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TM3x: Triploid Black Sigatoka–Resistant *Musa* Hybrid Germplasm

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The International Institute of Tropical Agriculture (IITA) targets the genetic improvement of plantain and banana (*Musa* spp. L.) through the utilization of all available *Musa* genetic resources and breeding methods (Vuylsteke et al., 1997). Black sigatoka–resistant tetraploid plantain hybrids and plantain-derived diploids have been produced by the Plantain and Banana Improvement Program (PBIP) of IITA and placed in the public domain by their registration in *HortScience* (Vuylsteke et al., 1993a, 1995; Vuylsteke and Ortiz, 1995). Also, improved diploid banana germplasm has been developed by the Fundacion Hondurena de Investigacion Agricola (FHIA, Honduras) and successfully utilized as parents of internationally released tetraploid hybrids, such as the dessert banana 'FHIA-1' (or 'Goldfinger') and the cooking banana 'FHIA-3' (Rowe and Rosales 1993). However, tetraploids are male fertile and capable of crossing with other euploids (either tetraploids or diploids), thereby setting hard seeds and making the fruit unpleasant to eat. Therefore, one more step of ploidy manipulations, i.e., the reduction of the chromosome number to the triploid level, was pursued vigorously at IITA to develop male-sterile hybrids (Ortiz, 1997).

Tropical *Musa* secondary triploid hybrids (hereafter TM3x) have been obtained from tetraploid–diploid crosses and selected after intensive testing at the IITA High Rainfall Station in Onne (southeastern Nigeria). The female parents of TM3x were black sigatoka–resistant primary tetraploid hybrids (hereafter TMPx) developed by IITA in Nigeria from triploid–diploid crosses (Vuylsteke et al., 1993b), while the male partners were either diploid hybrids selected by FHIA in Honduras (hereafter SH), or plantain-derived diploids (hereafter TMP2x) obtained by IITA in Nigeria from triploid–diploid crosses (Ortiz et al., 1995).

The TM3x germplasm was developed at IITA during the period 1992–96. Selected hybrids from this germplasm are available to breeders and geneticists interested in germplasm enhancement or for further testing and cultivar release in accordance with a country's specific variety release regulations.

Origin

'PITA-15' (tested as TM3x 15108-2), 'PITA-16' (tested as TM3x 15108-6), and 'PITA-19' (tested as TM3x 15108-1) are black sigatoka–resistant secondary triploid hybrids derived from the cross TMPx 4479-1 x SH-3362 (Fig. 1). TMPx 4479-1 is a selected primary tetraploid hybrid developed at IITA by crossing the French plantain landrace 'Bobby Tannap' with the wild diploid banana 'Calcutta 4' (Vuylsteke et al., 1993b), while SH-3362 is an improved diploid banana from FHIA (Rowe and Rosales, 1993, 1996).

The pedigrees of 'PITA-15', 'PITA-16', and 'PITA-19' reflect the importance of free availability of *Musa* genetic resources for the genetic improvement of banana and plantain. The diploid grandparental accessions of SH-2095, one of the diploid paternal great-grandparents of this secondary triploid germplasm, were collected in Papua New Guinea ('Sinwobogi'), Java in Indonesia ('Tjau Lagada'), Malaysia (wild *M. malaccensis*), and the Philippines ('Guyod'), whereas the other diploid great-grandparent 'Pisang jari buaya' was collected in Malaysia (Stover and Simmonds, 1987). The maternal grandparents were a medium-sized triploid French plantain landrace ('Bobby Tannap') from Cameroon and a wild diploid banana ('Calcutta 4') from Burma (or Myanmar) (Vuylsteke et al., 1993a). Thus, there were inputs from Africa (landrace triploid maternal germplasm and selection at IITA), America (development of diploid paternal parent by FHIA), Asia (wild and cultivated diploid germplasm), and the Pacific (cultivated germplasm) for the development of these secondary triploid hybrids. Hence, this improved germplasm developed by IITA should be regarded as a tetra-continental output.

'PITA-20' (tested as TM3x 14604-35), another black sigatoka–resistant secondary triploid hybrid, was obtained from crossing the selected full-sibs TMPx 6930-1 (tetraploid) and TMP2x 1549-7 (diploid) (Fig. 2). Both primary euploid hybrids were derived

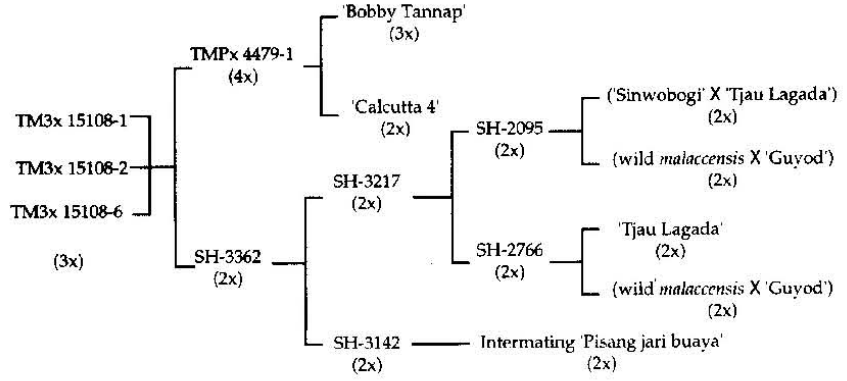

Fig. 1. Pedigree of 'PITA-15' (or TM3x 15108-2), 'PITA-16' (or TM3x 15108-6), and 'PITA-19' (or TM3x 15108-1).

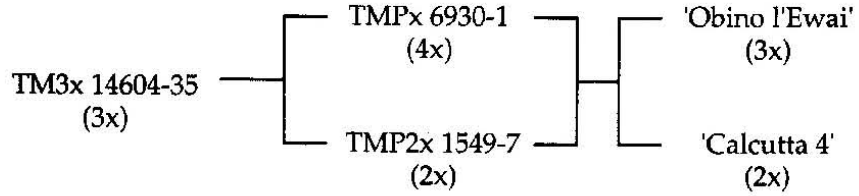

Fig. 2. Pedigree of 'PITA-20' (or TM3x 14604-35).

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from 'Obino l'Ewai' x 'Calcutta 4'. The maternal grandparent of 'PITA-20' is a medium-sized triploid French plantain landrace from Nigeria.

TMPx 4479-1 was hand-pollinated with pollen of SH-3362. This cross produced 100 seeds, from which nine triploid seedlings were germinated in vitro (Vuylsteke et al., 1990). Similarly, TMPx 6930-1 was artificially pollinated with pollen of TMP2x 1549-7 to obtain 134 seeds, which produced 48 seedlings after in vitro embryo culture. These seedlings were planted in 1992 in an early evaluation trial (EET) at Onne and fruit were harvested in the 1993–94 season (PBIP, 1994). The four secondary triploids selected from this EET exhibited partial resistance to black sigatoka, using the method of Vakili (1968), and had significantly greater bunch mass (11.0–16.4 kg per plant) than the population mean (4.5 kg ± 0.3) (Ortiz and Vuylsteke, 1994). All selected hybrids were advanced to a preliminary yield trial in 1994, along with their parents and grandparents (Plantain and Banana Improvement Program, 1995).

Description and performance

The selected secondary triploid hybrids outyielded their polyploid parents and grandparents at Onne (Fig. 3), but fruit of 'PITA-19' was smaller than that of the French plantain landraces (Table 1). 'PITA-16', the secondary triploid with the heaviest bunch, has a pendulous dense bunch with curved angular bottlenecked fruits and short pedicels. It has an imbricated male bud and sometimes shows a few persistent neutral flowers in its inflorescence. This male-sterile triploid hybrid exhibits regulated suckering behavior. 'PITA-16' has been advanced for testing in multilocational trials at IITA stations in Nigeria and, together with 'PITA-15', has been sent to Uganda for local testing.

All selected TM3x showed partial resistance to black sigatoka, as measured by the number of standing functional leaves without spots at flowering (Table 1). 'PITA-16', like its female parent TMPx 4479-1, appears to be virus tolerant at Onne (Plantain and Banana Improvement Program, 1996), where banana streak virus and cucumber mosaic virus have been observed. In addition, 'PITA-16' and its full-sibs could have resistance to fusarium wilt [*Fusarium oxysporum* Schlecht. f. sp. *cubense* (E.F. Smith) Snyd. & Hans.] and burrowing nematode [*Radopholus similis* (Cobb) Thorne] based on their pedigree (Fig. 1). Their diploid parent has been reported to be resistant to fusarium wilt, and their paternal grandparent, SH-3142, is an improved nematode-resistant diploid. SH-3142 was developed by FHIA from the highly nematode-resistant diploid banana 'Pisang jari buaya' (Rowe and Rosales, 1993, 1996).

'PITA-15' had the best fruit quality among the secondary triploids tested in Nigerian taste panels on boiled unripe fruits (Plantain and Banana Improvement Program, 1996). Furthermore, 17% of the panelists preferred the boiled unripe fruits of this hybrid to those of the widespread False Horn plantain landrace 'Agbagba'. A similar number favored boiled unripe fruits of 'PITA-15' to those of the registered TMPx 4479-1. In general, panelists considered that 'PITA-15' had boiled unripe fruit of acceptable taste. However, 63% of the same panelists preferred the fried ripe fruits of 'PITA-16' in a Nigerian dish called "dodo." This discrepancy between taste panels shows the importance of fruit end-use in the development of improved *Musa* germplasm. Such situations will always occur when breeding a crop like *Musa,* which has multiple end-uses.

'PITA-20' had significantly greater bunch mass than its polyploid parent and grandparent in the plant crop (Table 1). Because of its shorter growth cycle, the yield potential of 'PITA-20' (20.0 $t \cdot ha^{-1} \cdot year^{-1}$) was higher than that of 'PITA-16' (19.0 $t \cdot ha^{-1} \cdot year^{-1}$) and double that of 'Bobby Tannap' (9.9 $t \cdot ha^{-1} \cdot year^{-1}$). Although taste panel assessment has not been carried out on 'PITA-20', preliminary results showed that panelists preferred "dodo" made from fruits of secondary triploids obtained by intercrossing plantain-derived euploid hybrids, to that made from secondary triploids such as 'PITA-15' and 'PITA-16' (Plantain and Banana Improvement Program, 1996). This result was not surprising, since hybrids like 'PITA-20' have more plantain alleles than 'PITA-15' and 'PITA-16', which inherited at

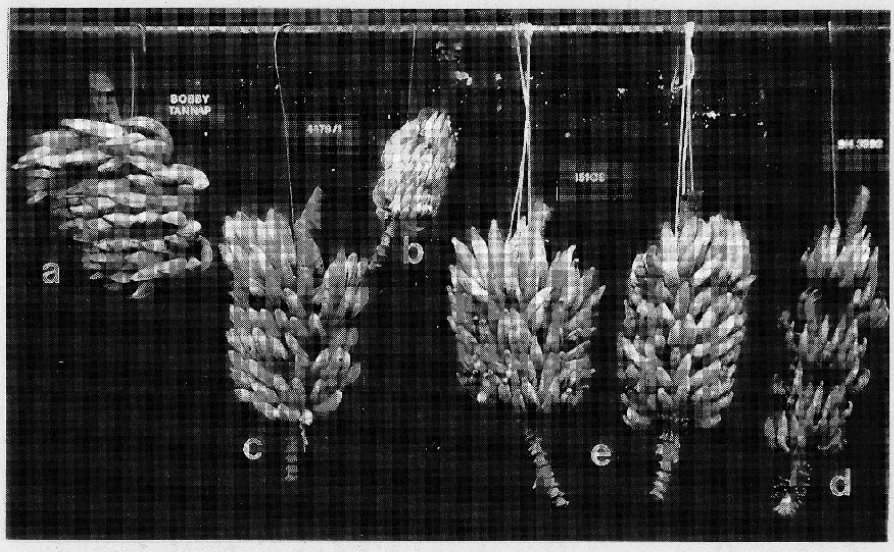

Fig. 3. Ploidy manipulation in *Musa*: Black sigatoka–resistant secondary triploid hybrids TM3x 15108-2 and TM3x 15108-6 (**e**), obtained from crossing the black sigatoka partially resistant tetraploid TMPx 4479-1 (**c**) and the improved diploid banana SH-3362 (**d**). The tetraploid hybrid parent was obtained by crossing the susceptible triploid plantain 'Bobby Tannap' (**a**) with the highly resistant wild diploid banana 'Calcutta 4' (**b**).

Table 1. Preliminary yield trial of *Musa* secondary triploid hybrids, primary tetraploid hybrid parents, and triploid landraces at Onne, Nigeria (1994–96).

Clone	Days to flowering	Plant height (cm)	Standing leaf count (no.)	Youngest leaf spotted (no.)	Tallest sucker height (cm)	Days for fruit filling	Bunch mass (kg)	No. hands	No. fruits	Fruit length (cm)	Fruit girth (cm)
Black sigatoka–resistant (BSR) secondary triploids (TM3x)											
'PITA-15'	321	268	14	12	47	102	12.7	7	106	22	12
'PITA-16'	329	239	9	7	223	133	14.4	7	127	18	13
'PITA-19'	333	215	12	9	64	116	11.6	8	119	16	11
'PITA-20'	302	275	14	13	209	125	14.0	7	112	21	13
BSR primary tetraploid (TMPx) parents of selected TM3x											
4479-1	321	276	11	9	229	111	9.0	6	74	20	13
6930-1	339	238	12	8	151	124	8.9	7	84	17	12
Black sigatoka–susceptible maternal grandmother plantain landraces											
'Bobby Tannap'	353	298	8	6	74	89	7.2	6	65	21	13
'Obino l'Ewai'	396	290	8	5	70	90	9.0	7	70	19	13
$LSD_{0.05}$	33	19	1	2	35	13	2.0	1	15	2	1

Fig. 4. Bunch of a secondary triploid plantain hybrid with persistent neutral flowers. This bunch type resembles that of its maternal grandparent, the French plantain 'Obino l' Ewai'.

least one-third of their alleles from an unrelated banana parent.

'PITA-20', a male-sterile triploid with regulated suckering behavior, shows a pendulous dense bunch with short pedicels, angular bottlenecked fruits, and imbricated male bud. 'PITA-20' sometimes shows a small number of persistent neutral flowers in its inflorescence at the beginning of fruit filling. An inflorescence type with persistent neutral flowers (Fig. 4) may enable selected TM3x to be more attractive to farmers who are used to cultivating French plantains. The recovery of this grandparental inflorescence phenotype is expected from intercrossing plantain-derived hybrids, since each of the full-sib parents has at least one of the two dominant genes with complementary gene action that control this characteristic in *Musa* (Ortiz, 1995).

The development of TM3x suggests that heterosis for bunch mass and yield potential can be achieved either by maximizing heterozygosity through crossing unrelated parents (e.g., 'PITA-16' from the cross TMPx 4479-1 X SH-3362) or by proper selection within populations of hybrids derived from full-sib parents (e.g., 'PITA-20') (Ortiz, 1997). However, the latter approach, also known as "progressive heterosis" in alfalfa (Groose et al., 1989), will require a relatively larger segregating population size (about 50 sibs) to identify the right genetic combination based on phenotype.

Molecular characterization

Molecular genetic analysis has been proposed as an effective means of identifying cultivars and establishing the boundaries of ownership in order to help protect plant breeders' rights (Melchinger et al., 1994). Simple sequence length polymorphisms (SSLP) are particularly well suited to this application as they are abundant and multiallelic. As this marker system relies upon the polymerase chain reaction, it is suitable for high throughput applications and automation (Bates et al., 1996). In addition, the co-dominant nature of SSLP markers allows the allelic contribution of each parent to be determined for each hybrid. SSLP analysis has already been used for variety identification in grapevine and soybean (Rongwen et al., 1995; Thomas and Scott, 1993), but this is the first case of its utilization in the registration of *Musa* hybrids.

DNA was isolated from plants growing in field plots at Onne Station, Nigeria, as previously described (Gawel and Jarret, 1991). Primer pairs (hereafter primers) for specific genomic regions containing microsatellites were developed as described previously (Jarret et al., 1994). The PCR amplification conditions were adapted from those described by Yu et al. (1994). A random selection of 33 primers was screened on the grandparental genotypes 'Obino l'Ewai' and 'Calcutta 4', followed by the screening of 12 of these primers on 14 full-sib tetraploid hybrids (including parental genotypes TMPx 548-4 and TMPx 6930-1). Based on this prescreening information, three primers were selected for the analysis of the germplasm described above. Two of these primers were found to be highly informative in distinguishing this germplasm, and their characterization is shown in Table 2.

A single primer (Ma-3-139) was identified that generated unique SSLP fingerprints for each hybrid (Fig. 5). However, the accumulation of banding patterns generated by two primers (Ma-3-139 and Ma-0-48) was necessary to distinguish all hybrids from all parental and grandparental genotypes. This molecular genetic characterization serves to demonstrate the uniqueness of these hybrids from each other and from the progenitor germplasm. Comparable screening with RAPD assays failed to identify any single primer generating unique fingerprints for each hybrid. This observation suggests that SSLP is indeed the more appropriate assay for fingerprinting *Musa* hybrid germplasm.

Availability

IITA shares germplasm with partners in developed and developing countries under the agreement that this material will not be licensed for commercial purposes. Hence, TM3x germplasm cannot be patented or registered for restricted release. Researchers interested in using TM3x germplasm should write to the Leader, Plantain and Banana Improvement Program, IITA, c/o Lambourn and Co., 26 Dingwall Rd., Croydon, CR9 3EE, England; e-mail: IITA@CGNET.COM. Virus-indexed in vitro stocks of TMPx 4479-1, the maternal parent of 'PITA-15', 'PITA-16', and 'PITA-19', are available upon request to the Director,

Table 2. Characterization of PCR primers used for generating unique fingerprints of secondary triploid *Musa* hybrids (TM3x).

Marker	Primer sequence (5′ -> 3′)	Tm (°C)	Repeat motif
Ma-0-48	CCCGTCCCATTTCTCA TTCGTTGTTCATGGAATCA	58	$(GA)_{15}$
Ma-3-139	ACTGCTGCTCTCCACCTCAAC GTCCCCCAAGAACCATATGATT	62	$(GA)_{14}$

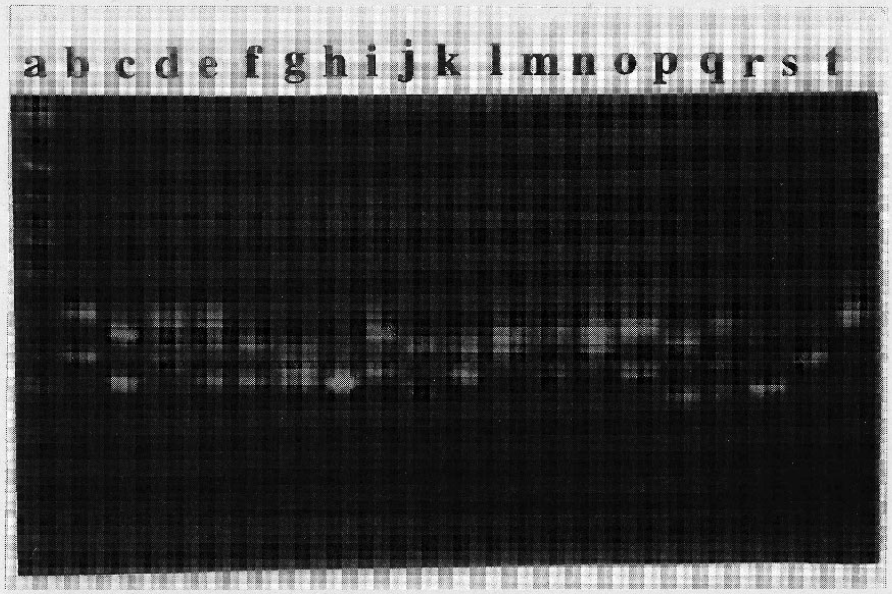

Fig. 5. PCR amplification of simple sequence length polymorphisms in 'Bobby Tannap', 'Calcutta 4', TMPx 4479-1, TM3x 15108-1, TM3x 15108-2, TM3x 15108-6, SH-3362; 'Obino l'Ewai', 'Calcutta 4', TMPx 6930-1, TM3x 14604-35, 14064-37, TMP2x 1549-7; 'Obino l'Ewai', 'Calcutta 4', TMPx 548-4, TM3x 14123-5, TM3x 14123-6, 'Galeo'; (b-t, respectively; a = 100 b.p. DNA ladder) using primer Ma-3-139, electrophoretically separated on 4% Metaphor agarose gel containing 0.3 mg·mL^{-1} ethidium bromide.

INIBAP, Parc Scientifique Agropolis, 34397 Montpellier Cedex 5, France; e-mail: INIBAP@CGNET.COM. Recipients are asked to give appropriate recognition of the germplasm source if it is used in developing a new germplasm, parental line, or cultivar.

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