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Cucumis melo L. New Breeding Lines Tolerant to Melon Vine Decline

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In this article, we describe two melon (*Cucumis melo* L.) breeding lines with the genetic background of a traditional nonclimacteric *inodorus* cultivar that show fruit characteristics similar to climacteric types. The breeding lines described here were identified during a breeding program aimed at introgressing the tolerance to melon vine decline (MVD) of 'Pat 81' (*C. melo* ssp. *agrestis* var. *conomon*) into the genetic background of 'Piel de Sapo' (*C. melo* ssp. *melo* var. *inodorus*; Fita et al., 2009). MVD is a melon soilborne disease caused primarily (but not only) by the fungus *M. cannonballus* Pollack et Uecker, which rots the roots. Melon vine decline and/or collapse results from a damaged root that is unable to supply the vine's demands for nutrients and water. The occurrence and intensity of collapse is highly dependent on environmental conditions and cultural practices (Fita et al., 2007). 'Pat 81' is one of the most tolerant melon entries reported to date. This is because of its long and branched root system, which is able to explore vast soil areas, and its low level of infection by the MVD-causing fungus. The combination of these two characteristics is crucial to overcoming the disease (Dias et al., 2002, 2004; Fita et al., 2006, 2008). After the introgression of genetic tolerance of 'Pat 81' into the 'Piel de Sapo' genome, the first MVD-tolerant 'Piel de Sapo' breeding lines were recently released (Fita et al., 2009). However, there is a lack of genetic tolerance to MVD in other melon types.

During the introgression of the tolerance into 'Piel de Sapo', some plants with new, unexpected transgressive fruit phenotypes appeared. The fruits of these plants were round and showed a ripening process with certain traits similar to those of climacteric melons, namely changes in rind color, flesh softening, fruit abscission, and subtle aroma (Pech et al., 2008). However, neither 'Piel de Sapo' nor 'Pat 81' are considered climacteric. 'Piel de Sapo' is a cultivar group that belongs to the large *inodorus* group and is a model of nonclimacteric ripening, like other *inodorus* types (honeydew, amarillo, piel de sapo, tendral, kirkagac, yuva, and casaba). It lacks the ethylene peak associated with ripening and does not exhibit the changes associated with climacteric maturation, which is typical of *cantalupensis* types. 'Pat 81' belongs to the same botanical group as PI 161375, whose ripening process has been studied in detail and is considered to be nonclimacteric (Périn et al., 2002). Other experiments made by crossing two nonclimacteric melons have generated offspring with climacteric ripening phenotypes with increased respiration and ethylene levels that results in flesh softening, changes in the fruit color, abscission, and aroma production (e.g., the segregant populations derived from crosses between 'Piel de Sapo' and PI 161375). Those parents are supposed to be nonclimacteric phenotypes caused by mutations in several different genes (Monforte et al., 2004; Moreno et al., 2008). In fact, when we phenotypically characterized 'Pat 81' and PI 161375 along with different nonclimacteric *inodorus* types, including 'Piel de Sapo', we observed differences in the ripening process between the *inodorus* and the *conomon* types such as late-season fruit abscission for the latter group.

The breeding lines presented here could be the starting point for the development of new cultivars with different characteristics from the *inodorus* group and more similarities to muskmelons or other climacteric cultivars and possessing the advantage of being tolerant to MVD, a soilborne disease of increasing significance worldwide.

Origin

The breeding lines described in this article were derived from a backcross breeding program (Fita et al., 2009). In brief, that program consisted of obtaining MVD-tolerant lines from a cross between the cultivar Piñonet Piel de Sapo (PS) and the Korean accession 'Pat 81' used as a donor of resistance to MVD, both maintained in the Genebank of the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia (COMAV-UPV). The plants in each backcross generation were grown in pots filled with naturally infested soil and then selected (with a 5% to 10% selection pressure) for their root system architecture and their low level of lesions. At the third backcross generation (BC_3), 10 selected families were tested for their agronomic traits in two fields. In this experiment, we selected a line that had very good performance in terms of resistance to MVD and showed fruits with climacteric-like characteristics that did not fit the features of any of the parent lines. That line, 3234, showed segregation for fruit shape (round, ovate, and elliptical) and rind netting along with fruit changes usually associated with a climacteric ripening process. Most of the fruits exhibited a change of color (turning yellow, with or without dark green spots), flesh softening, and formation of the abscission layer and were aromatic when mature.

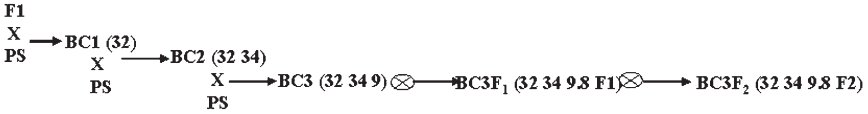

Fig. 1. Pedigree of melon vine decline (MVD)-tolerant lines. Each cross step was followed by a selection for root architecture and resistance to MVD; a 5% to 10% selection pressure was applied.

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

Lines	Yield ($t \cdot ha^{-1}$)	Number of fruits per plant	Fruit wt (kg)	Shape index^z	Flesh percentage^y	Flesh firmness (N)^x	Soluble solids content (°Brix)
				Segorbe			
$32349.8F_1$	23	3.5*	1.01*	1.34*	0.47*	30.2*	10.0*
Pat 81	19*	3.9*	0.22 *	1.49*	0.40*	12.9*	7.9*
Piel de Sapo	23	2.5	1.47	1.53	0.60	39.4	11.6
				Almenara			
$32349.8F_1$	31	3.1*	1.53*	1.28*	0.58*	25.1*	13.4
$32349.8F_2$	23*	3.3*	1.09*	1.27*	0.55*	30.2	13.7
Piel de Sapo	30	2.5	1.95	1.76	0.67	32.1	13.7

^{z}Shape index, calculated as fruit length divided by fruit diameter.

^{y}Flesh percentage, measured as the length of the equatorial diameter occupied by flesh compared with the equatorial diameter.

^{x}Flesh firmness (N), measured twice in a cross-section of the flesh of the fruit with an 8-mm diameter probe at 0.3 $mm \cdot s^{-1}$ (Instron Firmness Tester 5544; Instron Corporation, Canton, MA).

*Significantly different from 'Piel de Sapo' using a Dunnet contrast with Type I error $P \leq 0.05$.

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This family was selected to derive new melon types resistant to MVD. Line 3234 plants were grown under infestation conditions to select the best parents for self-pollination or cross-pollination among siblings. One of the best performers in terms of resistance was 32349.8, whose self-pollination formed the 32349.8F_1 line. In this article, we present lines 32349.8F_1 and 32349.8F_2 (Fig. 1).

Description

Lines 32349.8F_1 and 32349.8F_2 develop andromonoecious plants, intermediate in size, with vines of 1.70 m and a growing cycle of 95 d. The agronomic behavior of line 32349.8F_1 was tested in two trials: one in Segorbe (Valencia, Spain) and another in Almenara (Valencia, Spain). In both trials, 'Piel de Sapo' was included as a control; in the Segorbe trial, 'Pat 81' was also included. Line 32349.8F_2 was only studied in the Almenara trial. In each field, a completely randomized 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 on beds. Production and quality features were evaluated.

Field performance and fruit quality. Field results showed great differences between the lines and both parents in terms of fruit features. In terms of yield, line 32349.8F_1 did not show any differences from 'Piel de Sapo' in any of the field trials, whereas 32349.8F_2 showed a significantly lower yield (Table 1). Both breeding lines developed smaller fruits than 'Piel de Sapo' with a fruit weight between 1 and 1.5 kg, which was not as small as 'Pat 81' fruits (with an average of 220 g). Line 32349.8F_1 reached a comparable production to that of 'Piel de Sapo' as a result of an increase in the number of fruits per plant. Both lines showed some fixed traits such as the round-to-ovate shape (Table 1) or rind color. Rind color was light green with spots similar to 'Piel de Sapo' with the difference that 'Piel de Sapo' shows pale green or yellow as a secondary color, whereas the lines showed light yellow and sometimes white as a secondary color (Fig. 2). Residual variability was still observed for other traits such as rind netting. Line 32349.8F_1 predominantly showed fruits with low or intermediate rind netting, whereas the netted rind phenotype was fixed in 32349.8 F_2 (Fig. 3). Regarding fruit quality, the lines showed a lower flesh percentage than 'Piel de Sapo', which was nonetheless acceptable from a commercial standpoint. Also, the level of soluble solids, from 10 to 13.7, is appropriate for market demands (Table 1). The flesh color was white to light cream in all cases. Fruits of lines 32349.8F_1 and 32349.8F_2 showed a climacteric-like behavior with a decrease in flesh firmness and a change of rind color during ripening. At the ripe stage, and especially when overripe, the fruit appearance was very different from 'Piel de Sapo', which always retains its green rind color, whereas lines 32349.8F_1 and 32349.8F_2 showed a yellow–orange rind color (Fig. 2). Most of the mature fruits of the studied lines had a sweet aroma.

As part of an ecotilling project, we are studying the natural variability in genes involved in the ripening process in melon (Esteras et al., 2009). We have checked the haplotype of breeding lines 32349.8F_1 and 32349.8F_2 for two genes: the ACO1 gene (ACC oxidase, involved in the conversion of ACC into ethylene during fruit ripening; Lasserre et al., 1996) and the melon ortholog of the nonripening NOR gene in tomato (related to the impaired regulation in early ethylene biosynthesis steps in tomato; Giovannoni, 2007). The lines display the *conomon* haplotype from 'Pat 81' for the ACO1 gene, whereas they display the *inodorus* haplotype from 'Piel de Sapo' for the NOR gene. Moreno et al. (2008) mapped a QTL in chromosome III involved in the climacteric phenotype of one near-isogenic line (NIL) derived from the cross between 'Piel de Sapo' and PI 161375. They also mapped several other candidates involved in ethylene metabolism and cell wall degradation, including ACO1, but they found no relationship between them and the climacteric phenotype of the NIL. Despite these results, a possible role of these genes in the regulation of climacteric ripening, when

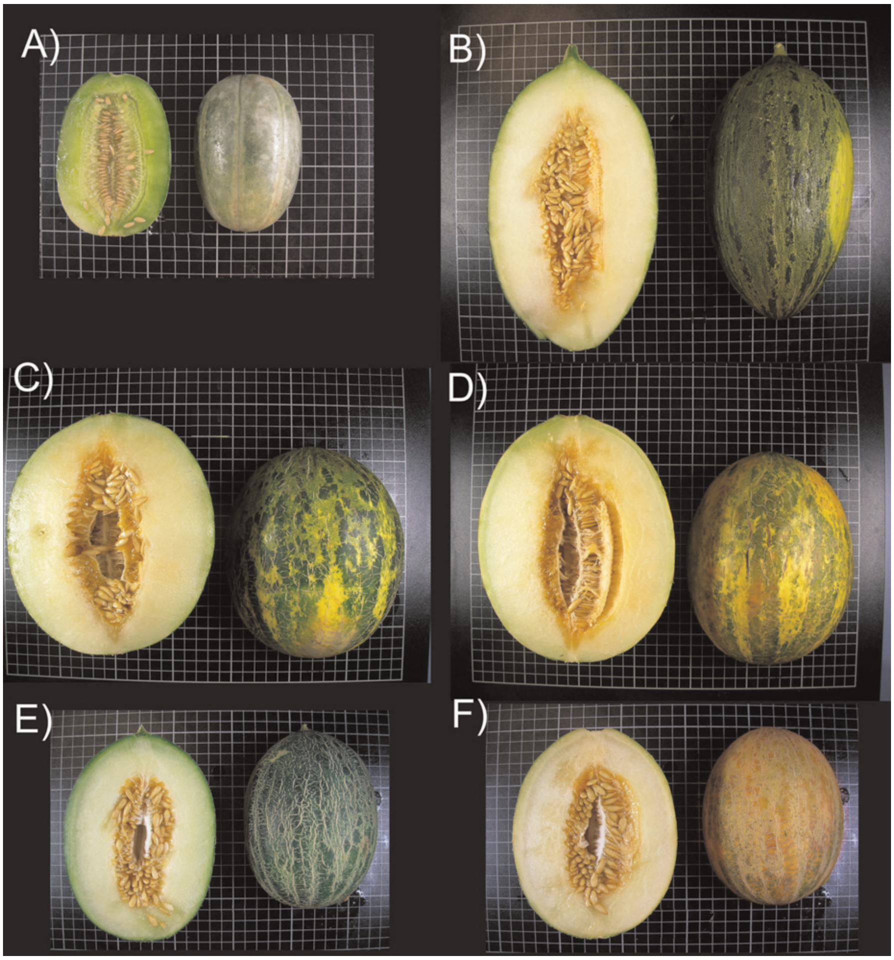

Fig. 2. Fruits of the parents and lines. (**A**) 'Pat 81', (**B**) 'Piel de Sapo', (**C–D**) line 32349.8F_1 at two ripening stages, and (**E–F**) line 32349.8F_2 at two ripening stages.

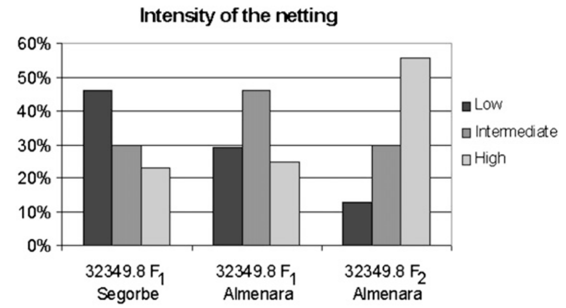

Fig. 3. Intensity of the netting of the lines in the field trials.

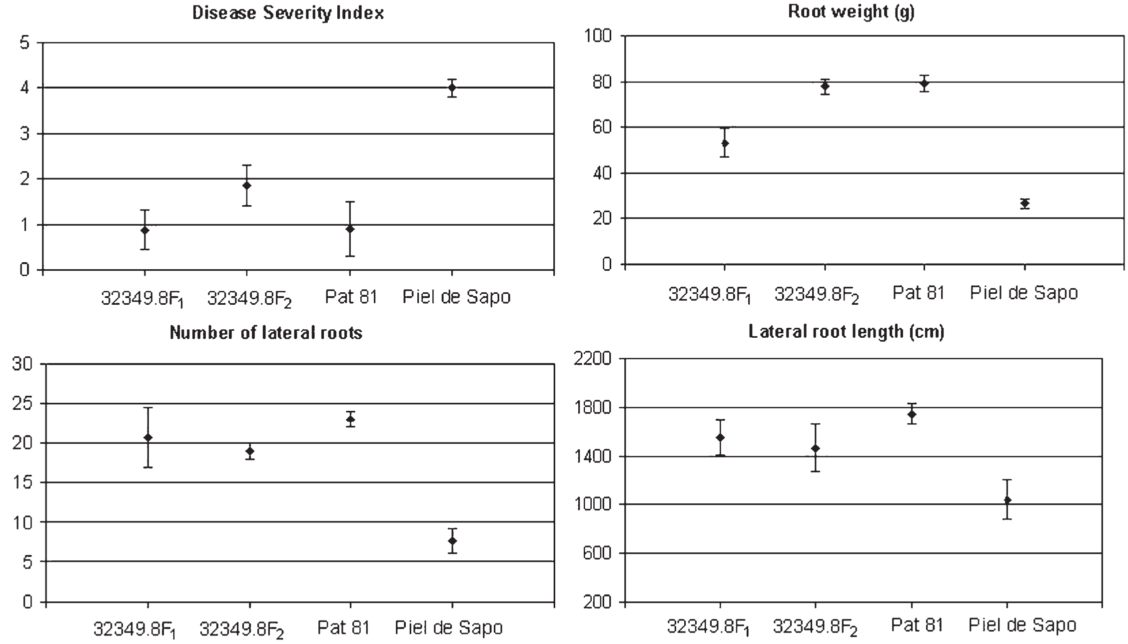

Fig. 4. Root evaluation of melon vine decline-tolerant breeding lines grown for 90 d in 18-L pots filled with naturally infested soil. The ‘Piel de Sapo’ original line and the ‘Pat 81’ donor of the tolerance were included as controls. DSI = disease severity index (as an average of the disease index in hypocotyls, lateral roots, and fine roots, ranging from 0 = healthy roots to 4 = totally necrotic roots).

introgressed into a mixed *inodorus–conomon* genetic background, cannot be discarded. Moreno et al. (2008) suggests than nonclimacteric phenotypes from ‘Piel de Sapo’ and PI 161375 are produced by different genes. It is also possible that different and complex genetic regulation exists for the climacteric character and further research is needed to determine if these or other genes are involved in the climacteric ripening of these lines.

Tolerance to melon vine decline. Under infested field conditions, breeding lines 323449.8F$_1$ and 32349.8F$_2$ exhibited no symptoms of MVD. However, to verify the tolerance level of the 32349.8F$_1$ line and its offspring, line 32349.8F$_2$, six plants of each line, along with ‘Pat 81’ and PS, were grown in 18-L pots filled with naturally infested soil. Roots were extracted 90 d after transplanting and evaluated by their lesions and root architecture. Each root was scanned after removing the fine roots as explained in Fita et al. (2006) to better evaluate the root architecture. Root length of the scanned roots was measured with 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). A disease severity index (DSI) was used to evaluate the root damage. DSI ranges from 0, referring to healthy root tissue, to 4 for extremely damaged roots, taking into account the level of discoloration, browning, rot and necrosis in hypocotyls, lateral roots, and rootlets. Plants with a DSI equal to or less than 2.5 were considered resistant (Dias et al., 2002, 2004; Fita et al., 2008). Regarding the DSI, the lines 323449.8F$_1$ and 32349.8F$_2$ showed a very high degree of tolerance (DSI 2.5 or less; Fig. 4). In addition, they exhibited a high number and length of lateral roots similar to the MVD-resistant ‘Pat 81’ (Fig. 4). The root weight was intermediate for line 323449.8F$_1$ and high for 323449.8F$_2$, indicating that both genotypes can retain a large part of their root mass after infection (Fig. 4).

In summary, lines 32349.8F$_1$ and 32349.8F$_2$ exhibited a high level of tolerance to MVD and a root system with a great capability for exploring the soil and also have ovate–round climacteric fruits that may be very attractive to the market. The remaining variability makes these lines suitable for fixing traits and deriving new commercial lines tolerant to MVD.

Availability

These lines are deposited in the Genebank of the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia (COMAV-UPV), Camino de Vera, SN, 46022, Valencia, Spain. Seeds will be available for research purposes, including the development and commercialization of new cultivars, and can be obtained, through concession agreement, from F. Nuez; e-mail: fnuez@btc.upv.es. It is requested that appropriate recognition of the source be given when this germplasm contributes to the research or development of a new breeding line or cultivar.

Literature Cited

Dias, R.C.S., B. Picó, A. Espinós, and F. Nuez. 2004. Resistance to melon vine decline derived from *Cucumis melo* ssp. *agrestis*: Genetic analysis for root structure and root response. Plant Breed. 123:66–72.

Dias, R.C.S., B. Picó, J. Herraiz, A. Espinós, and F. Nuez. 2002. Modifying root structure of cultivated muskmelon to improve vine decline resistance. HortScience 37:1092–1097.

Esteras, C., L. Pascual, M. Saladie, C. Dogimont, J. García-Mas, F. Nuez, and B. Picó. 2009. Use of Ecotilling to identify natural allelic variants of melon candidate genes involved in fruit ripening. Plant Gem Lisbon 2009 Plant Genomics European Meeting. p. 213.

Fita, A., B. Picó, and F. Nuez. 2007. Melon roots under stress: Melon vine decline. Plant Stress 1: 93–104.

Fita, A., B. Picó, R.C.S. Dias, and F. Nuez. 2008. Effects of root architecture on response to melon vine decline. J. Hort. Sci. Biotechnol. 83:616–623.

Fita, A., B. Picó, R.C.S. Dias, and F. Nuez. 2009. ‘Piel de Sapo’ breeding lines tolerant to melon vine decline. HortScience 44:1458–1460.

Fita, A., B. Picó, and F. Nuez. 2006. Implications of the genetics of root structure in melon breeding. J. Amer. Soc. Hort. Sci. 131:372–379.

Giovannoni, J.J. 2007. Fruit ripening mutants yield insights into ripening. Curr. Opin. Plant Biol. 10:283–289.

Lasserre, E., T. Bouquin, J.A. Hernandez, J. Bull, J.C. Pech, and C. Balague. 1996. Structure and expression of three genes encoding ACC oxidase homologs from melon (*Cucumis melo* L.). Mol. Gen. Genet. 251:81–90.

Monforte, A.J., M. Oliver, M.J. Gonzalo, J.M. Alvarez, R. Dolcet-Sanjuan, and P. Arús. 2004. Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.). Theor. Appl. Genet. 108: 750–758.

Moreno, E., J.M. Obando, N. Dos-Santos, J.P. Fernández-Trujillo, A.J. Monforte, and J. García-Mas. 2008. Candidate genes and QTLs for fruit ripening and softening in melon. Theor. Appl. Genet. 116:589–602.

Pech, J.C., M. Bouzayena, and A. Latchéa. 2008. Climacteric fruit ripening: Ethylene-dependent and independent regulation of ripening pathways in melon fruit. Plant Sci. 175:114–120.

Périn, C., M.C. Gomez-Jimenez, L. Hagen, C. Dogimont, J.C. Pech, A. Latche, M. Pitrat, and J.M. Lelievre. 2002. Molecular and genetic characterization of a non-climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol. 129:300–309.