

‘Piel de Sapo’ Breeding Lines Tolerant to Melon Vine Decline

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Melon vine decline (MVD) results in severe economic losses in melon crops (*Cucumis melo* L.) throughout the Mediterranean area and in the United States. MVD is a complex disease produced by many soilborne pathogens such as *Monosporascus cannonballus* Pollack et Uecker, *Acremonium cucurbitacearum* Alfaro-García, W. Gams et García-Jimenez, *Macrophomina phaseolina* (Tassi) Goidanich, and *Rhizopycnis vagum* DF Farr (Bruton et al., 1995; Chilosi et al., 2008; Fita et al., 2007a; García-Jiménez et al., 2000). Occurrence and pathogenicity of fungi associated with MVD is variable depending on the climate area and the cultural practices (Aegerter et al., 2000; Bruton et al., 1999; Makam et al., 2005; Martyn, 2007). Despite this complex situation, *M. cannonballus* is the pathogen most frequently associated with severe outbreaks of MVD. It has great virulence and produces severe root damage in artificial inoculation alone or combined with other fungi, comparable to that found in naturally infested roots (Armengol et al., 2003; Beltrán, 2006; Fita et al., 2008; Iglesias et al., 2000a). Also, real-time polymerase chain reaction has demonstrated that the roots of genotypes reported to be resistant to MVD have lower levels of *M. cannonballus* in comparison with susceptible genotypes (Picó et al., 2008). *M. cannonballus* causes brown discoloration of the roots, which can evolve into rot and even lead to necrosis of the whole root system. Affected roots are unable to supply the vine with the necessary nutrients and water, leading to plant wilt and collapse. Vine decline and/or collapse are usually observed in the late

season when the level of water required by fruit increases (Fita et al., 2007a).

The occurrence and intensity of these aboveground symptoms are highly dependent on the environmental conditions and cultural practices such as temperature, fruit load, water management, and so on. Consequently, disease assessment has been specifically centered on the extent of root damage. This methodology requires root extraction from the soil, which makes breeding programs highly difficult and time-consuming because all the plants must first of all be artificially pollinated and then, at the adult stage, evaluated by their root systems (Fita et al., 2007a). The limited number of sources of resistance to MVD reported to date (Cohen et al., 1996; Crosby, 2001; Esteva and Nuez, 1994; Wolff and Miller, 1998) is also a major obstacle in the development of resistant cultivars.

One of the most promising accessions for its tolerance to MVD was ‘Pat 81’, which belongs to the subsp. *agrestis* of *C. melo* (Pitrat, 2008). ‘Pat 81’ displayed very low levels of vine collapse in field assays and exhibited root lesions that were less widespread and less severe than susceptible cultivars after artificial inoculations with *M. cannonballus* and *A. cucurbitacearum* (Dias et al., 2002, 2004; Iglesias et al., 2000a, 2000b, 2000c). In addition, ‘Pat 81’ develops a highly branched root system with long laterals and a high regeneration ability, which has proven to be crucial in overcoming the disease. The performance of melon plants against MVD is highly dependent on two factors: the intrinsic tolerance to the fungus infection and the architecture of the root system before and after infection (Fita et al., 2006, 2008). ‘Pat 81’ has these two features and has proven to be a satisfactory rootstock for the grafting of melons in the battle against MVD (Fita et al., 2007b). For these reasons, it was selected as a donor

parent to breed melon lines resistant to MVD (Dias, 2003).

The ‘Piel de Sapo’ melon is very popular in the Mediterranean area, especially in Spain, and is becoming increasingly important in the European market. To cover the market demand during winter, Brazil is also producing this melon type. Unfortunately, ‘Piel de Sapo’ melons are highly susceptible to MVD, which seriously limits output in the main producing areas of the world.

Origin

‘Piel de Sapo’ resistant lines were obtained through a backcross breeding program started in 2000. ‘Piel de Sapo’ Piñonet accession (PS), from the Genebank of the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia (COMAV, UPV), was used as the recurrent parent and ‘Pat 81’ accession as the donor of resistance to MVD (Figs. 1 and 2). For each backcross stage, a 5% to 10% selection pressure was applied over an average of 80 plants. To evaluate the tolerance to MVD in each backcross generation, plants were grown individually in 25-L pots filled with naturally infested soil. Each plant was handcrossed and/or selfed, and at 120 d after planting (after harvesting), all roots were extracted for examination. Root damage was scored using the disease severity index (DSI), the average of different scores for discolorations, browning, rot, and necrosis being assigned to the different parts of the root system (hypocotyls, lateral roots, and rootlets). The scores range from 0, being healthy root tissue, to 4, representing extremely damaged roots. Plants with a DSI equal or lower to 2.5 were considered to be resistant (Dias et al., 2002, 2004; Fita et al., 2008). Fungal isolations were made to verify the presence of fungi in roots. *M. cannonballus* was found in all samples, whereas *A. cucurbitacearum* was only found in some damaged roots. Other fungi were isolated sporadically, i.e., some species of the genera *Rhizoctonia*, *Macrophomina*, *Rhizopycnis*, *Pythium*, *Trichoderma*, and *Fusarium*. In addition, root architecture after infection was also studied in all plants. The parameters used to evaluate root architecture were 1) root weight (g); 2) number of lateral roots; 3) root length (cm), usually measured as the length of the three longest roots; and 4) vigor indices, ranging from 0 (very bad root architecture) to 4 (a vigorous and well-distributed root system). At the third backcross generation (BC₃), 10 selected families were tested in two fields, one located in Puzol (Valencia, Spain) and the other in Turis (Valencia, Spain). The Puzol field had a long history of MVD, whereas the Turis field was free from disease because no cucurbits had ever been planted there. Those families with the best performance in terms of agronomic traits and resistance were selected for reproduction (Dias, 2003). Plants belonging to these families were grown in pots and evaluated by their root systems as previously explained to

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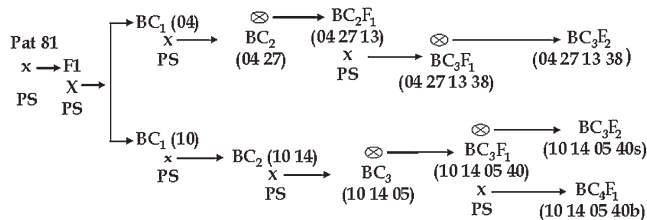


Fig. 1. Pedigree of 'Piel de Sapo' (PS) resistant breeding lines. Each cross stage was followed by a selection for root architecture and resistance to melon vine decline; a 5% to 10% selection pressure was applied.

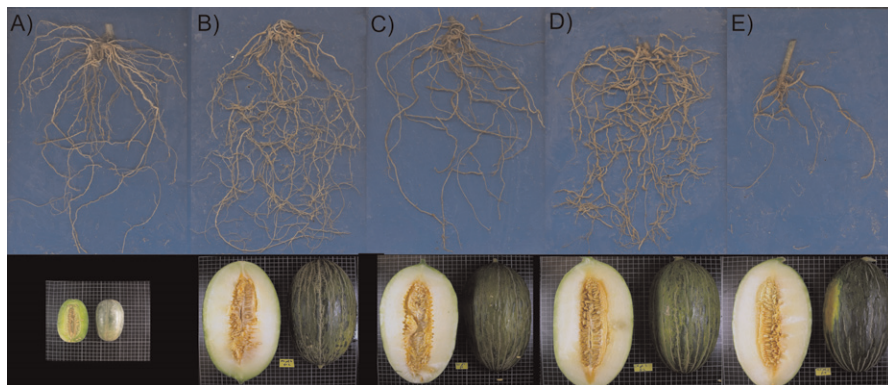


Fig. 2. Roots (from plants grown for 90 d in 18-L pots filled with naturally infested soil) and fruits (from plants grown in the field) of the parental lines and the 'Piel de Sapo' breeding lines resistant to melon vine decline. (A) 'Pat 81' resistant line; (B) line 04271338; (C) line 10140540s; (D) line 10140540b; and (E) 'Piel de Sapo'.

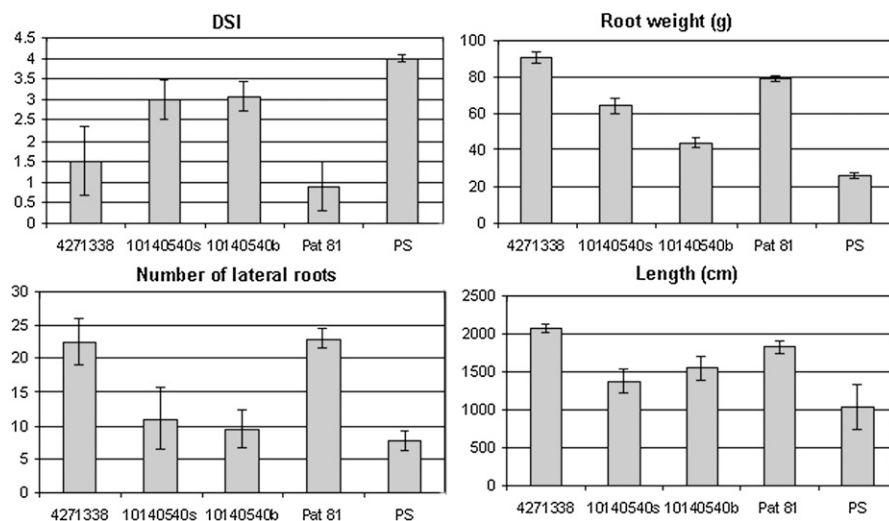


Fig. 3. Root evaluation of 'Piel de Sapo' (PS) resistant breeding lines grown for 90 d in 18-L pots filled with naturally infested soil. The 'Piel de Sapo' recurrent parent and the 'Pat 81' donor of resistance are included as controls. DSI = disease severity index (as an average of the disease index in hypocotyls, lateral roots, and fine roots, ranging from 0 = healthy roots to 4 = totally necrotic roots).

obtain the lines presented in this article. Lines 04271338 and 10140540s are BC_3F_2 selections from a different initial BC_1 , whereas 10140540b is a BC_4F_1 derivative from the same family as line 10140540s (Fig. 1).

Description

Line performance was tested in comparison with 'Piel de Sapo' in two locations

within the Valencia Province (Segorbe and Almenara) using common practices of commercial melon production. The presence of MVD has never been reported in the Segorbe field, whereas the Almenara field has a long history of MVD. In each field, a randomized complete block design, with eight replications, was used. Each plot consisted of four plants per genotype with 1 m between plants and 1.5 m between rows in beds. In the

Segorbe field, 'Pat 81' was included to provide a reference of fruit and yield features introduced by the tolerance donor. Production and quality features were evaluated in both fields. The number of collapsed plants in the Almenara field was recorded. Furthermore, to verify the resistance level of the selected lines, six plants from each line were grown in 18-L pots filled with naturally infested soil. Roots were extracted at 90 d and evaluated by their lesions and root architecture as described for the backcross selection. Each root was scanned after removing the fine roots as explained in Fita et al. (2006) to offer a better evaluation of the root architecture. Root length was measured over the scanned roots using the software WinRhizo Pro 2003 (Regent Instruments Inc., Québec, Canada). Data were analyzed by analysis of variance using the software package SPSS 16.0.1 (SPSS, Inc., Chicago, IL).

Field performance. Field results reflected the lines great recovery of the 'Piel de Sapo' agronomic traits despite the unfavorable features initially introduced by the tolerant donor 'Pat 81', which bears small fruits (≈ 200 g) with reduced sugar content, flesh percentage, and firmness (Table 1). Lines are andromonoecious vigorous plants with a growing cycle of 105 d. They have sweet and juicy, oval to elliptic fruits with firm white flesh and a dark green maculated rind typical of 'Piel de Sapo'. Under our conditions, they produce medium-sized fruits with a mean weight of 1.6 kg and an average plant yield of 4 kg. In general, the lines with the 'Piel de Sapo' recurrent parent did not display any differences in terms of yield per plant and fruit weight, except for line 04271338, which showed a plant yield significantly greater than the 'Piel de Sapo' in the Almenara field. In lines 04271338 and 10140540s, some variation was found for the external appearance of the fruits in terms of shape (fruits were either round or more elongated than the 'Piel de Sapo') or color (green blotched or maculated with a lighter background than the 'Piel de Sapo'). Nevertheless, the percentage of fruits that clearly differed from the 'Piel de Sapo' was less than 20%. Flesh percentage was significantly reduced in lines 10140540s and 10140540b in both assays. These two lines also showed a reduction in flesh firmness in the Segorbe field. Despite their high levels of soluble solid content, in general, these lines did not equal 'Piel de Sapo' quality, except for line 10140540b in Almenara. The genotype of these lines is not totally fixed for some traits. Interestingly, variation in all lines included plants with high soluble solid content, reaching 17 °Brix (data not shown), which can be used to generate sweeter melon lines through further selection.

Level of resistance. None of the plants from the selected lines collapsed in the infested Almenara field in comparison with 40% of the plants from the 'Piel de Sapo' line collapsing. As previously stated, the symptom of plant collapse is highly dependent on environmental conditions (Fita et al., 2007a). Regarding the DSI, line 04271338 gave the

Table 1. Field performance of 'Piel de Sapo' resistant breeding lines in comparison with the 'Piel de Sapo' recurrent parent.

Lines	Yield (t·ha ⁻¹)	Fruit wt (kg)	Flesh percentage	Flesh firmness (N)	Soluble solids content (°Brix)
Segorbe					
04271338	20	1.41	0.53 ^z	39.9	10.0 ^z
10140540s	22	1.23	0.51 ^z	33.1 ^z	10.2 ^z
10140540b	20	1.46	0.54 ^z	35.5 ^z	11.3 ^z
Pat 81	19 ^z	0.22 ^z	0.40 ^z	12.9 ^z	7.9 ^z
Piel de Sapo	23	1.47	0.60	39.4	11.6
Almenara					
04271338	39 ^z	1.96	0.7	36.4	12.04 ^z
10140540s	27	1.88	0.62 ^z	36.7	12.25 ^z
10140540b	30	2.10	0.61 ^z	35.7	13.27
Piel de Sapo	30	1.95	0.67	32.1	13.72

^zSignificantly different from 'Piel de Sapo' using a Dunnett contrast with Type I error ($P \leq 0.05$).

best performance with 100% of the plants proving resistant (DSI 2.5 or less), whereas lines 10140540s and 10140540b showed signs of segregation for the DSI, although as lines, they can be considered tolerant (Fig. 3). All lines showed a root weight significantly higher than the 'Piel de Sapo' with line 04271338 also having higher root weight than 'Pat 81'. Line 04271338 also stands out as a result of its high number of lateral roots, the length of which was greater than that of 'Pat 81'. Lines 10140540s and 10140540b developed not only a greater number of but also longer laterals than the 'Piel de Sapo'.

In summary, line 04271338 displayed the desired features of both parents, these being the fruit traits and yield of the commercial 'Piel de Sapo' in addition to a high level of resistance to the infection and a root system with a high capacity to explore the soil typical of 'Pat 81'. These results make this line ideal for fixing the genotype to give rise to a new 'Piel de Sapo' line resistant to MVD. Lines 10140540s and 10140540b also performed satisfactorily but did not achieve quite as favorable root and fruit properties as line 04271338. However, the range of variation present in these lines revealed that these can be useful to derive additional 'Piel de Sapo' lines resistant to MVD.

Availability

These lines will be deposited in the Genebank of the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia (COMA VUPV), Camino de Vera, SN, 46022, Valencia, Spain. Seeds will be made available for research purposes, including the development and commercialization of new cultivars and can be obtained, through concession agree-

ment, from F. Nuez, e-mail: fnuez@btc.upv.es. It is requested that the source be given the appropriate recognition whenever this germplasm contributes to research or the development of a new breeding line or cultivar.

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