

A new source of resistance to bacterial wilt of eggplants obtained from a cross: *Solanum aethiopicum* L x *Solanum melongena* L

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Summary — As the eggplant hybrid F₁ Kalenda no longer showed enough resistance to *Pseudomonas solanacearum* EF Smith in Guadeloupe and Martinique (French West Indies), a new breeding program was undertaken using *Solanum aethiopicum* as source of resistance. Despite sterility problems encountered during the first generations, selection was conducted under an artificial inoculation test according to a recurrent selection scheme including backcrosses by *Solanum melongena*. From the second backcross it was possible to obtain families with a high level of resistance to bacterial wilt, as well as a wide variation in the shape and colour of the fruits.

Solanum melongena / *Solanum aethiopicum* / *Pseudomonas solanacearum* / resistance / recurrent selection

Résumé — Une nouvelle source de résistance au flétrissement bactérien obtenue à partir d'un croisement entre *Solanum aethiopicum* L et *Solanum melongena* L. Un nouveau programme de sélection d'aubergines utilisant *Solanum aethiopicum* L comme source de résistance à *Pseudomonas solanacearum* EF Smith a été lancé en Guadeloupe et en Martinique; l'hybride F₁ Kalenda ne manifestant plus une résistance suffisante. La sélection a pu être réalisée malgré d'importants problèmes de stérilité rencontrés pendant les premières générations. Elle fut conduite généralement sous inoculation artificielle selon un modèle de sélection récurrente avec des phases de backcross par *Solanum melongena* L. Après le second backcross, il fut possible de sélectionner des familles manifestant un haut degré de résistance à la bactérie et possédant une très large variabilité pour la forme et la coloration du fruit.

Solanum melongena / *Solanum aethiopicum* / *Pseudomonas solanacearum* / résistance / sélection récurrente

INTRODUCTION

Bacterial wilt caused by *Pseudomonas solanacearum* EF Smith (Kelman, 1953) leads to important losses in solanaceous crops in Martinique and Guadeloupe (French West Indies, Carribean area) (Messiaen, 1975). As in other tropical and subtropical regions of the world (Buddenhagen, 1985), this disease is a limiting factor to the development of eggplant cultivation.

A breeding program conducted by Daly (1972, 1973) produced the line Madinina with a good level of resistance to the bacterium. Then, fruit anthracnosis, a new disease caused by *Colletotrichum gloeosporioides* P (Fournet, 1973) led Kaan (1973) to breed a new variety, Aranguéz, which was resistant to anthracnosis. The well shaped and coloured variety was crossed with Madinina in order to produce the F₁ hybrid, Kalenda (Daly, 1986). Kalenda is highly resistant to anthracnosis and shows good tolerance to bacterial wilt.

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In 1982, damage caused by *P solanacearum* led to extensive losses in eggplant plantations on both islands (Messiaen, 1983). The F₁ hybrid Kalenda, still cultivated today, did not show sufficient resistance to bacterial wilt, especially in conditions such as hot season planting (July–August) (Bereau and Messiaen, 1975) or planting in poorly drained fields.

Hebert (1985) showed that a high level of resistance to bacterial wilt is available in the *Solanum aethiopicum* Aculeatum group. This species is often described under the name *S integrifolium* (Lester and Niakan, 1986). Beyries (1979) used it as rootstock for eggplant and tomato in naturally infected fields.

Until now, *S aethiopicum* species as a source of bacterial wilt resistance has only been used in the cross with *S melongena* because of infertility problems in order to obtain F₁ hybrids used as rootstock (Sakaï, 1984).

This paper reports on the first phase of the breeding program conducted at INRA (French West Indies) in order to transfer the *S aethiopicum* Aculeatum group's resistance to *P solanacearum* into commercial eggplant varieties.

MATERIALS AND METHODS

Inoculation techniques

Since our objective was to find a better resistance level than that of Kalenda, this variety was chosen as the susceptible control. Two techniques were used: infected field, in order to test the progenies of the crosses with the susceptible parent Aranguéz; and artificial infection, to identify resistant families in the following generation.

Infected field

Trial plots were used which had received an artificial inoculation 1 or 2 years earlier. Under these conditions, the inoculum is considered to be homogeneously distributed over the plot and selecting pressure is intermediate.

Artificial infection

So that all the plants could be exposed to the same strong infectious conditions, screening for resistant plants was completed according to the method described by Prior *et al* (1989a). This method is based

on artificial inoculation with a mixture of 3 strains (GA1, GA3, GA4) isolated from eggplant. These strains were collected from different locations in Guadeloupe and have been deposited in the Collection Nationale de Bactéries Phytopathogènes (CNBP, INRA Station de Pathologie Végétale, 49000, Angers, France). All these strains belong to Race 1 (Buddenhagen *et al*, 1962) and Biovar III (Hayward, 1964), but they differ in host range (Prior and Steva, 1989) and in aggressiveness (Prior *et al*, 1990). Virulent fluidal type colonies of the bacterium were grown on tetrazolium chloride (TZC) medium (Kelman, 1954). After 48 h incubation at 30 °C, single colonies were cultivated for 18 h in liquid TZC-free medium at 30 °C, then harvested and rinsed with sterile distilled water by centrifugation (4 000 g, 20 min). Final concentration of the mixed suspension was optically adjusted to 2 × 10⁷ CFU/ml. At planting time (60 days after sowing) 2 ml of inoculum were placed close to the roots of each plant.

All the tested families were planted in a randomized complete-block design with 4 replicates (15 plants per replicate). Considering that in the case of eggplant a wilted plant dies rapidly, percentages of dead or wilted plants (WP) were observed 70 days after inoculation.

Breeding for resistance to *P solanacearum* (fig 1)

In 1982, several crosses were made between different *S aethiopicum* entries from the INRA collection (Station d'Amélioration des Plantes Maraîchères, 84140 Montfavet, France) used as female parents and tropi-

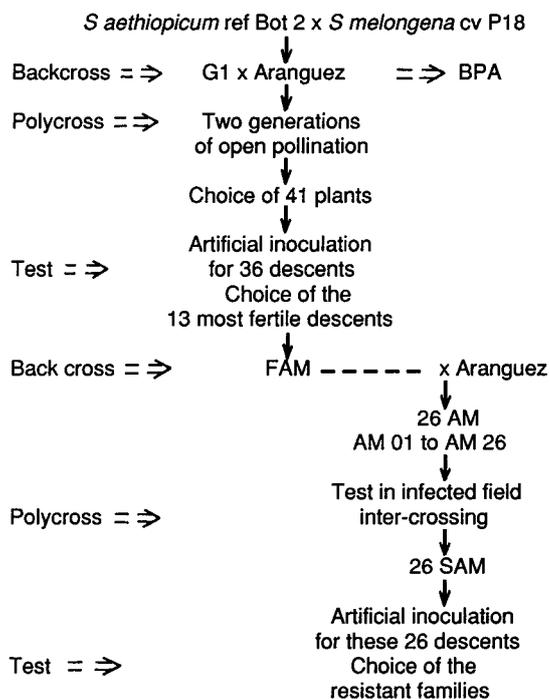


Fig 1. Pedigree of the SAM families.

cal varieties of *S melongena*. Embryo culture with V3 medium (Schoch and Sibi, 1978) was used in order to obtain more viable F_1 plants from these crosses. Inter-specific hybrids were crossed with Aranguéz. A very low fertility level was observed during this first phase of the program, and only the progenies of the cross, named BPA, – (*S aethiopicum* ref Bot 2 x *S melongena* cv P18) x Aranguéz – were obtained in 1983. P18, a line of Turkish origin, has a good resistance to fruit anthracnosis and bacterial wilt (Vincourt, unpublished observations) but its fruit color is not suitable because of the sun's influence on the purple shade. The progenies of the BPA cross were allowed to open-pollinate for 2 generations. At the end of 1984, this material had recovered a better though not total fertility. Non-viable combinations had been eliminated.

Later on, recurrent selection with backcrosses was performed. Selection and intercrossing were followed by a backcross phase with Aranguéz in order to improve the quality of the fruit (colour, shape, size).

After the 2 generations of open pollination, equivalent to a polycross phase, fruits from 41 plants resulting from the BPA cross were harvested. In November 1984, the offsprings (named FAM families) of these 41 plants were screened by artificially inoculating plants with *P solanacearum*. Only 36 families gave a sufficient number of plants for the test. Some flowers from resistant plants were pollinated with Aranguéz. Not more than 20% of these crosses were successful, but the aging of plants (4–5 months after planting) led to an increase in fertility. Only 13 families yielded progenies, generally 5 plants per family were crossed. We thus obtained 26 progenies, named AM 01 through AM 26. They were tested for resistance to *P solanacearum* in the infected field.

After elimination of the 2 most susceptible families (AM 02 and AM 25), the AM families were crossed in pairs at random. Twenty-six families were obtained, numbered from SAM 01 to SAM 26. They were submitted to a wilt resistance test in 1986 under artificial inoculation.

Statistical analysis

The Friedman 2-way analysis of variance by ranks was used (Friedman, 1937, 1940) because of significant variance differences within families. The "tests non paramétriques" procedure of the STATITCF micro-computer program (ITCF, 1988) was used to test the null hypothesis and to compare the families with the Kalenda control.

RESULTS

Results for the 36 FAM families are presented in table I. A wide variation between families was observed, together with a large heterogeneity within families. Out of 36 families, 20 had a resistance

level which was significantly better than that of Kalenda. This F_1 hybrid, with 88% dead or wilted plants provides evidence of the efficiency and strength of the artificial inoculation.

Concerning the AM families tested in the infected field (table II), a good level of resistance was observed, since 19 families exhibited < 0% wilted plants. With 82% dead or wilted plants, Kalenda exhibits the highest susceptibility.

Table I. Ranking of FAM families issued from the first polycross for bacterial wilt resistance.

| Family | Mean % plants wilted (X) | Sums of ranks |
|---------|--------------------------|---------------|
| FAM 41 | 0 (Y) * | 134.5 |
| FAM 29 | 3 * | 123.5 |
| FAM 15 | 3 * | 122.5 |
| FAM 26 | 3 * | 124.0 |
| FAM 09 | 5 * | 116.5 |
| FAM 04 | 7 * | 111.0 |
| FAM 23 | 8 * | 104.0 |
| FAM 24 | 8 * | 105.5 |
| FAM 32 | 10 * | 108.5 |
| FAM 37 | 10 * | 104.5 |
| FAM 38 | 10 * | 98.5 |
| FAM 39 | 12 * | 103.5 |
| FAM 02 | 12 * | 94.5 |
| FAM 12 | 12 * | 111.0 |
| FAM 30 | 12 * | 93.0 |
| FAM 35 | 13 * | 89.5 |
| FAM 31 | 13 * | 93.5 |
| FAM 10 | 15 * | 82.5 |
| FAM 17 | 15 * | 82.5 |
| FAM 25 | 17 * | 76.0 |
| FAM 20 | 18 * | 80.5 |
| FAM 07 | 22 * | 60.5 |
| FAM 22 | 22 * | 66.5 |
| FAM 08 | 22 * | 67.5 |
| FAM 18 | 23 * | 62.0 |
| FAM 27 | 23 * | 60.5 |
| FAM 16 | 25 * | 50.5 |
| FAM 14 | 27 * | 53.5 |
| FAM 21 | 27 * | 48.0 |
| FAM 28 | 32 * | 36.5 |
| FAM 40 | 33 * | 40.0 |
| FAM 03 | 35 * | 35.5 |
| FAM 06 | 37 * | 27.0 |
| FAM 36 | 45 * | 20.5 |
| FAM 01 | 50 * | 13.0 |
| KALENDA | 88 | 7.5 |
| FAM 19 | 100 | 4.5 |

X : Percentage of dead or wilted plants 70 days after inoculation calculated from 60 plants per family. Y : Means within columns followed by an asterisk are significantly different from the control Kalenda at the 0.05 level of probability, according to the Friedman 2-way analysis of variance by ranks test.

The SAM families generally showed a very good resistance to *P solanacearum*; 15 had a resistance level significantly better than that of Kalenda (table III). All the SAM families were characterized by a good resistance to fruit anthracnosis (data not shown).

With regard to the shape and colour of the fruit, this material has a very wide variability. Fruits are long or round, and green, pink, purple or black in colour. The variability also shows up in the colour of leaves (from green to purple) and flowers (from white to purple).

Table II. Ranking of AM families issued from the second back-cross for bacterial wilt resistance.

| Family | Mean % plants wilted (X) | Sums of ranks |
|---------|-----------------------------|------------------|
| AM 01 | 0 (Y) * | 69.5 |
| AM 03 | 0 * | 69.5 |
| AM 04 | 0 * | 69.5 |
| AM 05 | 0 * | 69.5 |
| AM 06 | 0 * | 69.5 |
| AM 07 | 0 * | 69.5 |
| AM 08 | 0 * | 69.5 |
| AM 09 | 0 * | 69.5 |
| AM 10 | 0 * | 69.5 |
| AM 11 | 0 * | 69.5 |
| AM 12 | 0 * | 69.5 |
| AM 13 | 0 * | 69.5 |
| AM 15 | 0 * | 69.5 |
| AM 16 | 0 * | 69.5 |
| AM 19 | 0 * | 69.5 |
| AM 20 | 0 * | 69.5 |
| AM 21 | 0 * | 69.5 |
| AM 23 | 0 * | 69.5 |
| AM 26 | 0 * | 69.5 |
| AM 17 | 5 | 46.0 |
| AM 22 | 5 | 36.5 |
| AM 18 | 13 | 31.5 |
| AM 14 | 13 | 22.5 |
| AM 24 | 18 | 30.5 |
| AM 02 | 42 | 12.5 |
| AM 25 | 55 | 8.0 |
| KALENDA | 82 | 4.0 |

X : Percentage of dead or wilted plants under natural infestation 110 days after plantation, calculated from 60 plants per family. Y : Means within columns followed by an asterisk are significantly different from the control Kalenda at the 0.05 level of probability, according to the Friedman 2-way analysis of variance by ranks test.

Table III. Ranking of SAM families, issued from the second polycross for bacterial wilt resistance.

| Family | Mean % plants wilted (X) | Sums of ranks |
|---------|-----------------------------|------------------|
| SAM 07 | 0 (Y) * | 88.0 |
| SAM 20 | 0 * | 88.0 |
| SAM 21 | 2 * | 78.5 |
| SAM 06 | 3 * | 75.0 |
| SAM 10 | 3 * | 70.5 |
| SAM 18 | 3 * | 71.5 |
| SAM 22 | 3 * | 70.0 |
| SAM 26 | 3 * | 74.0 |
| SAM 05 | 5 * | 65.0 |
| SAM 08 | 5 * | 64.4 |
| SAM 09 | 5 * | 71.5 |
| SAM 25 | 5 * | 61.5 |
| SAM 01 | 7 * | 57.0 |
| SAM 14 | 7 * | 62.0 |
| SAM 16 | 7 * | 57.5 |
| SAM 24 | 7 | 56.0 |
| SAM 12 | 8 | 53.5 |
| SAM 15 | 8 | 50.0 |
| SAM 23 | 8 | 53.5 |
| SAM 04 | 10 | 46.0 |
| SAM 03 | 10 | 51.0 |
| SAM 11 | 12 | 41.5 |
| SAM 13 | 12 | 39.0 |
| SAM 17 | 15 | 35.0 |
| SAM 19 | 20 | 20.0 |
| SAM 02 | 43 | 8.0 |
| KALENDA | 97 | 4.0 |

X : Percentage of dead or wilted plants 70 days after inoculation calculated from 60 plants per family. Y : Means within columns followed by an asterisk are significantly different from the control Kalenda at the 0.05 level of probability, according to the Friedman 2-way analysis of variance by ranks test.

DISCUSSION

The first 2 generations after the first generations issued from the interspecific hybrid were always found to be sterile. We noticed that aging of plants had a favorable effect on spontaneous fertility of interspecific hybrids under tropical conditions. Similar results such as phenotypic variation in the offspring of interspecific hybrids have frequently been observed over the course of time.

The Aranguéz parent being very susceptible to *P. solanacearum*, the results obtained indicate the average dominance of genes governing the resistance character in the selected FAM families.

It is impossible to determine the respective roles of the *S. aethiopicum* and of the P18 parent in the genetic control of the resistance in the SAM families, the 2 original parents being resistant. The recurrent selection allowed the recombination of genes governing genetic control of bacterial wilt resistance from the 2 parents. This genetic recombination gave progenies with a significantly better resistance than that of Kalenda.

The selection method used here has proved to be very efficient in such a context where the genetic variability is wide and probably implies several partly dominant genes. The polycross phase allows the expression of various gene combinations, maintaining a large proportion of the variability induced by interspecific crosses.

A severe screening test was adopted in order to eliminate susceptible and weakly resistant plants. This test appears to be as effective as the natural infectious process, as previously stated by Schmit (1978) and to result in a better differentiation between resistant and susceptible plants, as reported by Winstead and Kelman (1952). Another advantage is that wilt symptoms were observed earlier than with natural contamination. We believe that the validity of this inoculation procedure (mixed cultures of bacterial strains) for breeding programs is increased by using representative strains of the pathogen in term of host range (race classification) and biochemical characteristics (biovar classification). This strategy is consistent with other eggplant (Goth *et al*, 1986; Li *et al*, 1988) and potato (Swanepoel and Young, 1988) breeding programs.

P. solanacearum strains originating from the French West Indies appear to be highly pathogenic compared with strains from other countries (Prior *et al*, 1989b), so that the genetic material obtained should have a resistance suitable for many countries. This resistance will be evaluated in a world - wide experimental system together with the original parents and with other sources of resistance.

The satisfactory shape and colour of the fruit of resistant genotypes makes their direct use possible after fixation by self-pollination. The most resistant families can also be used as a source of resistance to improve existing varieties, or in future breeding programs.

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