64.3c	9	90.0b	3.9b,c	9.4b,c	4.1a	4	100
	E8[b]	5.0g–m	5.7e–j	12.3o–q	5.3r	5.1e–g	P	L	O	6.0h–n	6	19.5c,d	3.0c–e	6.1b,c	2.3d–h	12	0
	E9	4.7i–n	5.1k–p	12.7k–p	8.2c–i	5.1e–g	P	M	O	3.2j–n	–	–	–	–	–	6	100
	E11	6.0b–h	5.6f–k	23.4a	7.6i–m	5.0f–h	W	L	O	0.7m,n	–	–	–	–	–	9	100
	E12	5.4e–l	6.0e–g	13.4i–l	8.6b–d	5.6c–e	P	L	O	9.0f–m	4	38.7c	3.9b	5.2b,c	2.7b–e	5	100
	E14	6.1b–g	4.8o–r	12.5m–q	8.6b–e	5.1e–g	W	M	O	8.1g–n	–	–	–	–	–	7	100
	E23[a]	5.6d–k	5.4h–n	12.8j–p	8.0e–k	5.4c–f	P	L	O	3.5i–n	–	–	–	–	–	7	100
D-Sa 2/2	E1	3.6n–p	6.6c,d	12.5m–q	7.3l–o	6.3a	W	L	G	16.7f	–	–	–	–	–	15	0
	E2	3.3o,p	7.4b	13.6i–k	7.7h–m	5.9a–c	W	L	G	4.6i–n	5	10.5c,d	2.9d,e	3.6b,c	2.3d–h	9	100
	E3	5.0g–m	5.2j–o	12.7k–p	5.6r	5.7b–d	W	L	O	2.7k–n	56	3.7d	1.6i–l	4.1b,c	3.0b–d	16	100
	E5	6.0b–h	5.0m–q	11.6q,r	6.8o–q	5.4c–f	P	M	O	7.9g–n	8	2.8d	1.4l	4.0b,c	2.5b–h	10	100
	E6	5.9b–i	4.6p–s	4.2s,t	5.2r	5.1e–g	V	L	O	2.2k–n	25	9.8c,d	2.6e–g	4.5b,c	3.1b,c	12	100
	E7	6.9a–c	4.2s–t	12.6l–p	7.1m–p	5.0f–h	P	L	O	1.9l–n	15	5.8d	1.7h–l	4.0b,c	1.8h–i	13	100
	E10[a]	4.6j–n	5.6f–l	13.6i–k	7.4l–o	5.6c–e	P	L	G	1.7l–n	–	–	–	–	–	25	100
	E11	5.6d–k	4.8o–r	13.2i–n	7.6j–n	5.6c–e	W	M	O	2.9j–n	13	6.2c,d	1.8g–l	3.7b,c	2.5b–h	6	0
	E12	6.6a–e	4.2s,t	13.2i–n	7.9f–l	5.0f–h	P	L	O	8.6f–n	15	15.9c,d	3.0d,e	4.3b,c	2.5b–h	8	100
	E13	4.6j–n	5.1l–p	12.4n–q	7.0n,p	5.1e–g	P	L	O	10.2f–l	–	–	–	–	–	11	100
	E14	6.0b–h	4.8o–r	12.6l–p	6.9o–q	5.3d–f	P	M	G	3.3j–n	71	3.5d	1.5k,l	2.5c	2.2e–i	9	100
	E15	4.9h–m	5.5g–m	13.3i–m	7.8g–l	5.7b–d	W	L	O	47.8d	–	–	–	–	–	6	100
	E16	4.3l–o	5.9e–h	13.2i–n	6.6p,q	6.1a,b	P	M	G	3.1j–n	37	7.3c,d	2.4e–k	3.4c	2.0f–i	9	100
	E24	5.4e–l	5.5g–m	10.9r,s	7.7h–m	5.3c–f	P	L	O	2.7k–n	8	34.5c,d	3.0c–e	6.7b,c	2.3d–h	5	100
	E25	4.9h–m	5.2j–o	12.9j–o	7.3l–o	5.3d–f	W	L	O	7.2g–n	9	19.9c,d	2.5e–i	4.8b,c	2.4d–h	NT	–
	E26	5.6d–k	6.9b,c	16.0e,f	8.8a–c	5.0f–h	V	L	O	25.3e	–	–	–	–	–	3	0
	E28	5.3f–m	5.3i–o	13.9i	7.4l–o	5.1e–g	W	L	O	–	41	11.5c,d	2.7e,f	5.5b,c	3.1b	4	100
	E29[a]	5.9b–i	5.8e–i	10.7s	5.4r	5.0f–h	W	L	O	13.4f–h	5	5.3 d	1.8f–l	3.0c	1.8h,i	1	100
	E34	4.3l–o	6.1e,f	12.4n–q	7.5k–n	5.4c–f	P	L	O	3.0j–n	7	6.0c,d	2.1e–l	3.6c	2.7b–e	6	100
	E35	6.4a–f	4.6p–s	12.4n–q	8.0e–k	5.0f–h	P	L	O	–	–	–	–	–	–	15	100
	E38	4.6j–n	4.9n–r	14.0h,i	8.3c–h	5.4c–f	W	M	O	6.5g–n	8	10c,d	2.4e–k	4.4b,c	2.3d–h	7	0
	C3	4.6j–n	4.9n–q	15.5f,g	8.5b–e	4.6h	V	M	O	0.1n	3	5.8d	1.6j–l	4.4b,c	4.2a	2	0
	C7[b]	5.2f–m	5.5g–m	13.6i–k	7.6i–m	5.3c–f	W	L	O	–	3	26.0c,d	3.7b–d	5.6b,c	2.4c–h	11	100
	C12	4.6j–n	4.6p–s	13.6i–k	7.6i–m	4.6h	W	M	O	0.8m,n	39	6.4c,d	2.2e–l	3.5c	2.5b–h	10	100
	C17	4.1m–o	5.0m–q	12.5m–q	7.5k–n	4.7g,h	W	L	O	6.2h–n	85	10.7c,d	2.5e–i	3.7b,c	2.6b–g	5	100

Table 4 Continued

Genotype		Flowers								Fruits					Resistance to *Fusarium*	
		Number of flowers per inflores	Flower width (mm)	Flower length (mm)	Anther length (mm)	Number of anthers per flower	Petal colour	Type of pistil	Stigma colour	Pollen viability (%)	Number of fruits	Average weight (g)	Equatorial diameter (cm)	Polar diameter (cm)	Peduncle length (cm)	Number of inoculated plants	*Fusarium* resistance (%)
D-Sa 2/3	E1	5.3f–m	5.3i–o	13.3i–m	7.8g–l	5.4c–f	P	L	O	3.5i–n	–	–	–v	–	–	23	
	E2	7.6a	5.6f-l	14.1h,i	8.1d–k	5.1e–g	P	M	O	14.8f,g	7	6.8c,d	2.3e–l	3.1c	2.4c–h	19	100
	E3	6.7a–d	5.3i–o	13.6i,j	6.9o–q	5.7b–d	P	M	O	14.7f,g	43	5.9d	2.1e–l	3.6c	2.9b–d	10	100
	E5	7.0a,b	5.3i–o	14.1h,i	8.6b–d	5.3d–f	P	M	O	12.0f–i	35	9.2c,d	2.1e–l	4.2b,c	2.4c–h	19	100
	E7	6.0b–h	4.5q–t	12.9j–o	7.4l–o	5.1e–g	P	L	O	4.8i–n	6	3.3d	1.6h–l	3.5c	2.8b–e	6	100
	C1	4.4k–o	5.2j–o	13.9i	9.4a	5.0f–h	P	L	O	4.8i–n	75	98.9b	4.6b	11.7a,b	4.3a	10	100
	C2	2.0q	5.5g–m	15.0g	8.3c–g	5.0f–h	W	M	O	114f–j	69	12.5c,d	2.4e–k	4.0b,c	2.7b–g	6	100

[a] Presence of spines
[b] Absence of green stripes in unripe fruits

Molecular analysis

Out of the 100 UBC primers tested, 50 gave polymorphisms between cv. Dourga and *S. aethiopicum*. Sixty-seven clear polymorphic bands were identified: 32 derived from *S. melongena* and 35 from *S. aethiopicum*. The polymorphic primers were tested in the somatic hybrids, which showed an identical pattern with a concomitant presence of specific bands of eggplant and *S. aethiopicum*, thereby confirming the hybridity of the individuals regenerated after electrofusion. A representative picture of the amplification products is shown in Fig. 4A. In the same figure it can be observed that most of the species-specific fragments of *S. aethiopicum* and *S. melongena* are present in the dihaploids. Polymorphisms among dihaploid plants were evidenced both as a different intensity of amplified bands with identical molecular weights (e.g. weak 1,700-bp fragment in lane 9 – see arrow) and as a segregation of bands that were polymorphic in the parental species (e.g. the 1,700-bp fragment is absent in lanes 8 and 10; the 2,200-bp fragment is absent in lanes 16 and 18 – see arrows).

Of the 14 primers tested, only four (OPD15, OPG12, L15 and L18) gave good and reliable RAPD profiles. A high level of polymorphism between eggplant and *S. aethiopicum* was detected; a representative picture is shown in Fig. 4B. The somatic hybrids inherited PCR products from both the cultivated (6 bands) and wild parents (17 bands). The nine dihaploids analysed revealed patterns identical to those of the somatic hybrid donor of the anthers.

Isozyme analysis

Isozyme analysis was performed on the fusion parents, the three somatic hybrids and 27 androgenic plants. SKDH zymograms showed two zones of activity: the cultivated fusion parent presented two anodal bands showing identical activity (Fig. 5 A, lane 1); *S. aethiopicum* zymogram presented two slow-migrating bands, in which the most anodal band was as active as the most cathodal one (lane 2). The somatic hybrids exhibited the parental SKDH bands with no intermediate mobility bands, suggesting that these enzymes are active as monomers (lanes 3, 4, 5). G-6-PDH zymograms of eggplant and *S. aethiopicum* presented two and four zones of activity, respectively (Fig. 5B, lanes 1, 2). All somatic hybrids showed four activity bands (lanes 3–5), thereby confirming the monomeric structure of the enzymes involved.

In both cases, dihaploid plants showed either the same bands evidenced in the somatic hybrids (lanes 6, 7) or a certain degree of segregation (lanes 8–12).

Test for *Fusarium* resistance

A reproducible response to the *Fusarium* inoculation was observed in the fusion parents and in the derived

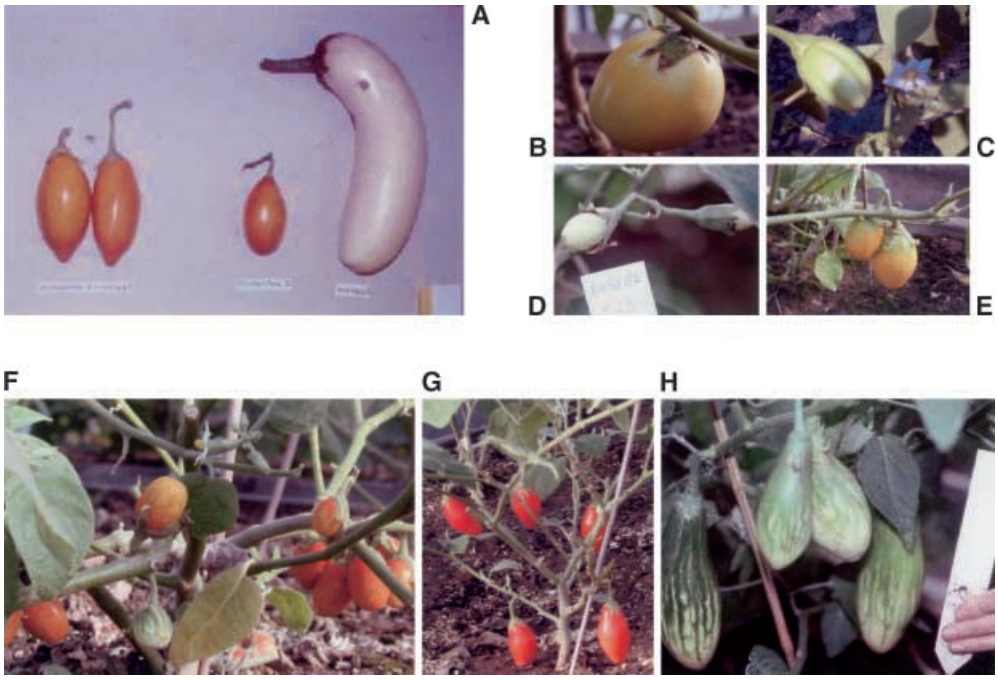

Fig. 3 **A** Fruit of a somatic hybrid (*left*), of *Solanum aethiopicum* (*middle*) and of *S. melongena* cv. Dourga (*right*). **B–H** Variability in the fruits of dihaploid plants. Note: the absence (**B**, **C**) or the presence (**E**, **F**, **H**) of green stripes in unripe fruits, the good production of fruits (**F–H**) and the variation in size and shape of fruits

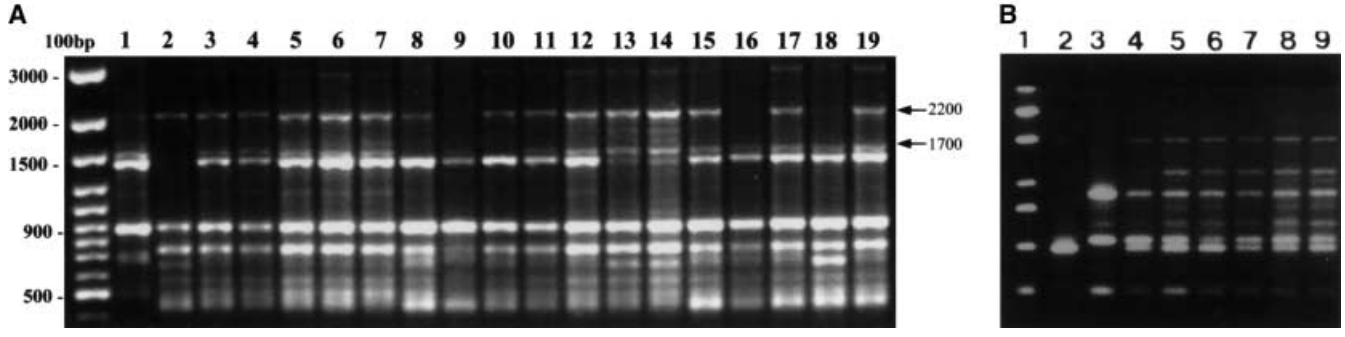

Fig. 4 **A** I-SSR analysis (primer UBC 807): *lane 1 Solanum melongena* cv. Dourga, *lane 2 S. aethiopicum* gr. *gilo*, *lanes 3–5* somatic hybrids D-Sa 2/1, D-Sa 2/2, D-Sa 2/3, *lanes 6–19* some androgenic plants. **B** RAPD analysis (primer OPD15): *lane 1* 1-kb ladder, *lane 2 S. melongena* cv. Dourga, *lane 3 S. aethiopicum* gr. *gilo*, *lane 4* the somatic hybrid D-Sa 2/2, *lanes 5–9* some dihaploid plants

somatic hybrids employed as anther donors. Symptoms were evident 2 weeks after inoculation in susceptible eggplant cv. Dourga plants, whose leaves subsequently became yellowish and wilted. Within 4 weeks following inoculation, these plants were completely wilted and dead, whereas *S. aethiopicum* as well as the somatic hybrids were resistant, showing 100% survival (Table 4; Fig. 6A). The 392 cuttings of the dihaploids inoculated with *Fusarium* showed a clear response: out of the 41 genotypes analysed, seven were susceptible and died within 4 weeks, while 34 were symptomless with 100% healthy plants (Table 4). Therefore, the resistance trait segregated in the androgenetic dihaploids (Fig. 6B).

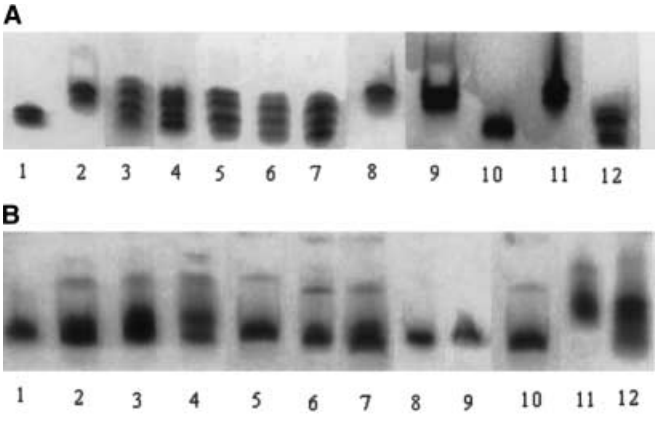

Fig. 5 SKDH (**A**) and G-6-PDH (**B**) phenotypes detected in: *Solanum melongena* cv Dourga (*lane 1*), *S. aethiopicum* gr. *gilo* (*lane 2*), somatic hybrids D-Sa 2/1, D-Sa 2/2, D-Sa 2/3 (*lanes 3–5*), two androgenic non-segregant plants (*lanes 6, 7*), five androgenic segregant plants (*lanes 8–12*)

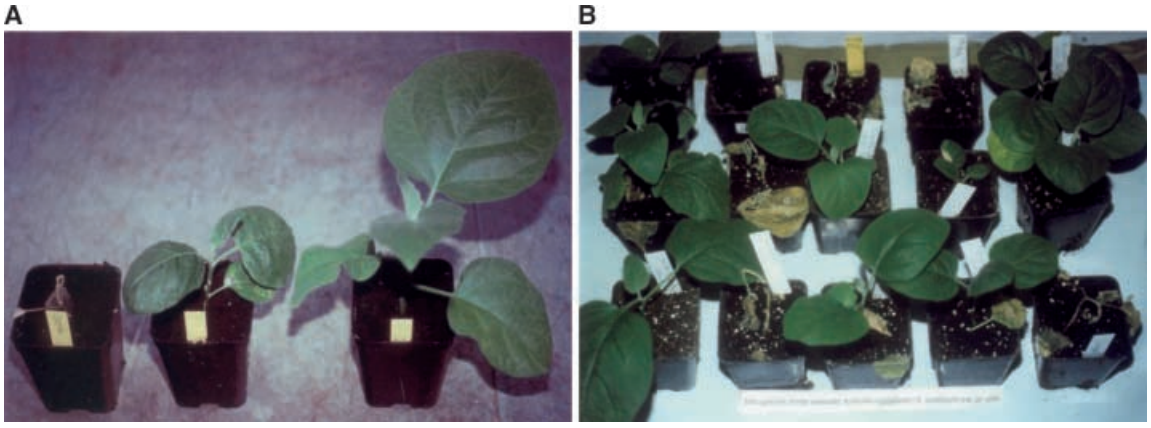

Fig. 6 **A** Response to artificial infection with *Fusarium oxysporum* f. sp. *melongenae* in *Solanum melongena* cv. Dourga (*right*), *S. aethiopicum* group *gilo* (*middle*) and their somatic hybrid (*left*). **B** Segregation for *Fusarium* resistance in different ex vitro dihaploid cuttings

Discussion

The results obtained from the investigation reported here suggest that the somatic hybrids obtained by protoplast electrofusion between *Solanum melongena* cv. Dourga and *S. aethiopicum* group *gilo* represent a valuable source of *Fusarium* wilt resistance for eggplant improvement. *S. aethiopicum* group *gilo* was totally resistant to an Italian *Fusarium* isolate that was lethal for the cultivated eggplant. The resistance trait was expressed in all of the somatic hybrids and segregated in the dihaploid generated through anther culture.

Androgenic plants from somatic hybrids were obtained for the first time by anther culture from an interspecific somatic hybrid between *Solanum tuberosum* and *S. brevidens* (Rokka et al. 1995). Interestingly, only hexaploid somatic hybrids showed androgenic competence and produced triploid anther-derived plants. Gavrilenko et al. (2001) recently obtained androgenic regenerants from two intergeneric somatic hybrids between *Lycopersicon esculentum* and *Solanum etuberosum*. In our study, anther culture was efficient since a satisfactory number of androgenic plants were produced, corresponding to 2.7% of the cultured anthers. An FDA count of the chloroplasts in stomata guard cells – which we determined to be a reliable indirect method for the rapid determination of ploidy level – demonstrated that these plants were mostly diploid (81.9%). The reduced ploidy level of the dihaploid plants, whose genome is probably formed by a single partially recombined chromosomal set of each species, may also explain the lower pollen viability and the morphological variability.

The morphological traits observed in the somatic hybrids were prevalent in the androgenic plants. However, the dihaploids showed variability in their growth habit, leaf shape and flower and fruit characteristics. Single individuals of dihaploid plants also displayed new associations of traits inherited from the parental species (e.g. violet petals from eggplant and orange skin fruit from *S. aethopicum* in D-Sa 2/2-E6 and -E26; the absence of spines from *S. aethiopicum* and susceptibility to *Fusarium* from eggplant in D-Sa 2/1-E1; a white flower and susceptibility to *Fusarium* in D-Sa 2/2-E1 and -E11, etc.) or the appearance of new traits (e.g. a higher number of flowers per inflorescence, prostate growth habit, etc). Conversely, the seed-derived tetraploid plants of the somatic hybrids were highly homogeneous (data not shown). The phenotypic variability expressed in the androgenic plants probably derived from segregation and interspecific genetic recombination during meiosis in the somatic hybrids.

The isoenzymatic (SKDH and G-6-PDH) and molecular (I-SSR and RAPDs) markers were suitable for characterising the hybrids because of the large number of polymorphisms between the fusion parents. These markers provide appropriate tools by which to check the hybrid condition of the fusion products and the androgenic origin of plants obtained by anther culture. They were inherited in the dihaploid population where segregation was also detected. Isozyme and molecular markers may be usefully employed to follow and accelerate the introgression of traits of interest from *S. aethiopicum* into eggplant, as has been demonstrated in other *Solanaceae* (Brown et al. 1996; Fulton et al. 1997).

S. aethiopicum can be sexually crossed with eggplant, but the resulting F_1 hybrids are only partially fertile with a pollen viability significantly lower than that of somatic hybrids (Daunay et al. 1993). Recombination between homeologous chromosomes is the prerequisite for the introgression of traits from donor to the recipient. Our results from the morphological, biological, molecular and biochemical analyses as well as the *Fusarium* resistance data of the androgenic dihaploids analysed are consistent with the possibility that our somatic hybrids behave as segmental allopolyploids. Similar conclusions, based on cytogenetic and isozyme analyses, were gathered in previous studies on sexual amphidiploids of eggplant with *S. indicum* L. (Rajasekaran 1970) and *S. integrifolium* (Isshiki et al. 2000), both species belonging to the same section (Oliganthes) of *S. aethiopicum* gr. *gilo*. Indeed, a low frequency of quadrivalent formation was observed at meiosis in the amphidiploid between eggplant and

S. integrifolium, species also referred to as *S. aethiopicum* gr. *aculeatum* (Issiki et al. 2000).

For introgression of the *Fusarium* resistance into eggplant, both the somatic hybrids and the resistant dihaploids may be employed in the backcrosses. Dihaploids offer the advantage of being readily employable in crosses with the diploid cultivated eggplant, and the backcrossed progenies can promptly enter in the breeding programme. In contrast, somatic hybrids require the use of an induced tetraploid eggplant and a subsequent reduction in the ploidy of the backcrossed progenies. However, the low fertility of the dihaploids will probably require more attempts in order to achieve a successful cross with eggplant.

Besides their usefulness as potential valuable breeding materials, the dihaploids may be utilised to determine the inheritance of morphological and agronomical traits. Moreover, two of the somatic hybrids employed here as anther donors were significantly more tolerant to the bacterial wilt caused by *Ralstonia* (Collonnier et al. 2001). This opens the perspective of exploitation of the dihaploid population to identify molecular markers linked to *Fusarium* and *Ralstonia* resistance, thereby facilitating the introgression of these traits into eggplant via marker-assisted selection. To achieve these aims, we are currently carrying out the molecular characterisation of the whole population of dihaploids.

Acknowledgements EU financial support (contract no. IC18CT-970187) is acknowledged. The authors thank Mrs. Maria Grazia Tacconi and Mr. Domenico Platia for their excellent technical assistance and Dr. V. Souvannavong, CNRS-UPS-France, for the flow cytometry analysis.

References

Austin S, Lojkowska E, Ehlenfeldt MK, Kelman A, Helgeson JP (1988) Fertile interspecific somatic hybrids of *Solanum*: a novel source of resistance to *Erwinia* soft rot. Phytopathology 78:1216–1220

Brown CR, Mojtahedi H, Santo GS (1995) Introgression of resistance to Columbia and Northern root-knot nematodes from *Solanum bulbocastanum* into cultivated potato. Euphytica 83:71–78

Brown CR, Yang C-P, Mojtahedi H, Santo GS, Masuelli R (1996) RFLP analysis of resistance to Columbia root-knot nematode derived from *Solanum bulbocastanum* in a BC_2 population. Theor Appl Genet 92:572–576

Cappelli C, Stravato VM, Rotino GL, Buonaurio R (1995) Sources of resistance among *Solanum* spp. to an Italian isolate of *Fusarium oxysporum* f. sp. *melongenae*. In: Andrásfalvi A, Moór A, Zatykó (eds) EUCARPIA, 9th Meet Genet Breed Capsicum Eggplant. SINCOP, Budapest, pp 221–224

Chambonnet D (1985) Culture d'anthères in vitro chez trois Solanacées maraichères: le piment (*Capsicum annuum* L.), l'aubergine (*Solanum melongena* L.), la tomate (*Lycopersicon esculentum* L.) et obtention de plantes haploides. PhD thesis, Academy of Montpellier

Chen Y-KH, Palta JP, Bamberg JB (1999) Freezing tolerance and tuber production in selfed and backcross progenies derived from somatic hybrids between *Solanum tuberosum* L. and *S. commersoni* Dun. Theor Appl Genet 99:100–107

Collonnier C, Mulya K, Fock I, Mariska I, Servaes A, Vedel F, Siljak-Yakovlev S, Souvannavong V, Ducreux G, Sihachakr D (2001) Source of resistance against *Ralstonia solanacearum* in fertile somatic hybrids of eggplant (*Solanum melongena* L.) with *Solanum aethiopicum* L. Plant Sci 160:301–313

Daunay MC, Lester RN, Laterrot H (1991) The use of wild species for the genetic improvement of brinjal eggplant (*Solanum melongena*) and tomato (*Lycopersicon esculentum*). In: Hawkes JC, Lester RN, Nee M, Estrada N (eds) Solanaceae 3: taxonomy, chemistry, evolution, vol 27. Royal Botanic Gardens Kew and Linnean Soc, London, pp 389–413

Daunay MC, Chaput MH, Sihachakr D, Allot M, Vedel F, Ducreux G (1993) Production and characterisation of fertile somatic hybrids of eggplant (*Solanum melongena* L.) with *Solanum aethiopicum* L. Theor Appl Genet 85:841–850

Deimling S, Zitzisperger J, Wenzel G (1988) Somatic fusion for breeding of tetraploid potatoes. Plant Breed 101:181–189

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Fock I, Collonnier C, Purwito A, Luisetti J, Souvannavong V, Vedel F, Ambroise A, Kodja H, Ducreux G, Sihachakr D (2000) Resistance to bacterial wilt in somatic hybrids between *Solanum tuberosum* and *S. phureja*. Plant Sci 160:165–176

Fulton TM, Nelson JC, Tanksley SD (1997) Introgression and DNA analysis of *Lycopersicon peruvianum*, a wild relative of the cultivated tomato, into *Lycopersicon esculentum*, followed through three successive backcross generations. Theor Appl Genet 95:895–902

Gavrilenko T, Thieme R, Rokka VM (2001) Cytogenetic analysis of *Lycopersicon esculentum* (+) *Solanum etuberosum* somatic hybrids and their androgenetic regenerants. Theor Appl Genet 103:231–239

Gleddie S, Keller WA, Setterfield G (1986) Production and characterisation of somatic hybrids between *Solanum melongena* L. and *S. sisymbrifolium*. Theor Appl Genet 71:613–621

Guri A, Sink KC (1988) Interspecific somatic hybrid plant between eggplant (*Solanum melongena*) and *Solanum torvum*. Theor Appl Genet 76:490–496

Hébert Y (1985) Résistance comparée de 9 espèces du genre *Solanum* au flétrissement bactérien (*Pseudomonas solanacearum*) et au nématode *Meloidogyne incognita*. Intéret pour l'amélioration de l'aubergine (*Solanum melongena* L.) en zone tropicale humide. Agronomie 5:27–32

Isshiki S, Okubo H, Fujieda K (2000) Segregation of isozymes in selfed progenies of a synthetic amphidiploid between *Solanum integrifolium* and *S. melongena*. Euphytica 112:9–14

Jarl CI, Rietveld EM, De Haas JM (1999) Transfer of fungal tolerance through interspecific somatic hybridisation between *Solanum melongena* and *S. torvum*. Plant Cell Rep 18:791–796

Kennet R, Barkai-Golan R, Chorin M, Dishon I, Katan Y, Netzer D, Palti J, Volcani Z (1970) A revised checklist of fungal and bacterial diseases of vegetable crops in Israel. Spec Publ Volcani Inst Agric Res Bet Dagan, Bet Dagan, pp 39

Kishi K (1974) Disease and pest control in enclosed environments in Japan. Outlook Agric 8:100–104

Laferriere LT, Helgelson JP, Allen C (1999) Fertile *Solanum tuberosum* + *S. commersonii* somatic hybrids as source of resistance to bacterial wilt caused by *Ralstonia solanacearum.* Theor Appl Genet 98:1272–1278

Lefrancois CY, Chupeau Y, Bourgin JP (1993) Sexual and somatic hybridisation in the genus *Lycopersicon*. Theor Appl Genet 86:533–546

Murashige T, Skoog F (l962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 473–497

Rajasekaran S (1970) Cytogenetic studies of the F_1 hybrid *Solanum indicum* L. × *S. melongena* L. and its amphidiploid. Euphytica 19:217–224

Rokka VM, Valkonen JPT, Pehu E (1995) Production and characterisation of haploids derived from somatic hybrids between *Solanum brevidens* and *S. tuberosum* through anther culture. Plant Sci 112:85–95

Rotino GL (1996) Haploidy in eggplant. In: Jain SM, Sopory SK, Veillux RE (eds) In vitro production of haploids in higher plants, vol 3. Kluwer, Dordrecht, pp 115–124

Sihachakr D, Haicour R, Serraf I, Barrientos E, Herbreteau C, Ducreux G, Rossignol L, Souvannavong V (1988) Electrofusion for the production of somatic hybrid plants of *Solanum melongena* L. and *Solanum khasianum* CB Clark. Plant Sci 57:215–223

Sihachakr D, Daunay MC, Serraf I, Chaput MH, Mussio I, Haicour R, Rossignol L, Ducreux G (1994) Somatic hybridization of eggplant (*Solanum melongena* L.) with its close and wild elatives. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 1. Somatic hybridization in crop improvement. Springer, Berlin Heidelberg New York, pp 255–278

Steekelenburg NAM van (1976) *Fusarium* wilt of eggplant in the Netherlands. Neth J Plant Pathol 82:191–192

Stravato VM, Cappelli C, Polverari A (1993) Attacchi di *Fusarium oxysporum* f. sp. *melongenae* agente della tracheofusariosi della melanzana in Italia centrale. Inf Fitopatol 43:51–54

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TS (eds) Isozymes in plant genetics and breeding. Elsevier, Amsterdam, pp 469–516