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SOMATIC CELL GENETICS

Genotyping and metabolite characterization of somatic hybrids between *Arabidopsis thaliana* and *Swertia mussotii*

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Abstract Symmetric and polyethylene glycol (PEG)-induced asymmetric fusions between *Swertia mussotii* and *Arabidopsis thaliana* protoplasts generated >100 putative hybrid cell lines. Of these, 65 were shown to have a hybrid origin, based on molecular markers, chromosome number, and morphology. An assay directed at the *A. thaliana* CACTA transposon family detected only three positive lines among the 65 hybrid calli tested. The CACTA sequence amplified from clone D14 was highly homologous with CAC2, indicating that the chromosome content of the hybrid cell lines was largely inherited from *S. mussotii*. Profiles of secondary metabolites identified a number of *S. mussotii*- or *A. thaliana*-specific compounds, as well as significant proportions of compounds not represented in the profile of either parent.

Keywords CAC transposons · Genotyping · New markers · GC-MS · Chromosome elimination

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Introduction

Somatic hybridization, unlike conventional crossing, is not restricted by sexual incompatibility and has been used to obtain hybrids involving parents drawn from a wide range of species (Dudits *et al.* 1987; Fahleson and Glimelius 1999; Xiang *et al.* 2004; Davey *et al.* 2005; Liu *et al.* 2005; Xia 2009). Hybrids between *Bupleurum scorzonerifolium* and several Gentianaceae species have recently been produced with the aim of synthesizing specific valuable secondary metabolites in a fast-growing species (Wang *et al.* 2010; Jiang *et al.* 2012; Yu *et al.* 2012). The annual species *Swertia mussotii* Franch. (Gentianaceae) is adapted to the high altitude environment of Tibet and Qinghai. Extracts of the whole plant are functional against various forms of hepatitis (Yamahara *et al.* 1978; Kikuzaki *et al.* 1996), and the major active molecules have been identified as oleanolic acid, mangiferin, swertiamarin, and amarogentin (Brahmachari *et al.* 2004). How these molecules are synthesized *in planta* has not yet to be determined.

Arabidopsis thaliana is an important model plant with facile genetics and small genome. As whole genome sequencing has been completed, *A. thaliana* has a relatively well understood genetic background and thus provides unique opportunities for genome and functional genome analysis.

The initial aim of the current research was to generate somatic hybrids between *S. mussotii* and *A. thaliana*. Asymmetric hybrids which harbored the smallest contribution of *S. mussotii* DNA could be used to identify the genes underlying the synthesis of the key secondary metabolites. However, most of the hybrids were dominated by DNA from the *S. mussotii* parent. Here, we describe the production and characterization of a number of independent asymmetric somatic hybrids between *S. mussotii* and *A. thaliana*. The secondary

metabolite profiles of the hybrids and parents were evaluated, and the analysis of CACTA transposon movement was used to identify pericentromeric and centromeric regions insertion events among the asymmetric somatic hybrids.

Materials and Methods

Origin of parental protoplasts, protoplast fusion, and regeneration. A cell suspension of *A. thaliana* ecotype Col-0 was maintained in D1 liquid medium on a shaker at 150 rpm with a 10–12-h photoperiod under 25°C with 7-d subculture intervals (Wang *et al.* 2005). The *S. mussotii* cells were obtained from cultured compact calli maintained on KT medium (Wang *et al.* 2010). Both cell types were converted to protoplasts after incubation in an enzyme solution (0.6 M mannitol, 5 mM $CaCl_2$, 1.5% cellulase Onozyka RS, and 0.3% pectolyase Y-23) for 3–4 h as described by Xu *et al.* (2003). The *S. mussotii* protoplasts were used either directly for fusion (combination I) or were exposed to 300 W/cm^2 ultraviolet (UV) light for 30 s prior to fusion (combination II). For both combinations, the *S. mussotii* protoplasts were subsequently mixed with those of *A. thaliana* in a ratio of 1.5:1 and fused using PEG. Briefly, the protoplast mixture was precipitated for 30 min in culture dish, then four drops of PEG solution [0.11 M glucose, 0.09 M $Ca(NO_3)_2$, 40% (*w*/*v*) PEG 6000] were added to the border of the protoplast mixture. Fusing protoplasts were then incubated for 15–20 min. Next, four drops of 0.27 M $Ca(NO_3)_2$ were added in two subsequent steps, with incubation for 10 min each time. The solution was replaced twice with washing buffer with incubation for 10 min each time. Finally, the washing buffer was replaced with P5 liquid medium (Xu *et al.* 2003), incubated for 10 min, after which the P5 medium replaced (Xu *et al.* 2003). The fusion products were cultured in P5 liquid medium in darkness at 25°C. Developing calli ~2 mm in diameter were transferred to D1 medium to encourage proliferation, then to IB and B1 medium to promote differentiation (Jiang *et al.* 2012). All the media (Table 1) used were MB basal media supplemented with 2 mg/l glycine, 146 mg/l glutamine, and 300 mg/l casein hydrolysate. All cultures were incubated in darkness or in a chamber with a 10–12-h photoperiod. A light intensity of 18–20 μmol m^{-2} s^{-1} was provided by cool-white fluorescent lamps.

Table 1 Culture media

Code	Basal media	Growth regulators (mg/L)	Sugar (g/L)	Agar (g/L)
D1	MB	1 mg/L 2,4-D	30 sucrose	7.5
KT	MB	2 mg/L 2,4-D+0.5 mg/L KT	30 sucrose	7.5
B1	MB	1 mg/L BA	30 sucrose	7.5
IB	MB	0.5 mg/L IAA+0.5 mg/L BA	30 sucrose	7.5
P5	MB	1 mg/L 2,4-D	90 glucose 10 sucrose	–

Genotypic analysis. Genomic DNA was isolated from putatively hybrid calli and both parents using a modified CTAB method (Doyle and Doyle 1990). A set of 18 random-sequence decamer oligonucleotides obtained from OperonTechnology (Huntsville, AL) were used as PCR primers, as described elsewhere (Xia *et al.* 1998). Each reaction was subjected to 94°C/5 min, 45 cycles of 94°C/10 s, 36°C/30 s, 72°C/50 s, and 72°C/7 min, and the amplicons were separated through 1.5% (*w*/*v*) agarose gels and detected by ethidium bromide staining. In addition, the DNAs were also amplified using a primer pair targeting the 5S rDNA spacer sequence (Cox *et al.* 1992). These reactions were subjected to 94°C/5 min, 35 cycles of 94°C/40 s, 57°C/60 s, 72°C/90 s, and 72°C/7 min, and the amplicons were separated through 2% agarose gels and stained in ethidium bromide. For chloroplast simple sequence repeats (SSR) analysis, seven primer pairs were used (Ishii *et al.* 2001). These reactions were subjected to 94°C/5 min, 35 cycles of 94°C/60 s, 55°C/75 s, 72°C/120 s, and 72°C/7 min. These amplicons were separated by 6% polyacrylamide gel electrophoresis and visualized by silver staining (Xu *et al.* 2003). Finally, a pair of primers was designed based on the consensus sequence of the *A. thaliana* transposons CACTA1 (AB052792.1), CACTA2 (AB052793.1), CACTA3 (AB052794.1), and CACTA5 (AB095515.1). The primer sequences were 5′-ACGCTAAGACCGTAAATCC and 5′-AATCGCATCACA GACAAGT. These reactions were subjected to 94°C/5 min, 35 cycles of 94°C/30 s, 50°C/30 s, and 72°C/100 s, and the amplicons were gel-purified and cloned into pMD18-T (TaKaRa, Co., Dalian, China) for sequencing.

Chromosome counts. Chromosome counts were obtained from callus cells as detailed by Xia and Chen (1996).

Gas chromatography–mass spectrometry (GC-MS) analysis of secondary metabolites. A 30-mg aliquot of powdered shade-dried callus as described by Cai *et al.* (2009) was obtained from hybrids and parents and extracted in 10 ml methanol. The suspension was sonicated for 60 min and then filtered by vacuum through a 0.22-μm membrane. A GC-MS device (QP-5050A, Shimadzu Co., Kyoto, Japan) was fitted with a DB-5 MS column (0.25 mm×30 m, 0.25 μm film thickness) (J&W Scientific, Folsom, CA), through which the helium flow split ratio was 10:1, and operated at 70 eV ionization voltage with a scan range of 40–400 Da. The column temperature was 50°C for 2 min, raised thereafter by 8°C/min to 150°C, where it was held for 2 min, and then raised at 15°C/min to 250°C, where it was held for 20 min.

Results

Regeneration of fusion products and their morphology. The first division of fused cells was observed after a dark incubation period of 10 d, and tiny calli, ranging from 0.5 to 1 mm in diameter, appeared about 1 mo later. Proliferating calli reached a diameter of 2–3 mm after 6 wk. Twenty-six putative hybrid clones were produced using combination I (D1–26), and 75 were produced using combination II (B1–75) treatments. The majority of yellow-colored calli remained yellow and compact during their culture on D1 medium and went on to develop profuse numbers of roots and shoots after 2 mo on IB or B1 medium (Fig. 1*a, b* and Table 1). However, the watery, light yellow-colored hybrid clones, although they grew rapidly, were unable to form shoots or roots and eventually became necrotic (Fig. 1*c*). Protoplasts of neither parent were able to form callus.

Genotypic analysis of hybrid clones. The hybrid characteristics of the regenerated candidate hybrids callus were demonstrated by random amplified polymorphic DNA (random amplified polymorphic DNA (RAPD)), 5S rDNA, chloroplast SSR, and CACTA transposon analyses. The RAPD profiles produced by 4 of the 18 primers were consistent with the presence of DNA from both parents in 65 of the 101 hybrid calli (Fig. 2*a–c*). The profile of a few clones included fragments not present in either parent's RAPD profile. With respect to the 5S rDNA analysis, all hybrids showed amplicons that included fragments from *S. mussotii*. In clones B16 and B40, a non-parental fragment was also present (Fig. 2*d*). Only one chloroplast SSR marker target within the intron of *atp*F gave stable amplicons (Fig. 2*e*). Most hybrids had *S. mussotii* fragments, and several new bands were amplified in B18, B30, and B40 (Fig. 2*e*). When the primers designed to detect CACTA transposons were employed, the amplicons from hybrids D14, B16, and B19 each included a fragment of the same size as one present in the profile of *A. thaliana*, while *S. mussotii* and the rest of the hybrids failed to yield an amplicon (Fig. 2*f*). When the sequence of the D14 amplicon was subjected to BLAST analysis, an 88–96% level of homology with the CAC1–5 transposons was detected. The highest homology was with CAC2, disturbed by a 28-nt insertion and several single nucleotide substitutions (Fig. 3).

Chromosome number variation. Chromosome analysis was used to further examine the genetic constitution of the hybrids. The somatic chromosome number detected in the *S. mussotii* protoplasts was 18–22 and in *A. thaliana* protoplasts was 9–11, with few fragments (Fig. 4*a, b*). The *A. thaliana* chromosomes were much smaller than those of *S. mussotii*. In all of the combination I-derived and most of the combination II-derived hybrid clones, the somatic number ranged from 18 to 24 (Fig. 4*c*); however, in clone B40 (asymmetric hybrid), the number was much higher (38–40) (Fig. 4*d*). On the basis of the size of the chromosomes in the hybrid clones, most were concluded to be either complete *S. mussotii* chromosomes or recombined products.

Secondary metabolite content. Metabolite profiles of the hybrids and their parents showed that hybrids D1, B20, and B40 and the parents *S. mussotii* and *A. thaliana* produced 32, 36, 33, 31, and 26 peaks, respectively. Some of the compounds produced by the hybrid calli were similar to those of either *A. thaliana* or *S. mussotii* (Table 2), and some additional novel compounds were identified in some of the hybrids (Table 2). Pyrethrin, a terpenoid used as a plant-derived insecticide, was present in B40, although its synthesis was not detected in either of the parental species (Table 2).

Discussion

The genomic content of the hybrid calli. The hybrid lines recovered, whether from combination I or II treatments, were all highly asymmetric. Their chromosomes were uniformly large (like those of *S. mussotii* and unlike those of *A. thaliana*), and the number of RAPD products was greatly biased in favor of *S. mussotii* (83–87% of fragments) and against that of *A. thaliana* (12–15%). Only 3 of the 65 lines analyzed carried sequences homologous to the *A. thaliana* CAC transposons. Chromosome elimination is a common

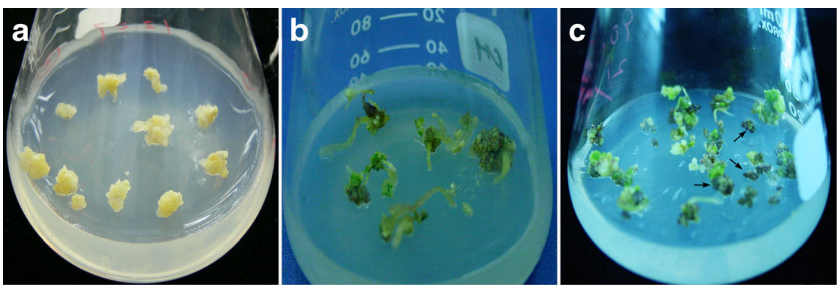
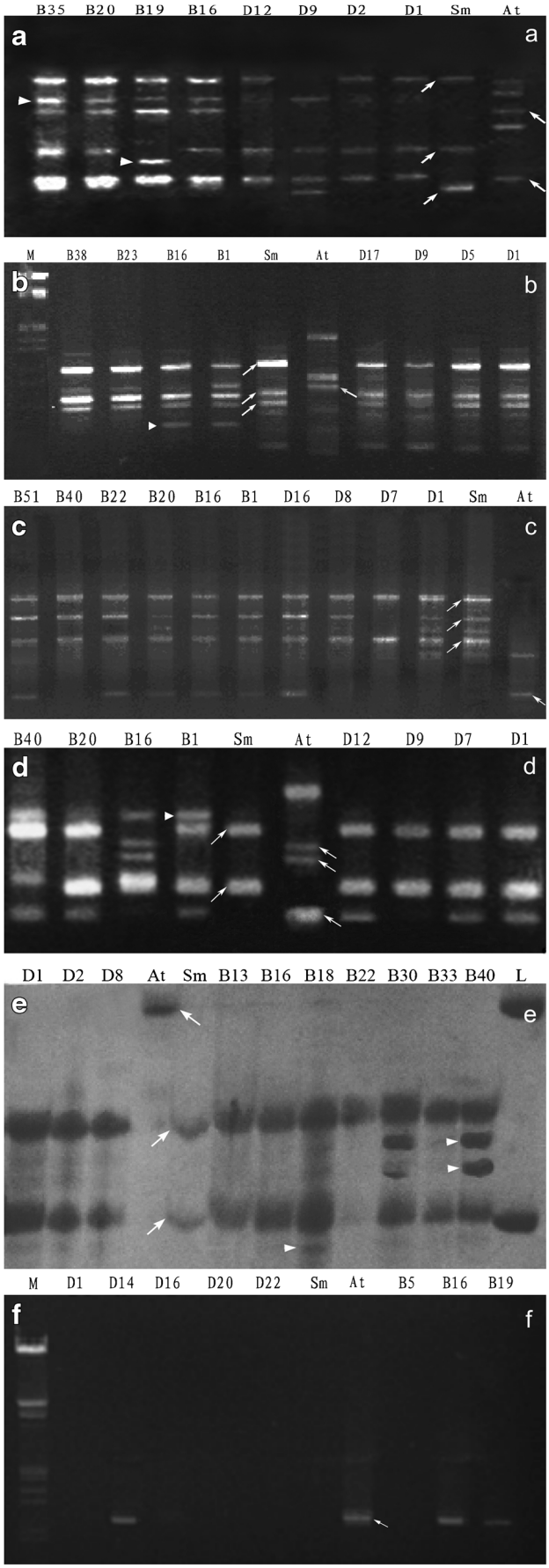


Fig. 1 Morphology of somatic hybrid calli and regenerants: (*a*) Hybrid calli expanding on D1 medium (*see* Table 1) from combination I after 10 d subculture; (*b*) Roots forming on hybrids from combination II on IB regeneration medium after 15 d subculture; (*c*) Necrotic, watery calli growing on B1 medium after 15 d subculture on regeneration medium. *Rightwards arrow*: necrotic calli.



◀ **Fig. 2** RAPD, 5S rDNA, chloroplast SSR, and locus-specific PCR markers profiles of the *A. thaliana* and *S. mussotii* hybrids from combination I and II treatments and parents: (*a–c*) RAPD profiles generated by primer OPU-11 (AGACCCAGAG), OPV-2 (AGTCAC TCCC), and OPJ-17 (ACGCCAGTTC); (*d*) 5S rDNA spacer variation; (*e*) chloroplast SSR profiles generated by Wct11 primers; (*f*) amplification profiles generated by locus-specific PCR primers recognizing the transposon CACTA family. *Sm*: *S. mussotii*; *At*: *A. thaliana*; *M*: molecular size markers (λDNA digested with *Hind*III and *Eco*RI). *L*: 100 bp DNA ladder; *D1*, *D2*, *D7*, *D9*, *D8*, *D12*, *D16*, and *D17*: combination I hybrids; *B1*, *B16*, *B18*, *B20*, *B22*, *B23*, *B30*, *B33*, *B35*, *B38*, *B40*, and *B51*: combination II hybrids. *Northeast arrow*: Fragments present in the profile of *S. mussotii*. *Northwest arrow*: Fragments present in the profile of *A. thaliana*. *White box*: Fragments not present in either parental profile.

event in symmetric somatic hybrids and occurs more frequently as the phylogenetic separation between the parents increases (Pental *et al.* 1986; de Vries *et al.* 1987; Endo *et al.* 1988; Xia 2009). Attempts have been made to minimize asymmetry by pretreating the protoplasts of the donor parent with radiation, while a general strategy applied to bias the elimination in favor of a particular parent has been to expose the protoplast of the non-favored protoplasts to UV irradiation prior to fusion (Liu *et al.* 2005; Cai *et al.* 2007; Jiang *et al.* 2012). In some cases, the outcome has been surprising in that chromosomes of the favored parent are eliminated (Wang *et al.* 2005; Zhou *et al.* 2006), perhaps because they are more sensitive to the presence of free radicals induced by the UV irradiation treatment applied to the non-favored parent protoplasts (Wang *et al.* 2005). An unexplained outcome from the present experiments was that the UV pre-treatment seemed to have no noticeable effect on the chromosomal constitution of the hybrids, which may suggest that the phylogenetic separation between *S. mussotii* and *A. thaliana* had a greater influence on the pattern of chromosome elimination than whether or not the *S. mussotii* protoplasts were exposed to UV irradiation.

The CACTA transposon PCR assay—an efficient method for centromeric regions identification. Various molecular markers are employed for chromosome identification in somatic hybrids, such as RAPD, inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and SSR (Cai *et al.* 2007; Xiao *et al.* 2009; Yang *et al.* 2009; Xiang *et al.* 2010). Markers that depend on arbitrarily primed amplicons such as RAPD, ISSR, and AFLP are not locus-specific, so it is difficult to determine which fragments from the parents are present in the hybrids. Locus-specific markers such as SSRs are codominant, sometimes species/genus-specific; however, their detectabilities are very limited, which means numerous marks are needed to identify locus from donor parent. As such, Xiang *et al.* (2010) reported only 11 of 58 SSR markers from wheat/oat somatic hybrids had the donor oat alleles.

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gi|b        ACGCTAAGACCGTAAATCCTCTAGCGGGAATGTAAAATACTTTAGTCCGCCAACACTTAGGCAAAATTATTCACTTCAGCGCCAACACTTAGGCAAAACT 100
gi|CAC2     ACGCTAAGACCGTAAATCCTCTAGCGGGAATGTAAAATACTTTAGTCCGCCAACACTTAGGCAAAATTATTCACTTCAGCGCCAACACTTAGGCAAAATT 100
Consensus   acgctaagaccgtaaatcctctagcgggaatgtaaaatactttagtccgccaacacttaggcaaaattattcacttcagcgccaacacttaggcaaaa t
gi|b        ATTCACTTCCCTCTACAGCTTCGTGAAAACCTAAGAAAATCGAGAAGATCAAAATTATTCACTTTTGCCTAAAAGCTAAGATCTGTCGAATCCACTCCCA 200
gi|CAC2     ATTCACTTCCCTCTACAGCTTCGTGAAAACCTAAGAAAATCGAGAAGATCAAAATTATTCACTTTTGCCTAAAAGCTAAGATCTGTCGAATCCACTCCCA 200
Consensus   attcacttccctctacagcttcgtgaaaacctaagaaaatcgagaagatcaaaattattcactttgcctaaaagctaagatctgtcgaatccactccca
gi|b        AAATCGATTCAACATTGTCGCTGGCCAGAAATTCGGATTCTCCGCCGCCACATTCTCCAGCTCAGCCACCGTTGAGCTCAAATTCAAGGTTAGTCTTTCT 300
gi|CAC2     AAATCGATTCAACATTGTCGCTGGCCAGAAATTCGGATTCTCCGCCGCCACATTCTCCAGCTCAGCCACCGTTGAGCTCAAATTCAAGGTTAGTCTTTCT 300
Consensus   aaatcgattcaacattgtcgctggccagaaattcggattctccgccgccacattctccagctcagccaccgttgagctcaaattcaaggttagtctttct
gi|b        TCCTTATTAATCCATTTCAGCTTCCATCTTACTCGCATTTTGACCTAATCTCATAGAATCGACTTCCATTTTGCAGCCACCAGTGAGCTCTAACCTTGTC 400
gi|CAC2     TCCTTATTAATCCATTTCAGCTTCCATCTTACTCGCATTTTGACCTAATCTCATAGAATCGACTTCCATTTT.......................... 372
Consensus   tccttattaatccatttcagcttccatcttactcgcattttgacctaatctcatagaatcgacttccatttt
gi|b        GCAGCCACCACTGAGCTCAAAATCAAGGCATGTCTTACTCTCAATTTGACCTAGTTCTTTGTGACGATTTGATTTTGGGGTTTATATGCATCGATTTGTT 500
gi|CAC2     GCAGCCACCACTGAGCTCAAAATCAAGGCATGTCTTACTCTCAATTTGACCTAGTTCTTTGTGACGATTTGATTTTGGGGTTTATATGCATCGATTTGTT 472
Consensus   gcagccaccactgagctcaaaatcaaggcatgtcttactctcaatttgacctagttctttgtgacgatttgattttggggtttatatgcatcgatttgtt
gi|b        ATATTTAGGGCAATGTGATTTGAATAGAATTAGGAAGTGAAACTTTTCTTCTTTCTTATACTGATTATCGTTTTGGGGTTTATATGCATCGATTTGTGAT 600
gi|CAC2     ATATTTAGGGCAATGTGATTTGAATAGAATTAGAAAGTGAAACTTTTCTTCTTTCTTATACTGATTATCGTTTTGGGGTTTATATGCATCGATTTGTGAT 572
Consensus   atatttagggcaatgtgatttgaatagaattag aagtgaaacttttcttctttctttatactgattatcgttttggggtttatatgcatcgatttgtgat
gi|b        TTGATTTAGAGTTCTTAAACGTTTTGCTTCAACTTATACTCATAATCGTTTTTTTTATCAAGTGTTGGTGATTGCAATGTCGAGGAAGTGAAACTTTTCT 700
gi|CAC2     TTGATTTAGAGTTCTTAAACGTTTTGCTTCAACTTATACTCATAATCGTTTTTTTTATCAAGTGTTGGTGATTGCAATGTCGAGGAAGTGAAACTTTTCT 672
Consensus   ttgatttagagttcttaaacgttttgcttcaacttatactcataatcgttttttttatcaagtgttggtgattgcaatgtcgaggaagtgaaacttttct
gi|b        TCTTCTATTCACACGACTACAGGTTAGTCACACGACAGATTCTTTTGTTCTGTTCTTTACTTATGAGTTGTATGAACTTAAGTTGTTGTGATTCTTTTGT 800
gi|CAC2     TCTTCTATTCACACGACTACAGGTTAGTCACACGACAGATTCTTTTGTTCTGTTCTTTACTTATGAGTTGTATGAACTTAAGTTGTTGTGATTCTTTTGT 772
Consensus   tcttctattcacacgactacaggttagtcacacgacagattcttttgttctgttctttacttatgagttgtatgaacttaagttgttgtgattcttttgt
gi|b        TCTGTTCTTTACTGAAGTTGATGTGATTGTTGTCGATTGGTTTTATTGGCTGAGATAGTGTAATTTGTTGGTCCTTAAACATGTGTCTTTGAATTAATCT 900
gi|CAC2     TCTGTTCTTTACTGAAGTTGATGTGATTGTTGTCGATTGGTTTTATTGGCTGAGATAGTGTAATTTGTTGGTCTTTAAACATGTGTCTTTGAATTAATCT 872
Consensus   tctgttctttactgaagttgatgtgattgttgtcgattggttttattggctgagatagtgtaatttgttggtc ttaaacatgtgtctttgaattaatct
gi|b        GAGATAGTATACTGTCGATTGTGTTCTTCATTGAAGTTGTTGTGATTGTTGTCGATTGGTTTTATTTGCTAAGATAGTGTAATTTGTTGGTCCTAAAACA 1000
gi|CAC2     GAGATAGTATACTGTCGATTGTGTTCTTCATTGAAGTTGTTGTGATTGTTGTCGATTGGTTTTATTTGCTAAGATAGTGTAATTTGTTGGTCCTAAAACA 972
Consensus   gagatagtatactgtcgattgtgttcttcattgaagttgttgtgattgttgtcgattggttttatttgctaagatagtgtaatttgttggtcctaaaaca
gi|b        TGTGTCTTTGAATTAATCTGAGATAGTATATTGTCGATTGTGTTTGCCATTGTTTATTTCTTAATTTGATGAACTTGATAAGAGATAAGTTTGTATATTG 1100
gi|CAC2     TGTGTCTTTGAATTAATCTGAGATAGTATATTGTCGATTGTGTTTGCCATTGTTTATTTCTTAATTTGATGAACTTGATAAGAGATAAGTTTGTATATTG 1072
Consensus   tgtgtctttgaattaatctgagatagtatattgtcgattgtgtttgccattgtttatttcttaatttgatgaacttgataagagataagtttgtatattg
gi|b        GTCCAAGTGCACTTGTCTGTGATGCGAT                                                                        1128
gi|CAC2     GTCCAAGTGCACTTGTCTGTGATGCTAT                                                                        1100
Consensus   gtccaagtgcacttgtctgtgatgc at
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Fig. 3 Sequence alignment of the amplicon generated by primers recognizing the transposon CACTA family from hybrid clone D14 with that of the CACTA2 transposon. The two sequences share 97.17% identity. *gi|CAC2*: CACTA2 sequence. *gi|b*: sequence from D14. The identical sequences between CAC2 and b are indicated with *green* background.

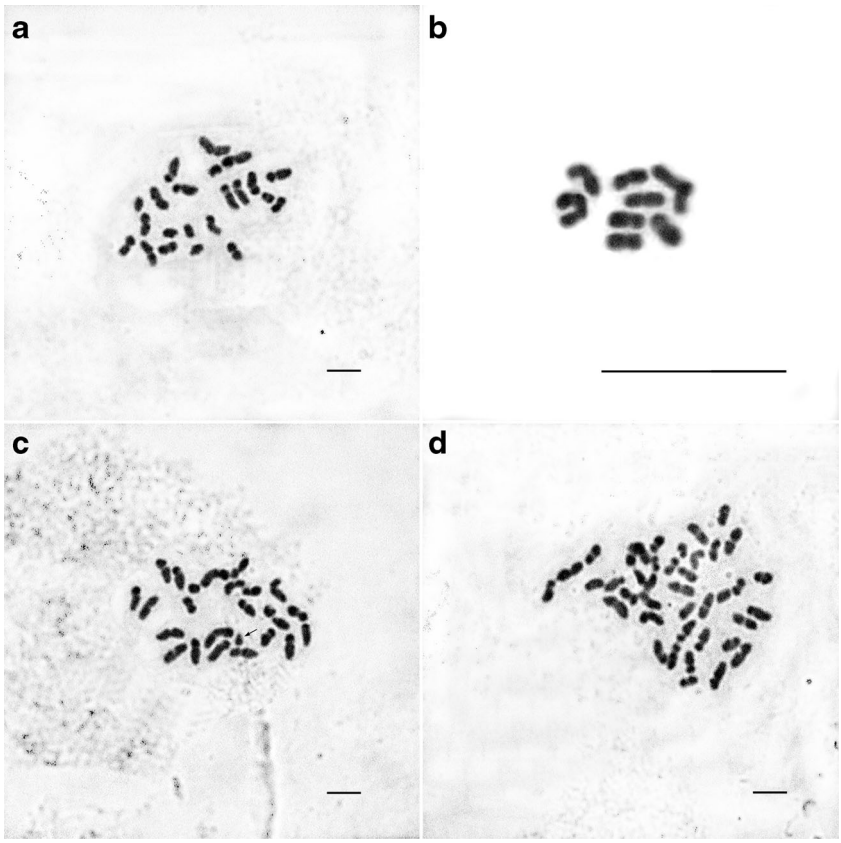

Fig. 4 Mitotic chromosomes of the parents and selected hybrid clones: (*a*) *S. mussotii* showing *2n*=22; (*b*) *A. thaliana* showing *2n*=10; (*c*) combination I hybrid D4 showing *2n*=24; (*d*) combination II hybrid B11 showing *2n*=40. *Rightwards arrow*: Chromosome fragments. *Bar*=5 μm.

Table 2 GC-MS analysis of secondary metabolite profiles of selected hybrid cell lines and the parents

No.	Ret. time/min	Compound	B20	B40	D1	At	Sm
1	3.185	Ethane amine				+	
2	3.238	Dihydroxyvitamin pyran					+
3	3.391	Valeric acid	+	+	+		+
4	3.450	Furaldehyde		+			
5	3.553	2-Hydroxy Ethyl isobutyrate	+			+	
6	3.561	2-Amino-pyran			+		
7	3.002	6-Oxygen-2-dicyclohexyl ketone	+		+		+
8	3.662	4-Hydroxy-4-methyl-2-pentanone		+			+
9	4.617	4-Hydroxyl butyric acid	+		+		
10	4.674	4-Hydroxyl butyric acid		+			
11	4.723	2,5-Dimethyl-4-hydroxy-3-furanone				+	
12	4.782	Cresol ester isobutyrate			+		
13	4.793	2,4-2-Dicarboxylic-5-dimethyl-3-furan-3-ketone	+		+		
14	4.817	5-Methyl furfural	+			+	
15	5.067	5-Methyl-2-furfural			+	+	
16	5.208	4-(2-Methyl-2-butyl) cyclohexanone	+		+		+
17	5.250	Dihydro-pyran-pentene anhydride	+			+	
18	5.439	Hexanoic acid	+	+	+	+	+
19	5.495	2-*sec*-Butylamine	+				
20	5.729	3-Pyrazolidone-3-pyrazolidone		+			+
21	5.835	1,3-Dioxolane					+
22	6.085	1-Ethyl-3-piperidinyl		+	+	+	
23	6.117	Decanoic acid		+			
24	6.307	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	+		+		
25	6.417	Coumaroyl alcohol					+
26	6.958	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-ones	+	+	+		+
27	7.011	2-Furan carboxylic aldehyde					+
28	7.010	Lauric acid				+	
29	7.130	Furanodiyl semicarbazide	+				
30	7.130	Methylpyridine				+	
31	7.250	Propyl cyclohexane	+				+
32	7.338	3-Ethyl-pyridine			+		
33	7.468	4-H-pyran-4-ones	+	+	+		+
34	7.550	Oxygen indene	+	+			
35	7.745	Caprylic acid		+			+
36	7.830	2-Hydroxymethyl-5-methyl furfural	+	+	+		+
37	7.882	4-Methyl-5-hydroxyethyl thiazole		+		+	
38	7.950	4-Vinyl-2-methoxy phenol		+			
39	8.155	Acetoxy-methoxy-pyrrolidone				+	
40	8.236	2-Methyl-thiazole	+		+		+
41	8.359	(E,E)-2,4-Heptadiene aldehyde	+				
42	8.467	2-Methoxy benzene	+		+		+
43	8.512	1-Amino-4-methylpiperazine		+			
44	8.523	Hydroquinone			+		+
45	8.526	Fumaric acid idebenone				+	
46	8.730	Amyl acetate 2 (5H)-furanone	+	+			+
47	8.750	2-Hexenyl valerate		+		+	
48	8.782	2-Octene aldehyde	+				
49	8.801	2,4-Thiazolidinedione			+		

Table 2 (continued)

No.	Ret. time/min	Compound	B20	B40	D1	At	Sm
50	8.873	Benzyl alcohol		+			
51	9.389	Glutamic acid methyl ester	+	+	+	+	+
52	9.400	2-Methoxy benzene		+			
53	9.444	Piperidone				+	
54	9.546	2,4,6-12C acid methyl ester	+				+
55	9.867	Ethyl *p*-hydroxybenzoate	+			+	
56	9.977	3,5-bis [1,1-Dimethyl ethyl]-phenol		+			
57	10.588	Acetyl-carnitine			+		
58	10.836	18 alkyl Decanal		+		+	
59	11.968	Pyrethrins	+				
60	12.258	*N*-butyl-thiophene ethidium-2-ones			+		
61	12.532	Stigmasterol acetate				+	
62	13.000	Nicotinic acid amine	+	+	+	+	+
63	13.140	Neophytadiene	+				
64	13.563	16 carbonate	+	+	+	+	+
65	15.753	*Trans*-phytol			+		
66	16.425	Octadecanoic acid	+	+	+	+	+
67	16.500	Linoleic acid	+				
68	17.488	8,11-Octadecadienoic		+			
69	18.008	Palmitic acid			+	+	+
70	18.279	9,12-Octadecadienoic		+			
71	18.533	3-Butyl-4-N-acid			+		
72	20.382	2(3H)-Furanone		+			
73	20.517	4-Oxygen-6-(1-piperidine-)-oleic acid	+			+	
74	21.025	Cyclohexyl ester acid 18					+
75	22.438	9,18-Tetrapropylene aldehyde	+	+	+	+	+
76	22.837	Linoleic acid ethyl ester	+	+			
77	22.962	Ammonia acid dimethyl amine	+		+		+
78	23.114	1,2-Benzene dicarboxylic acid					+
79	23.313	1H-indole acetic acid		+	+		
80	24.000	1-3,7,8-3-Xanthene-9 ketone					+
81	28.791	Squalene	+	+	+	+	+

At A. thaliana, *Sm S. mussotii*, *B20, B40, D1* hybrid clones

The CACTA transposon family is found in many plant genomes (Miura *et al.* 2001). The *A. thaliana* Col-0 genome harbors five members (CAC1–5), all located in the pericentromeric and centromeric regions (Kato *et al.* 2003; Miura *et al.* 2004). No sign of transposon activity has been demonstrable in an inter-ecotype hybrid or its subsequent generations of self-pollinated progeny (Kato *et al.* 2004). The rare recovery of CACTA transposons among the somatic hybrids (3/65 clones) is consistent with the transposons remaining in a silenced state, despite the genomic shock induced by the protoplast fusion process. Therefore, locus-specific PCR marker-based CACTA transposon analysis afforded us an efficient method to identify the introgressed pericentromeric and centromeric regions from *A. thaliana* in hybrids.

Metabolite profiles of the somatic hybrids. GC-MS was used to characterize the metabolic profiles of the two parents of the somatic hybridization events to identify the origin of the metabolites present in their hybrid offspring. GC-MS has been previously used as an efficient means of comparing the metabolic profiles of various *A. thaliana* genotypes in order to gain some insight into gene function (Fiehn *et al.* 2000). In this study, a significant proportion of metabolites were not present in the profile of either parent (13/32 in D1, 13/36 in B20, and 14/33 in B40). The production of the indole derivative 1H-indoleacetic acid (present in both D1 and B20) may have been a consequence of the more elevated level of auxin (IAA) in these hybrids, which contributed to their rapid growth, an important criterion for selection of putative hybrids (Xia *et al.*

2003; Cai *et al.* 2007). The presence or increase of the unsaturated fatty acids linoleic acid, 8,11-octadecadienoic acid, and 9,12-octadecadienoic acid in the hybrids has previously been noted by Wang *et al.* (2010) and Jiang *et al.* (2009). Although the terpenoid pyrethrin is well known to be produced in chrysanthemum (*Chrysanthemum cinerariaefolium*) (Crombie 1995), it has not been identified to date in either of the parental species. The novel production of so many compounds in these hybrids suggests that genomic shock may have occurred. Genomic shock, which can occur in somatic hybrids, is the nuclear response to an unusual challenge in which the genome becomes extensively restructured (McClintock 1984). The "unusual challenge" most frequently encountered is a wide sexual cross (Pikaard 1999; Comai 2000), but the outcome of somatic hybridization—the fusion of two heterologous nuclei—is in principle no different from this, and genomic shock-associated events, such as altered microsatellite profiles, gene silencing, and epigenetic changes, have been well documented for a number of somatic hybrids (Cai *et al.* 2007; Bassene *et al.* 2009; Shan *et al.* 2009).

Somatic hybridization provides a means to transfer chromosomal fragments and therefore in principle complete metabolic pathways, across a wide phylogenetic distance. This option is seldom available *via* the sexual route or requires transgenic approaches. The genome of the *S. mussotii–A. thaliana* somatic hybrid calli and regenerated plantlets was composed largely of *S. mussotii* DNA, but there was evidence of some introgression from *A. thaliana* that was sufficient to disturb the metabolic profile.

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