Plantain-derived Diploid Hybrids (TMP2x) with Black Sigatoka Resistance

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Plantain (*Musa* spp., AAB group), a triploid ($2n = 3x = 33$) giant perennial herb of the tropics, is a natural interspecific hybrid between the two wild banana species *M. acuminata* Colla. and *M. balbisiana* Colla. These diploid species contributed the A and B genomes, respectively (Simmonds, 1962). Triploidy makes plantain crossbreeding difficult but not impossible (Swennen and Vuylsteke, 1993). As such, plantain progenies were obtained when triploid plantains were crossed with diploid *M. acuminata* clones (Vuylsteke et al., 1993c). Genetic improvement of plantain is required because its sustainable production is threatened by increasing disease and pest pressure (Vuylsteke et al., 1993b). One of the major constraints is the black sigatoka leaf spot disease (*Mycosphaerella fijiensis* Morelet), which causes yield losses of at least 33% (Mobambo et al., 1993). Production and cultivation of resistant cultivars are generally considered the most appropriate means to control the disease.

The International Institute of Tropical Agriculture (IITA) targets the development of improved plantain germplasm. So far, 14 improved plantain-derived tetraploid (4x) hybrids with black sigatoka resistance have been selected for multilocal testing and further cultivar release by national agricultural research programs (Vuylsteke et al., 1993d). In addition to the tetraploid hybrids, progenies with other ploidies were also recovered from the $3x \times 2x$ crosses, diploids (2x) being most numerous (Vuylsteke et al., 1993c). Plantain-derived diploids play an important role in

plantain breeding because they provide opportunity for germplasm enhancement at the diploid level and are a source of plantain alleles (Vuylsteke et al., 1993a). Their occurrence also simplifies genetic analysis due to disomic inheritance, which facilitates and accelerates breeding. For example, the genetics of black sigatoka disease resistance, dwarfism, apical dominance, fruit parthenocarpy, bunch weight, and other yield components were established using these plantain-derived diploids (Ortiz and Vuylsteke, 1993). Most significant was the elucidation of the inheritance of black sigatoka resistance in plantain, which is controlled by a major recessive gene and two minor modifier genes with additive effects (Ortiz and Vuylsteke, 1992, 1994a). The diploid germplasm is currently being used by IITA and the South Atlantic Area Regional Plant Introduction Station of the U.S. Dept. of Agriculture–Agricultural Research Service to develop a saturated molecular map of *Musa*.

The diploid breeding/genetic stock was developed at IITA during the period 1988–93. These potential parental clones are ready for distribution to breeders and geneticists interested in either germplasm enhancement or genetic analysis of the plantain genome.

Origin

The plantain-derived diploid hybrids (Fig. 1), hereafter referred to as TMP2x (tropical *Musa* plantain-derived diploid hybrids), are F_1 progenies obtained from interspecific interploidy crosses (Vuylsteke et al., 1993c). The female parents were the triploid ($2n = 3x$

= 33) “French” plantain cultivars Bobby Tannap (Cameroon); Bungaoisan (Philippines), Ntanga-2 and Obino l’Ewai (Nigeria) (Swennen, 1990); and French Reversion, a somaclonal variant of the Nigerian False Horn plantain cultivar Agbagba (Vuylsteke et al., 1991). These cultivars were selected as parents due to their relatively high female fertility (Swennen and Vuylsteke, 1993). The diploid ($2n = 2x = 22$) male parent was the wild inedible banana ‘Calcutta 4’ (*M. acuminata* ssp. *burmannicoides*) from Burma/Myanmar (De Langhe and Devreux, 1960). ‘Calcutta 4’ has true-seeded fleshless nonparthenocarpic fruit because it lacks one of the three dominant complementary genes for parthenocarpy (Simmonds, 1953). Nevertheless, ‘Calcutta 4’ was used as male parent because it produces large quantities of fertile pollen, is black sigatoka resistant, and has been considered taxonomically related to plantain (De Langhe, 1969).

The $3x \times 2x$ crosses, which led to the production of the TMP2x germplasm, were made from 1988 to 1990. Several thousand seeds were harvested and embryos germinated in vitro. Tetraploid and diploid progenies were initially sorted out using stomatal size and density as discriminant function (Simmonds, 1948; Vandenhout, 1993). The ploidy level of some of these hybrids was later confirmed by chromosome counts using root tips. Diploid progenies resulted from the union of normal ($n = x$) gametes of both parents (Vuylsteke et al., 1993c).

Several hundred diploid hybrids were field-established in early evaluation trials (EETs) in 1989–90, and 81 promising hybrids advanced to preliminary yield trials (PYTs) in 1991–93 (IITA, 1993). The EETs were nonreplicated trials in which one to five plants per genotype were evaluated during one production cycle (≈ 1 year). The first PYTs were also nonreplicated trials in which four to five plants per genotype were observed in single-row plots during two production cycles (≈ 2 years). The selection criteria were partial resistance to black sigatoka disease, significantly higher bunch weight than the mean of the diploid breeding population, and general vigor. Genotype response to black sigatoka infection was measured using the method of Vakili (1968),

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'Bobby Tannap' x 'Calcutta 4'	→	TMP2x 1518-4, TMP2x 1657-4 TMP2x 2625-20, TMP2x 2629-62 TMP2x 4600-12, TMP2x 9722-1
'Bungaoisan' x 'Calcutta 4'	→	TMP2x 2348-6, TMP2x 2348-7
'French Reversion' x 'Calcutta 4'	→	TMP2x 1297-3
'Ntanga-2' x 'Calcutta 4'	→	TMP2x 1605-1
'Obino l'Ewai' x 'Calcutta 4'	→	TMP2x 597-2, TMP2x 1199-6 TMP2x 1448-1, TMP2x 1549-5

Fig. 1. Parentage of the 14 selected plantain-derived diploid hybrids (TMP2x) obtained from crossing the $3x$ plantains (*Musa* spp., AAB group) with the wild $2x$ ‘Calcutta 4’ banana.

which consists of recording the number of the youngest leaf spotted (YLS), counting down from the first (top) unfurled leaf, on plants at flowering, which is the time when leaf production stops. YLS correlates significantly with disease development time. Therefore, increasing YLS values indicate the presence of more healthy leaves on the plant and, hence, greater resistance to the fungus. Triploid black sigatoka-susceptible plantains or bananas have on average a YLS of four to six, whereas hybrids exhibiting partial resistance have a YLS ≥ 8 in the rainy season (Mobambo et al., 1993; Vuylsteke et al., 1993c). Growth and yield variables were evaluated at flowering and at harvest in EETs and PYTs, as described by Swennen and De Langhe (1985).

After intensive testing from 1989 to 1993 at Onne, IITA's High Rainfall Station in the humid forest zone near Port Harcourt, Nigeria, a total of 14 TMP2x clones was selected for further use as parental stock for primary tetraploids (in 3x x 2x crosses) or for secondary triploids (in 4x x 2x crosses).

Description and performance

The TMP2x clones are identified by their original cross/progeny number (Fig. 1). All

the TMP2x have higher levels of black sigatoka resistance than their susceptible plantain parents, as determined by an evaluation of YLS. Host response to black sigatoka in the partially resistant TMP2x germplasm is based on slower or delayed disease development. Less leaf spot damage ensues. Such host response seems to provide more durable resistance (Ortiz and Vuylsteke, 1994a).

Two exceptional 'Bungoisan'-derived hybrids, TMP2x 2348-6 and TMP2x 2348-7, were initially selected due to their greater bunch weight and large parthenocarpic fruit (Table 1). In addition, these two fertile, black sigatoka-resistant TMP2x have short to medium plants and weak apical dominance, i.e., improved suckering behavior. TMP2x 2348-7 has a subhorizontal bunch, while TMP2x 2348-6 has a pendulous bunch. The latter is an economically important character because pendulous bunches are more symmetrical and, therefore, better adapted for transportation. TMP2x 2348-6 has deciduous hermaphrodite flowers, while TMP2x 2348-7 has persistent hermaphrodite flowers and exhibits male bud imbrication.

Other promising diploid hybrids derived from cultivars Bobby Tannap and Obino I'Ewai were evaluated in PYTs in plant and ratoon

crops. Growth and yield characteristics of the average diploid population, the selected TMP2x, and of the male parent 'Calcutta 4' are listed in Table 2. Most of these selected TMP2x have subhorizontal bunch orientation, except TMP2x 597-2, 2829-62, and 9722-1, which have a pendulous bunch. Hermaphrodite flowers are deciduous like the male banana parent, except in TMP2x 1199-6, 1657-4, and 9722-1, which have persistent hermaphrodite flowers. This is a characteristic typical of the female "French" plantains. TMP2x 1448-1, 1549-5, 1657-4, 2829-62, and 9722-1 exhibit male bud imbrication. All selected TMP2x do not show the strong apical dominance of the plantains. Moreover, TMP2x 597-2, 1199-6, 2625-20, 2829-62, and 9722-1 develop only one or two suckers freely while further suckering is inhibited. Such regulated suckering is a highly desirable, dominant trait for perennial plantain production (Ortiz and Vuylsteke, 1994b). In contrast to their plantain parents, which are male-sterile, all the diploid hybrids, except TMP2x 9722-1, are male-fertile. Also, TMP2x 597-2 and TMP2x 1657-4 did not set seed when crossed as female partners in interdiploid crosses, suggesting female sterility. TMP2x 1518-4 has good bunch configuration (Fig. 2) and a greater number of

Table 1. Black sigatoka resistance and agronomic evaluation of four selected TMP2x clones compared with the average of the plantain-derived diploid population in early evaluation trials at Onne, Nigeria, 1989-90.^z

Clone ^v	YLS ^y at flowering	Plant ht (m)	HTS ^x at harvest (m)	Bunch wt (kg)	Hands (no.)	Fruit (no.)	Fruit			Filling time ^w (days)
							Wt (g)	Length (cm)	Girth (cm)	
TMP2x 2348-6	10	2.7	2.9	13.9	8	126	98	15	10	139
TMP2x 2348-7	9	2.2	2.9	15.2	7	117	130	15	10	165
TMP2x 597-2	9	3.6	1.9	7.8	7	108	72	18	10	118
TMP2x 1448-1	9	3.5	3.2	6.0	8	161	33	11	9	181
2x population ^a	8 $\pm$ 0.2	2.5 $\pm$ 0.1	2.5 $\pm$ 0.1	4.3 $\pm$ 0.7	6 $\pm$ 0.2	92 $\pm$ 6	46 $\pm$ 6	11 $\pm$ 1	10 $\pm$ 1	115 $\pm$ 6

^zGrowth and yield variables measured according to Swennen and De Langhe (1985).

^yGenotype response to black sigatoka disease measured using youngest leaf spotted (YLS) according to Vakili (1968).

^xHTS = height of tallest sucker (follower) at harvest of main pseudostem.

^wTime from inflorescence emergence to harvest.

^vSee Fig. 1 for parentage.

^aMean $\pm$ SE, n = 32 clones.

Table 2. Black sigatoka resistance and agronomic evaluation of 10 selected TMP2x clones compared with the performance of their diploid male parent 'Calcutta 4' and the average of the plantain-derived diploid population in preliminary yield trials at Onne, Nigeria, 1991-93 (plant and first-ratoon crops).^z

Clone ^w	YLS ^y at flowering	Plant ht (m)	HTS ^x at harvest (m)	Bunch wt (kg)	Hands (no.)	Fruit (no.)	Fruit			Filling time (days)
							Wt (g)	Length (cm)	Girth (cm)	
TMP2x 597-2	10	3.6 [*]	3.5 [*]	7.4 [*]	6	94	79 [*]	15 [*]	10 [*]	125 [*]
TMP2x 1199-6	10	3.1 [*]	2.5	8.3 [*]	7	125	65 [*]	16 [*]	9 [*]	115
TMP2x 1448-1	9	3.1 [*]	3.3	5.8 [*]	7	122	50 [*]	13 [*]	8 [*]	125 [*]
TMP2x 1518-4	10	3.4 [*]	2.6	5.6 [*]	9 [*]	146	54 [*]	13 [*]	6 [*]	103
TMP2x 1549-5	10	3.6 [*]	2.8	5.8 [*]	10 [*]	187 [*]	35 [*]	13 [*]	7 [*]	117 [*]
TMP2x 1657-4	9	3.1 [*]	2.7	7.9 [*]	8	155	48 [*]	13 [*]	8 [*]	125 [*]
TMP2x 2625-20	12	2.6	2.6	7.9 [*]	8	112	69 [*]	14 [*]	10 [*]	149 [*]
TMP2x 2829-62	11	3.4 [*]	2.8	5.5 [*]	8	137	35 [*]	14 [*]	7 [*]	123 [*]
TMP2x 4600-12	12	2.9 [*]	2.5	6.1 [*]	7	107	55 [*]	11 [*]	8 [*]	143 [*]
TMP2x 9722-1	12 ^v	3.0 [*]	2.8	5.7 [*]	9 [*]	142	39 [*]	14 [*]	7 [*]	87 [*]
2x population ^a	8 $\pm$ 0.2	2.6 $\pm$ 0.2	2.2 $\pm$ 0.1	3.0 $\pm$ 0.2	6 $\pm$ 0.1	95 $\pm$ 4	32 $\pm$ 2	11 $\pm$ 0.3	7 $\pm$ 0.2	117 $\pm$ 3
Calcutta 4	--- ^v	2.2 $\pm$ 0.2	2.8 $\pm$ 0.2	0.8 $\pm$ 0.3	7 $\pm$ 0.2	113 $\pm$ 15	6 $\pm$ 2	5 $\pm$ 1	3 $\pm$ 1	104 $\pm$ 4

^zGrowth and yield variables measured according to Swennen and De Langhe (1985).

^yGenotype response to black sigatoka disease measured using youngest leaf spotted (YLS) according to Vakili (1968).

^xHTS = height of tallest sucker (follower) at harvest of main pseudostem.

^wSee Fig. 1 for parentage.

^vHighly resistant to black sigatoka; leaf spots not readily observed.

^aMean $\pm$ SE, n = 81 clones.

^{*}Significant at $P \leq 0.05$ for multiple comparison within columns of TMP2x genotypes with their male diploid parent 'Calcutta 4'. Critical values of Student's t distribution were adjusted by Sidak's multiplicative inequality based on $\alpha^* = 1 - (1 - \alpha)/c$, in which α^* is the level of significance required for statistical testing and c is the number of comparisons between the hybrids and 'Calcutta 4', i.e., 10 (Sokal and Rohlf, 1981).

Fig. 2. Black-sigatoka-resistant, plantain-derived diploid hybrid TMP2x 1518-4 (center), obtained from crossing the 3x susceptible 'Bobby Tannap' plantain (left) with the highly resistant wild 2x 'Calcutta 4' banana (right).

hands and fruit than most of the other diploid selections (Table 2).

Another two clones, TMP2x 1297-3 and TMP2x 1605-1, were also selected in 1989–91. The black-sigatoka-resistant TMP2x 1297-3 and 1605-1 have weak apical dominance and high-yielding, subhorizontal (to almost pendulous) bunches with parthenocarpic fruit. These two diploid hybrids show striking morphological resemblance to the "French" plantains and may be genetically close to the lost diploid progenitor(s) that played a key role in the phylogeny of the triploid plantain germplasm (De Langhe, 1993). TMP2x 1605-1, a trisomic ($2n = 2x + 1 = 23$) derived from the giant plantain cultivar Ntanga-2, is male-sterile but female-fertile. TMP2x 1297-3 is a diploid derived from the 'French Reversion' somaclonal variant of 'Agbagba'. TMP2x 1297-3 offers the opportunity to breed in the previously inaccessible False Horn plantain gene pool (IITA, 1993). For example, TMP2x 1297-3 was crossed with its full-sib tetraploid TMPx 1112-1 to produce secondary triploid segregating offspring in which directional selection for large fruit size was applied. This breeding scheme aims to develop improved *Musa* germplasm with fruit size and quality similar to the preferred False Horn plantains.

Most of the improved plantain-derived diploids were crossed with primary TMPx tetraploids (Vuylsteke et al., 1993d) to assess their breeding value (IITA, 1993). Several secondary triploids, combining short to medium plant stature, high levels of black sigatoka resistance, weak apical dominance or regulated suckering, pendulous bunches with parthenocarpic fruit, and acceptable yields, were identified in the segregating populations. This was expected because promising tetraploid hybrids (TMPx) were selected earlier even when using a wild, nonselected male parent such as 'Calcutta 4' (Vuylsteke et al., 1993c). Segregation for plant size was expected because all hybrids are carriers of the recessive dwarfism (*dw*) gene (Ortiz and Vuylsteke,

1993). Moreover, TMP2x likely will produce offspring with more plantain-like characteristics than progenies derived from 'Calcutta 4', because TMP2x have 50% plantain genes in their genome. This genetic background may be important for improving fruit quality in the plantain breeding population. Also, plantain progenies derived from selected TMP2x likely have the same degree of black sigatoka resistance as progenies of 'Calcutta 4' because they have the same genotype for partial resistance to black sigatoka (Ortiz and Vuylsteke, 1994a).

Availability

Limited numbers of in vitro plantlets are available upon request. Researchers interested in using TMP2x germplasm as a source of plantain alleles for development of improved *Musa* populations in their breeding programs should write to the Leader, Plantain and Banana Improvement Program, IITA, c/o Lambourn & Co., Carolyn House, 26 Dingwall Rd., Croydon CR9 3EE, England. 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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