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Resistance to melon vine decline derived from *Cucumis melo* ssp. *agrestis*: genetic analysis of root structure and root response

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Abstract

Melon vine decline is a major soilborne disease that causes severe economic losses around the world. The fungal pathogens *Monosporascus cannonballus* Pollack et Uecker and *Acremonium cucurbitacearum* Alfaro-García, W. Gams et J. García-Jiménez have been reported as the main causal agents of this disease. A relatively high level of resistance has been reported in *Cucumis melo* L. ssp. *agrestis* (Naud.) Pangalo in the Asiatic accession Pat 81. Although specific resistance mechanisms controlling pathogen-induced root lesions have been characterized in Pat 81, the development of a vigorous and branched root system can also appreciably increase its tolerance to vine collapse. The genetics of root traits related to root vigour and structure were studied in the progeny derived from a cross between Pat 81 and the highly susceptible cultivar 'Piñonet' market class 'Piel de Sapo' (*C. melo* L. ssp. *melo*). The inheritance of specific resistance to soilborne fungi was also studied by analysing root response to inoculation employing naturally infested soil. Estimates of the broad-(H) and narrow-sense (h^2) heritabilities indicated that the majority of the variation for lesion resistance in lateral and fine roots, in the root weight, root length, and the root surface area could be explained by additive effects, whereas dominance is important in plant biomass and resistance to lesions in hypocotyls. Selection for improved root systems conferring resistance and tolerance to vine decline was successfully conducted in BC_1 progeny derived from a Pat 81 × 'Piñonet' mating. Moderate values of heritabilities (0.03–0.76) suggest that breeding strategies that combine family and individual selection could enhance genetic progress. As some of the traits analysed exhibited heterosis, breeding methods directed towards recovering hybrid vigour may increase gain from selection.

Key words: *Cucumis melo* — *Acremonium cucurbitacearum* — *Monosporascus cannonballus* — breeding for resistance/tolerance — root morphology — root damage

The complex disease of melon vine decline, also referred to as melon collapse, root rot, or sudden wilt, causes severe economic losses in many regions worldwide. Although other pathogens may be involved, *Monosporascus cannonballus* Pollack et Uecker and *Acremonium cucurbitacearum* Alfaro-García, W. Gams et J. García-Jiménez have been reported as the primary causal agent of this disease (García-Jiménez et al. 1994, 2000, Martyn and Miller 1996, Bruton et al. 1998). Current disease management is based mainly on methyl bromide soil fumigation prior to planting. As the use of methyl bromide will be prohibited in the near future, there is an urgent need to develop alternative strategies for disease control (Cohen et al. 2000).

The development and release of resistant/tolerant melon cultivars stems largely from difficulties in assessing field resistance which is due to the complex aetiology of the disease and environmental influences on the dynamics of plant collapse. Despite such difficulties, screening methods using natural and artificial inocula have been independently developed to select resistant/tolerant germplasm in severely affected countries such as Israel, USA and Spain (Cohen et al. 1996, 2000, Wolf and Miller 1998, Crosby et al. 2000, Iglesias et al. 2000a).

Genetic control of resistance/tolerance to vine decline has been investigated using progeny resulting from crosses between resistant/tolerant and susceptible genotypes. Cohen et al. (1996) studied tolerance to wilting derived from the Asiatic breeding line P6a by employing a plant collapse index scored in naturally infested fields, and suggested an additive mode of gene action for tolerance. The severity of plant collapse is, however, highly influenced by environmental stresses and cultural factors (i.e. high temperatures, dry winds, fruit load, etc.) (Cohen et al. 2000). Likewise, relationships between plant collapse and the state of infested root systems have been studied, and a resistance mechanism to root lesions caused by pathogenic fungi has been proposed in germplasm of different origins (Crosby et al. 2000, Dias et al. 2002). A large and vigorous root system coupled with a capacity to regenerate new rootlets after infection are traits, which have potential for increasing tolerance to vine decline. For these reasons, it would be desirable to accurately characterize the rooting systems of genotypes (resistant/tolerant and susceptible) to determine relative levels of resistance/tolerance to vine decline. Studies on root morphology and structure in cucurbits are scarce because of difficulties associated with root assessment by traditional analysis procedures (Walters and Wehner 1994).

Crosby (2000) estimated narrow-sense heritabilities for several melon root traits, and for root resistance to *M. cannonballus* in crosses of resistant melon cultivars (i.e. 'Doublon' and 'Deltex') and breeding lines, with highly susceptible melon cultivars. Heritability values as estimated by parent–offspring regression ranged from low (0.21) to extremely high (values exceeding 1.0).

In Spain, a relatively high level of resistance to vine decline has been documented in an Asiatic accession (Pat 81) of the wild species *Cucumis melo* L. ssp. *agrestis* (Naud.) Pangalo after inoculation under natural and artificial conditions (Esteva and Nuez 1994, Iglesias and Nuez 1998, Iglesias et al. 1999, 2000a,b). This accession confers tolerance to this disease through developing a root system that is more vigorous and branched than those of cultivated melons (Dias et al. 2002). The

high level of resistance/tolerance found in Pat 81 is currently being introgressed into susceptible Spanish cultivars of the market class 'Piel de Sapo' and 'Amarillo Canario' (Iglesias et al. 2000a,c). The genetics of the tolerance to plant collapse derived from Pat 81 has been studied by Iglesias et al. (2000a). They employed a new methodology based on the analysis of disease progress curves to minimize the stochastic fluctuation on the percentage of collapsed plants caused by environmental factors. Data from Pat 81 × 'Piel de Sapo' (and Pat 81 × 'Amarillo Canario') derived crosses were fitted to an additive model.

A genetic study of vine decline resistance/tolerance derived from Pat 81 has been conducted. This study required the genetic analysis of root traits related to root vigour and structure in progeny derived from a Pat 81 × 'Piel de Sapo' mating. The inheritance of resistance to the causal soilborne fungi responsible for the root lesions was also assayed as root response to artificial inoculation by using naturally infested soil. The genetic information obtained was then used in the selection of resistant plants in a segregating population.

Materials and Methods

Plant material: The susceptible commercial cultivar 'Piñonet, Selección Torpedo' (Semillas Battle, S.A, Barcelona, Spain) of C. melo L. ssp. melo (belonging to the cultivar group Inodorus, 'Piel de Sapo' market class) (P_1) was used as the female parent in a cross with the vine decline resistant accession Pat 81 of C. melo L. ssp. agrestis (Naud.) Pangalo (P_2; unadapted germplasm). The F_1 was self-pollinated (F_2), backcrossed to the susceptible parent (BC_1), and backcrossed to the resistant parent (BC_2).

Cultural techniques and experimental design: Resistance was assayed in two soil substrates (one pathogen inoculated and one uninoculated). The inoculated substrate consisted of a mixture of a soil (clayey-loamy) naturally infested with the pathogens of different genera responsible for collapse and pasteurized sand (1:1; vol/vol) (NS). The soil originated from a field traditionally used for melon cultivation in Valencia (Eastern Spain). The characteristics of this soil have been analysed in a previous study. The isolates of M. cannonballus and A. cucurbitacearum found in this field have proved to be more virulent than many other Spanish and American isolates (Iglesias et al. 2000c). The same mixture treated with methyl bromide was used as a sterilized control (SS). The NS effect on melon roots was compared with the effect of SS artificially inoculated with M. cannonballus and/or A. cucurbitacearum in previous studies (Iglesias et al. 2000b,c, Dias et al. 2002 and unpublished results). These studies revealed that A. cucurbitacearum is a less aggressive pathogen than M. cannonballus. Likewise, the root lesions were more severe and occurred earlier in NS than in artificially inoculated substrates. Therefore, natural soil inoculum (NS) that provides a more complete sample of the biotic composition of infested soil was employed for experimentation to ensure maximum root damage.

The number of plants evaluated for each generation in each soil substrate (SS and NS) was: 10 for the P_1, P_2 and F_1; 90 for the F_2, and 50 for the BC_1 and BC_2 families. Seeds were treated for 1 min with a solution of sodium hypochlorite (1.5%), and germinated in Petri dishes. After 4–5 days, they were sown in 0.6 kg pots with the soil substrates previously described (SS and NS). Plants were grown in a greenhouse in the summer season under controlled conditions (temperatures from 25 to 30°C day/20 to 25°C night, RH from 60 to 70% day/75 to 85% night, and a photoperiod of about 13–15 h natural light/11–19 h dark). Plants of the different generations were randomly arranged in the greenhouse. Plants were hand-watered daily and supplied with nutrient solutions (each plant received weekly 1 L of 15 N-10 P-15 K-2 mg, 200 mg/l, plus micronutrients).

Plant characterization: Plants were characterized about 40 days after planting (DAP). After cutting the vines, the root systems were carefully extracted from the pots. The substrate was flushed with tap water, then the roots were washed in distilled water. Roots were weighed, and placed on clear glass submerged in a thin layer of water and spread apart using dissecting needles. The roots were scanned (Epson 1680, Epson America, Long Beach, CA, USA), and the total surface area of the roots was estimated using the Adobe Photoshop 7.0 software (Adobe Systems Incorporated, San José, CA, USA). Isolations were made from randomly sampled plants of all generations (roots of approximately 50% of the plants assayed were analysed) to verify the presence of fungi in the hypocotyls and roots of different orders (i.e. primary, secondary, tertiary and higher) using the methodology reported in García-Jiménez et al. (1994).

For SS-treated plants, the following traits were evaluated at 40 DAP: biomass (B) (g), whole plant weight, root fresh weight (RFW) (g), root surface area (RSA) (cm^2) and the length of the three longest lateral roots (L1, L2 and L3) (cm). These traits were selected for family characterization based on information obtained in previous studies (Dias et al. 2002).

The plant biomass and RSA were evaluated at 40 DAP for plants grown in NS media. However, RFW was not measured as F_2 plants were transplanted into the greenhouse after characterization. These plants were self-pollinated to produce F_3 families, which will be employed for the identification of molecular markers linked to melon vine decline resistance in the future. To avoid excessive handling of the roots, only maximum root length (cm) was measured (L_{max}) at 40 DAP. Root lesions caused by pathogenic fungi were visually scored using three disease rating indices: (i) lesions on hypocotyls (LH) [0 = healthy with no lesions or discoloration, 1 = slight discoloration, 2 = moderate discoloration and/or with lesions, 3 = moderate maceration, and 4 = severe maceration (Biernacki and Bruton 2000)]; (ii) lesions on lateral roots (LLR) [0 = healthy with no lesions (browning and necrosis), 1 = mild lesions covering < 10% of the lateral root surface, 2 = mild to moderate lesions covering from 10 to 24% of the root surface, 3 = moderate to severe lesions covering from 25 to 50% of the root surface, and 4 = general root rot, where most of the lateral root's surface was affected (> 50%)]; and (iii) lesions on fine roots (LFR) [0 = healthy with no lesions (browning and necrosis), 1 = mild lesions on < 10% of fine root mass, 2 = mild to moderate lesions on 10–24% of root mass, 3 = moderate to severe lesions on 25–50% of root mass, and 4 = severe browning, necrosis and root rot, where most of the fine root mass was affected (> 50%)]. A relatively high correlation was observed between LLR and LFR ($r = 0.77$, $P < 0.001$) in previous studies (unpublished results). Therefore, the average of LLR and LFR was used as a disease severity index ($\bar{X}_{LLFR}$), which was then used to evaluate the response of the whole roots to the fungal damage. LH scores lesion severity in a specific region of the plant and is complementary to $\bar{X}_{LLFR}$.

Genetic analysis: To assess the effect of genotype on disease progression, data from genetically uniform classes (i.e. P_1, P_2 and F_1), were subjected to a unifactorial analysis of variance (ANOVA), and then means for root traits were separated by a Duncan's test. The correlations (Pearson correlation coefficient, r) between some traits were calculated using Statgraphics Plus 4.0 computational software (Statistical Graphics Corp., Rockville, MD, USA).

Traits were scored on individual plants to obtain generational variances. Broad-sense heritability (H) estimates were derived using population variance components by the formula described by Wright (1968) as $H = \{\sigma F_2 - [(\sigma P_1 + \sigma P_2 + 2\sigma F_1)/4]\}/\sigma F_2$. Narrow-sense heritability (h^2) was estimated using F_2 and backcross generation variance components as described by Warner (1952) as $h^2 = [2\sigma F_2 - (\sigma BC_1 + \sigma BC_2)]/\sigma F_2$, where σP_1 is the variance of P_1 (susceptible parent, cultivar 'Piñonet', 'Piel de Sapo'), σP_2 is the variance of P_2 (resistant parent, Pat 81), σF_1 and σF_2 are the variance of the F_1 and F_2 generations, respectively, and σBC_1 and σBC_2 are the variance of the F_1 backcrossed to P_1 and P_2, respectively. The least

effective factors involved in the traits analysed were estimated as described by Wright (1968) as $n = (\bar{P}_1 - \bar{P}_2)^2/8(\sigma F_2 - \sigma E)$, where $(\bar{P}_1 - \bar{P}_2)^2$ is the squared difference between the mean value of the parents, and σE is the environmental variance estimated as $\sigma P_1 + \sigma P_2 + 2\sigma F_1/4$.

Selection of resistant plants in segregating backcross populations: The disease assessment methodology and the genetic information obtained from the genetic analysis described above were used to evaluate a Pat 81 × 'Piñonet' 'Piel de Sapo'-derived BC_1 population. Fifty backcross plants were grown in a greenhouse in pots of 18 kg with a mixture of field soil and commercial standard peat (Dias et al. 2002) in a 3 : 1 ratio (v/v) during the spring-summer growing season, using drip-irrigation (temperatures ranged from 25 to 30°C day/20 to 25°C night, RH from 55 to 65% day/80 to 90% night, and 13–14/9–10 hours photoperiod). Five plants of the cultivar 'Piñonet', 'Piel de Sapo' and Pat 81 were included as susceptible and resistant controls, respectively. At the end of the growing cycle (110 DAP), the plants were sampled for RFW, RSA, L1, L2, L3 and lesion rating indices (i.e. LH, LLR and LFR). The branching order (BO) for L1, L2 and L3 (i.e. BO1, BO2 and BO3, respectively) was also evaluated according to Dias et al. (2002). A higher BO is associated with a greater capacity for soil penetration and with an increase in the water and nutrient uptake capacity of the plant (Crosby 2000, Iglesias et al. 2000b, Dias et al. 2002). However, such a measurement is laborious and for this reason BO was only evaluated in the backcross population, but not in the genetic analysis, which utilized a larger number of plants.

In previous studies (Iglesias et al. 2000b,c, Dias et al. 2002), Pat 81 plants and F1 progeny always exhibited $\bar{X}_{LLFR}$ values below 2.5, and therefore this value was used as a resistance threshold point. The root structure traits and LH scores of BC_1 progeny grouped by the aforementioned criterion were subjected to a unifactorial ANOVA test. Coefficients of variation were calculated for all traits using Statgraphics Plus 4.0 software.

Resistant BC_1 plants with large and branched root systems were selected and used for continued introgression of resistance genes into cultivated melon. A second backcross (BC_{1-2}) was obtained by crossing the BC_1 to the susceptible parent, and 20 plants of this backcross were grown and characterized for root traits and for resistance as described for the BC_1 above.

Results

Genetic analysis of root structure and root resistance

There were significant differences between the parents and the F_1 generation for most of the traits analysed (Tables 1 and 2). In the SS treatment, Pat 81 and the F_1 plants developed roots with greater fresh weight, RSA and length than the cultivar 'Piñonet', of the 'Piel de Sapo' market class (Table 1 and Fig. 1).

Inoculation of plants in the NS treatment caused lesions of variable severity in the roots of the different generations (Table 2). *Monosporascus cannonballus* was isolated from all the sampled roots. Perithecia of this fungus were observed associated with necrotic lesions in primary, secondary and tertiary roots. Other pathogenic fungi that could increase the severity of the lesions (i.e. *A. cucurbitacearum*, *Macrophomina phaseolina*, and *Pythium* sp. and *Fusarium* sp.) were also identified in approximately 80% of the roots sampled. Examination of Pat 81 and the F_1 revealed the presence of only mild lesions in hypocotyls, lateral roots and fine roots. Disease scores of these genotypes were significantly ($P < 0.001$) less than those describing roots of the cultivar 'Piñonet'. A high correlation ($r = 0.83$, $P < 0.001$) existed between LLR and LFR, using all generation data. Nevertheless, the correlation

Table 1: Plant and root traits evaluated for resistance to vine decline in progeny derived from a cross between *Cucumis melo* ssp. *agrestis* Pat 81 (P_2) and the melon cultivar 'Piñonet' (P_1), 'Piel de Sapo' market class, grown in sterilized soil 40 days after planting

Root traits	P_1 (N = 10)	P_2 (N = 10)	F_1 (N = 10)	P-value	F_2 (N = 90)	BC_1 (N = 50)	BC_2 (N = 50)	H/h²	n
Biomass (g)	8.8 a¹ ± 3.2	13.2 b ± 2.4	15.1 b ± 2.6	<0.001	14.4 ± 3.9	13.7 ± 4.4	15.3 ± 3.4	0.51/0.03	0.3
RFW (g)	1.45 a ± 0.62	3.08 b ± 0.89	2.86 b ± 0.84	0.001	2.64 ± 1.06	2.06 ± 0.92	3.07 ± 1.02	0.42/0.31	0.7
L1 (cm)	15.6 a ± 3.6	22.2 b ± 3.5	19.2 ab ± 3.9	0.009	19.5 ± 4.8	18.2 ± 4.2	20.6 ± 4.4	0.41/0.40	0.6
L2 (cm)	12.9 a ± 2.9	17.8 b ± 1.8	16.3 b ± 2.5	0.001	16.9 ± 3.9	16.0 ± 3.2	17.7 ± 3.5	0.6/0.51	0.3
L3 (cm)	10.0 a ± 2.0	15.1 b ± 2.8	14.7 b ± 2.4	<0.001	15.3 ± 2.7	15.2 ± 2.9	15.3 ± 2.3	0.2/0.12	2.2
RSA (cm²)	24.9 a ± 10.8	60.2 b ± 11.4	49.5 b ± 9.6	0.011	40.6 ± 13.8	36.4 ± 13.4	49.8 ± 12.7	0.43/0.21	1.9

N, number of plants characterized in each generation; RFW, root fresh weight; L1, L2, L3, length of the three longest lateral roots; RSA, root surface area; H, broad-sense heritability = $\{\sigma F_2 - [(\sigma P_1 + \sigma P_2 + 2\sigma F_1)/4]\}/\sigma F_2$ (Wright 1968); h², narrow-sense heritability = $[2\sigma F_2 - (\sigma BC_1 + BC_2)]/\sigma F_2$ (Warner 1952); n, number of least effective factors = $(P_1 - P_2)^2/8(\sigma F_2 - \sigma E)$ (Wright 1968).
Values are given as mean ± SD.
¹ Numbers in the same row followed by the same letter are not significantly different according to Duncan means comparison test at P = 0.05.

Table 2: Plant and root traits, and lesion rating indices evaluated for resistance to vine decline in the progeny derived from a cross between *Cucumis melo* ssp. *agrestis* Pat 81 (P_2) and the melon cultivar 'Piñonet' (P_1), 'Piel de Sapo' market class grown in naturally infested soil 40 days after planting

Root traits	P_1 (N = 10)	P_2 (N = 10)	F_1 (N = 10)	P-value	F_2 (N = 90)	BC_1 (N = 50)	BC_2 (N = 50)	H/h²	n
Biomass (g)	8.1 a¹ ± 2.9	11.2 b ± 2.6	13.7 b ± 2.9	0.009	11.4 ± 4.0	11.5 ± 3.9	11.9 ± 3.3	0.51/0.37	0.15
L_{max} (cm)	11.4 a ± 3.5	17.6 b ± 3.3	16.6 b ± 3.7	0.011	16.1 ± 4.9	14.9 ± 5.0	18.2 ± 4.7	0.50/0.04	0.42
LH	3.1 b ± 0.8	0.0 a ± 0.0	0.5 a ± 0.7	<0.001	1.1 ± 1.3	1.5 ± 1.3	0.6 ± 1.2	0.76/0.15	0.94
$\bar{X}_{LLFR}$	3.3 b ± 0.8	1.6 a ± 0.7	1.9 a ± 0.6	<0.001	2.3 ± 0.8	2.7 ± 0.8	2.1 ± 0.7	0.30/0.25	2.01
RSA (cm²)	11.5 a ± 5.3	33.8 b ± 6.2	29.2 b ± 7.1	<0.001	26.2 ± 11.1	18.7 ± 9.8	26.5 ± 8.4	0.66/0.65	0.76

N, number of plants characterized in each generation; L_{max}, maximum length of the roots; RSA, root surface area; LH, lesions in hypocotyls; $\bar{X}_{LLFR}$, average of the indices LLR and LFR [lesions were scored on a scale from 0 (no lesions) to 4 (severe root lesions)]; H, broad-sense heritability = $\{\sigma F_2 - [(\sigma P_1 + \sigma P_2 + 2\sigma F_1)/4]\}/\sigma F_2$ (Wright 1968); h², narrow-sense heritability = $[2\sigma F_2 - (\sigma BC_1 + \sigma BC_2)]/\sigma F_2$ (Warner 1952); n, number of least effective factors = $(P_1 - P_2)^2/8(\sigma F_2 - \sigma E)$ (Wright 1968).
Values are given as mean ± SD.
¹ Numbers in the same row followed by the same letter are not significantly different according to Duncan means comparison test at P = 0.05.
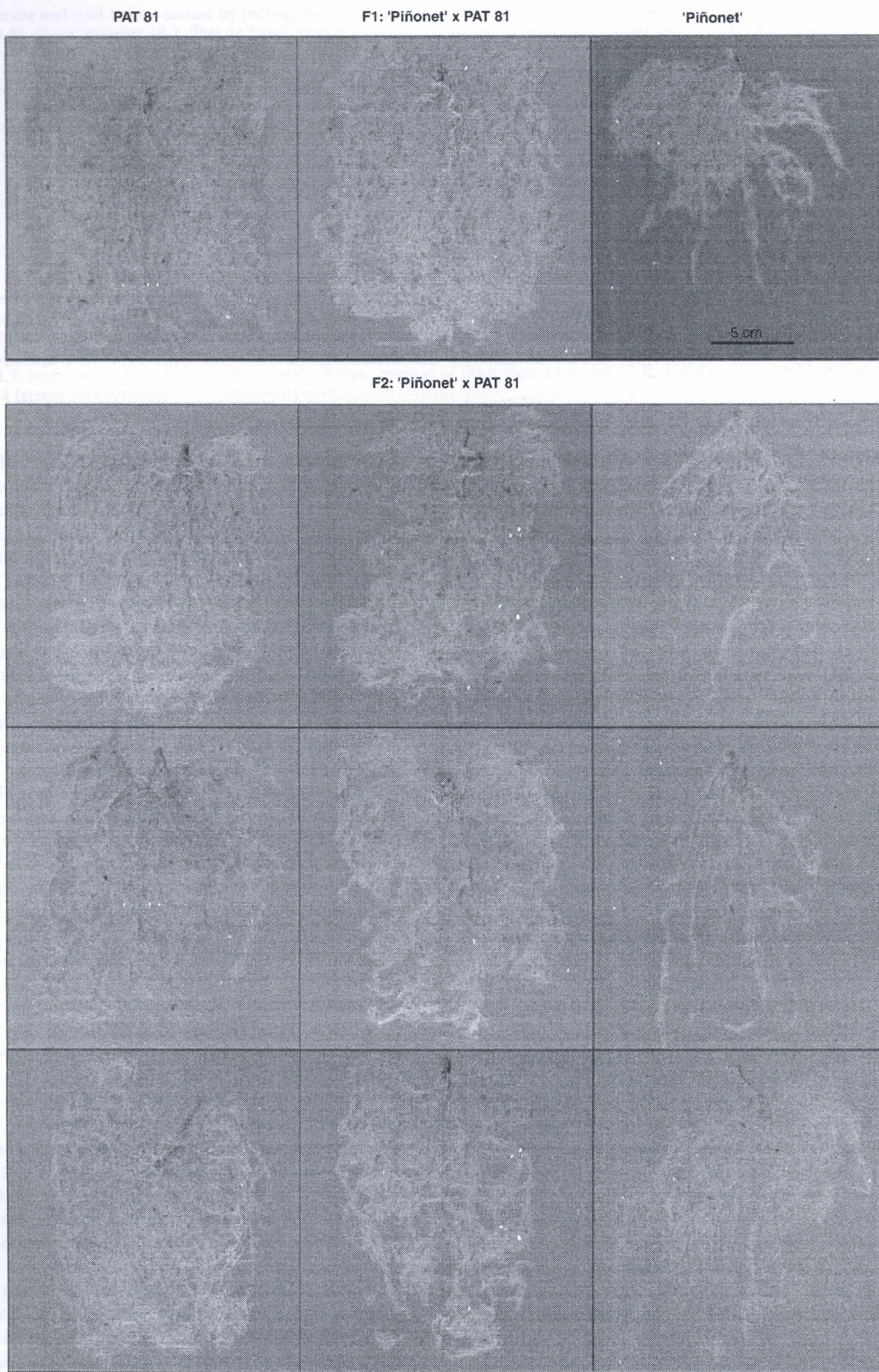
Fig. 1: Root topology and structure of the accession *Cucumis melo* ssp. *agrestis* Pat 81, the melon cultivar 'Piñonet', 'Piel de Sapo' market class, and their F_1 and F_2 progeny grown in sterilized soil 40 days after planting

Table 3: Root traits and root lesions caused by pathogenic fungi inciting vine decline as evaluated in backcrosses derived from a *Cucumis melo* ssp. *agrestis* Pat 81 (P_2) × 'Piñonet' (P_1), 'Piel de Sapo' market class mating grown in naturally infested soil 110 days after planting

Root traits	P_2	P_1	BC_1s^1	BC_1r^1	$BC_{1-2}s^1$	$BC_{1-2}r^1$	P-value	Coefficient of variation (%)
RFW (g)	$91.0\ b^2$	69.7 a	63.5 a	114.9 c	123.6 c	142.9 d	<0.001	67.0
RSA (cm^2)	37.2 b	29.9 ab	28.4 ab	48.8 b	48.3 b	60.6 c	0.002	44.4
L1 (cm)	108.0 c	29.6 a	44.4 a	75.5 b	81.0 b	154.4 c	<0.001	51.4
L2 (cm)	87.1 c	18.6 a	34.1 a	67.1 b	48.1 ab	92.2 c	<0.001	53.8
L3 (cm)	75.5 c	14.6 a	28.6 a	56.8 b	43.5 b	91.6 c	<0.001	57.9
BO1	5.7 b	3.5 a	3.9 a	4.8 ab	3.7 a	5.1 b	0.03	35.4
BO2	5.6 c	2.8 a	3.7 ab	4.6 bc	3.1 ab	5.0 c	0.003	37.8
BO3	6.0 c	2.7 a	3.1 a	4.6 b	3.6 a	5.0 c	<0.001	41.2
LH	0.0 a	4.0 c	3.6 c	1.9 b	4 c	1.8 b	<0.001	46.4
$\bar{X}_{LLFR}$	1.3 a	4.0 b	3.8 b	1.7 a	3.9 b	1.1 a	<0.001	41.2

[1] $BC_1s/BC_{1-2}s = BC_1/BC_{1-2}$ plants with $\bar{X}_{LLFR} > 2.5$; $BC_1r/BC_{1-2}r = BC_1/BC_{1-2}$ plants with $\bar{X}_{LLFR} \leq 2.5$.
[2] Numbers in the same row followed by the same letter are not significantly different according to Duncan means comparison test at $P = 0.05$. RFW, root fresh weight; RSA, root surface area; L1, L2 and L3, length of the three longest lateral roots; BO1, BO2 and BO3, branching order of L1, L2 and L3, respectively; LH, lesions in hypocotyls; $\bar{X}_{LLFR}$, average of the indices LLR and LFR. Lesions were scored on a scale from 0 (no lesions) to 4 (severe root lesions).

between LLR and LFR, and hypocotyl damage (LH) was comparatively lower ($r = 0.55$, $P < 0.001$; $r = 0.50$, $P < 0.001$, respectively).

Root damage of plants in the NS treatment resulted in a loss of biomass and a reduction of the RSA and root length in all generations (Tables 1 and 2). However, Pat 81 and the F_1 generation maintained a greater biomass and significantly longer roots with a greater surface area than the susceptible cultivar 'Piñonet' after infection (Table 2). Minor damage to the root structure of Pat 81 and F_1 plants allowed for regeneration of newly formed fine roots and root hairs which replaced those damaged by the fungi.

Means for most traits of the F_1 and F_2 were skewed towards the resistant parent, P_2 (Tables 1 and 2). Although heterosis was observed for some traits, with F_1 means being higher than Pat 81, these differences were not significant. In most cases, backcrosses to susceptible parents (BC_1) had mean values between F_1 and P_1, but these were skewed towards F_1. Inoculation of backcross progeny resulting from mating an F_1 to a resistant parent (BC_2) yielded mean values between F_1 and P_2.

Broad- and narrow-sense heritabilities could be estimated from generational variances (Tables 1 and 2). Broad-sense heritabilities varied from 0.76 to 0.20, which indicated that environment was important in the expression of most of the traits examined. The narrow-sense heritabilities were highly variable among traits, ranging from 0.65 to 0.03. These narrow- to broad-sense heritability values lend support to the existence of additive effects in root traits in SS (weight, surface area and length), RSA and disease severity index ($\bar{X}_{LLFR}$) in NS plants. The relatively low value of narrow-sense heritabilities (0.03–0.37) for the biomass trait, both in NS and in SS, and for LH (0.15) provides support for the existence of dominance and/or epistatic effects associated with these traits.

The least number of effective factors varied from 0.3 to 2.2 (Tables 1 and 2), suggesting that one or a few genes are responsible for the differences observed between parents for the traits analysed. However, the disparity in results found for some traits (e.g., L1 and L2 with $n < 1$, and L3 with $n > 2$) might be explained by the existence of large environmental variances. Likewise, the estimation method used assumes a lack of epistasis and equal genetic effects, which will underestimate n (Mather and Jinks 1971).

Selection of resistant plants in segregating backcross populations

The information obtained by Dias et al. (2002) and in the genetic analysis was used to identify resistant plants in a Pat 81 × 'Piñonet', 'Piel de Sapo' BC_1 segregating population. Significant differences ($P < 0.001$) in the LH and $\bar{X}_{LLFR}$ were detected between the parents, Pat 81 and 'Piñonet' (Table 3), and the BC_1 population segregated for root lesion severity. Inspection of the $\bar{X}_{LLFR}$ index allowed for grouping BC_1 plants into two groups: 24 partially resistant plants BC_1r ($\bar{X}_{LLFR} \leq 2.5$) and 26 susceptible plants, BC_1s ($\bar{X}_{LLFR} > 2.5$). Although significant differences ($P < 0.001$) were obtained between groups in LH, variability was high and plants rated as 0–4 were found in both the resistant and the susceptible group (Table 3). As a severely damaged hypocotyl can lead to plant death, LH must be considered complementarily to $\bar{X}_{LLFR}$ for the evaluation of melon genotypes against vine decline.

Significant differences ($P < 0.001$) were detected between parents for fresh weight, RSA, root length and BO (Table 3), thereby confirming data obtained from previous assays (Dias et al. 2002). Roots of BC_1r progeny were heavier, possessed a larger surface area, and were longer and more widely branched than roots of BC_1s. Therefore, despite the mild-to-moderate lesions ($\bar{X}_{LLFR} \leq 2.5$) of BC_1r plants, a considerable root mass was maintained after infection. Likewise, BC_1r roots displayed a higher RFW and RSA than the resistant parent, which was possibly because of the existence of heterosis for these traits.

The BC_{1-2} population also segregated for $\bar{X}_{LLFR}$, in which eight were partially resistant plants, $BC_{1-2}r$ ($\bar{X}_{LLFR} \leq 2.5$), and 12 were susceptible plants, $BC_{1-2}s$ ($\bar{X}_{LLFR} > 2.5$). LH was variable within each group (Table 3). As in the BC_1, significant differences ($P < 0.001$) were detected between $BC_{1-2}r$ and $BC_{1-2}s$ for all the root traits analysed. Moreover, roots of BC_{1-2} progeny were significantly ($P < 0.001$) more vigorous, longer and more branched than those of BC_1 progeny, thus confirming the effectiveness of selection. After infection, $BC_{1-2}r$ plants maintained a root mass larger than that of the resistant parent, which probably contributed to their tolerance to vine collapse.

Discussion

This is the first genetic analysis of resistance/tolerance to vine decline in progeny derived from Pat 81, based on root response to naturally infested soil. Although the diversity of the

Table 3: Root traits and root lesions caused by pathogenic fungi inciting vine decline as evaluated in backcrosses derived from a *Cucumis melo* ssp. *agrestis* Pat 81 (P$_2$) × 'Piñonet' (P$_1$), 'Piel de Sapo' market class mating grown in naturally infested soil 110 days after planting

Root traits	P$_2$	P$_1$	BC$_1$s[1]	BC$_1$r[1]	BC$_{1-2}$s[1]	BC$_{1-2}$r[1]	P-value	Coefficient of variation (%)
RFW (g)	91.0 b[2]	69.7 a	63.5 a	114.9 c	123.6 c	142.9 d	<0.001	67.0
RSA (cm^2)	37.2 b	29.9 ab	28.4 ab	48.8 b	48.3 b	60.6 c	0.002	44.4
L1 (cm)	108.0 c	29.6 a	44.4 a	75.5 b	81.0 b	154.4 c	<0.001	51.4
L2 (cm)	87.1 c	18.6 a	34.1 a	67.1 b	48.1 ab	92.2 c	<0.001	53.8
L3 (cm)	75.5 c	14.6 a	28.6 a	56.8 b	43.5 b	91.6 c	<0.001	57.9
BO1	5.7 b	3.5 a	3.9 a	4.8 ab	3.7 a	5.1 b	0.03	35.4
BO2	5.6 c	2.8 a	3.7 ab	4.6 bc	3.1 ab	5.0 c	0.003	37.8
BO3	6.0 c	2.7 a	3.1 a	4.6 b	3.6 a	5.0 c	<0.001	41.2
LH	0.0 a	4.0 c	3.6 c	1.9 b	4 c	1.8 b	<0.001	46.4
X̄$_{LLFR}$	1.3 a	4.0 b	3.8 b	1.7 a	3.9 b	1.1 a	<0.001	41.2

[1] BC$_1$s/BC$_{1-2}$s = BC$_1$/BC$_{1-2}$ plants with X̄$_{LLFR}$ > 2.5; BC$_1$r/BC$_{1-2}$r = BC$_1$/BC$_{1-2}$ plants with X̄$_{LLFR}$ ≤ 2.5.
[2] Numbers in the same row followed by the same letter are not significantly different according to Duncan means comparison test at P = 0.05. RFW, root fresh weight; RSA, root surface area; L1, L2 and L3, length of the three longest lateral roots; BO1, BO2 and BO3, branching order of L1, L2 and L3, respectively; LH, lesions in hypocotyls; X̄$_{LLFR}$, average of the indices LLR and LFR. Lesions were scored on a scale from 0 (no lesions) to 4 (severe root lesions).

between LLR and LFR, and hypocotyl damage (LH) was comparatively lower ($r = 0.55$, P < 0.001; $r = 0.50$, P < 0.001, respectively).

Root damage of plants in the NS treatment resulted in a loss of biomass and a reduction of the RSA and root length in all generations (Tables 1 and 2). However, Pat 81 and the F$_1$ generation maintained a greater biomass and significantly longer roots with a greater surface area than the susceptible cultivar 'Piñonet' after infection (Table 2). Minor damage to the root structure of Pat 81 and F$_1$ plants allowed for regeneration of newly formed fine roots and root hairs which replaced those damaged by the fungi.

Means for most traits of the F$_1$ and F$_2$ were skewed towards the resistant parent, P$_2$ (Tables 1 and 2). Although heterosis was observed for some traits, with F$_1$ means being higher than Pat 81, these differences were not significant. In most cases, backcrosses to susceptible parents (BC$_1$) had mean values between F$_1$ and P$_1$, but these were skewed towards F$_1$. Inoculation of backcross progeny resulting from mating an F$_1$ to a resistant parent (BC$_2$) yielded mean values between F$_1$ and P$_2$.

Broad- and narrow-sense heritabilities could be estimated from generational variances (Tables 1 and 2). Broad-sense heritabilities varied from 0.76 to 0.20, which indicated that environment was important in the expression of most of the traits examined. The narrow-sense heritabilities were highly variable among traits, ranging from 0.65 to 0.03. These narrow- to broad-sense heritability values lend support to the existence of additive effects in root traits in SS (weight, surface area and length), RSA and disease severity index (X̄$_{LLFR}$) in NS plants. The relatively low value of narrow-sense heritabilities (0.03–0.37) for the biomass trait, both in NS and in SS, and for LH (0.15) provides support for the existence of dominance and/or epistatic effects associated with these traits.

The least number of effective factors varied from 0.3 to 2.2 (Tables 1 and 2), suggesting that one or a few genes are responsible for the differences observed between parents for the traits analysed. However, the disparity in results found for some traits (e.g., L1 and L2 with n < 1, and L3 with n > 2) might be explained by the existence of large environmental variances. Likewise, the estimation method used assumes a lack of epistasis and equal genetic effects, which will underestimate n (Mather and Jinks 1971).

Selection of resistant plants in segregating backcross populations

The information obtained by Dias et al. (2002) and in the genetic analysis was used to identify resistant plants in a Pat 81 × 'Piñonet', 'Piel de Sapo' BC$_1$ segregating population. Significant differences (P < 0.001) in the LH and X̄$_{LLFR}$ were detected between the parents, Pat 81 and 'Piñonet' (Table 3), and the BC$_1$ population segregated for root lesion severity. Inspection of the X̄$_{LLFR}$ index allowed for grouping BC$_1$ plants into two groups: 24 partially resistant plants BC$_1$r (X̄$_{LLFR}$ ≤ 2.5) and 26 susceptible plants, BC$_1$s (X̄$_{LLFR}$ > 2.5). Although significant differences (P < 0.001) were obtained between groups in LH, variability was high and plants rated as 0–4 were found in both the resistant and the susceptible group (Table 3). As a severely damaged hypocotyl can lead to plant death, LH must be considered complementarily to X̄$_{LLFR}$ for the evaluation of melon genotypes against vine decline.

Significant differences (P < 0.001) were detected between parents for fresh weight, RSA, root length and BO (Table 3), thereby confirming data obtained from previous assays (Dias et al. 2002). Roots of BC$_1$r progeny were heavier, possessed a larger surface area, and were longer and more widely branched than roots of BC$_1$s. Therefore, despite the mild-to-moderate lesions (X̄$_{LLFR}$ ≤ 2.5) of BC$_1$r plants, a considerable root mass was maintained after infection. Likewise, BC$_1$r roots displayed a higher RFW and RSA than the resistant parent, which was possibly because of the existence of heterosis for these traits.

The BC$_{1-2}$ population also segregated for X̄$_{LLFR}$, in which eight were partially resistant plants, BC$_{1-2}$r (X̄$_{LLFR}$ ≤ 2.5), and 12 were susceptible plants, BC$_{1-2}$s (X̄$_{LLFR}$ > 2.5). LH was variable within each group (Table 3). As in the BC$_1$, significant differences (P < 0.001) were detected between BC$_{1-2}$r and BC$_{1-2}$s for all the root traits analysed. Moreover, roots of BC$_{1-2}$ progeny were significantly (P < 0.001) more vigorous, longer and more branched than those of BC$_1$ progeny, thus confirming the effectiveness of selection. After infection, BC$_{1-2}$r plants maintained a root mass larger than that of the resistant parent, which probably contributed to their tolerance to vine collapse.

Discussion

This is the first genetic analysis of resistance/tolerance to vine decline in progeny derived from Pat 81, based on root response to naturally infested soil. Although the diversity of the

pathogens isolated from NS confirms the complexity of melon vine decline aetiology, *M. cannonballus* plays a prominent role in disease development. Results support the existence of a difference in response of resistant and susceptible plants to hypocotyl pathogen attack (LH), when compared with responses observed in lateral and fine roots ($\bar{X}_{LLFR}$). The severity of lesions in hypocotyls is reported to be highly influenced by environmental and cultural factors (i.e. sowing depth, contact surface area and uneven distribution of inoculum in the soil) (Bruton et al. 2000). Likewise, different pathogens could be associated with lesions in different parts of the roots. For instance, *M. phaseolina*, as well as some other soilborne pathogens, attacks the crown, whereas *M. cannonballus* and *A. cucurbitacearum* predominantly attack primary or secondary roots. Although the use of NS for screening melons against vine decline has its shortcomings, it seems difficult to represent the biotic complexity of the disease using artificial inoculations of sterile soil. The complementary use of LH and $\bar{X}_{LLFR}$ is suggested in order to evaluate the resistance of the different melon genotypes to melon vine decline.

The resistance mechanism of plants against the pathogenic fungi in Pat 81 is associated with a vigorous and branched root system (Dias et al. 2002). Previous studies documented a reduction of root area after infection (even in partially resistant genotypes) mainly due to the loss of fine roots (Crosby et al. 2000). However, Pat 81 maintained a higher root mass after infection when compared with the susceptible cultivar 'Piñonet'. This trait could potentially confer tolerance to vine collapse in melon germplasm despite the development of mild-to-moderate root lesions.

The resistance to fungi and root vigour were also observed in Pat 81 progeny. The mild infection detected on F_1 roots is consistent with previous field results (Iglesias et al. 2000a), where plants did not collapse or exhibited a lower collapse incidence than the susceptible parent.

Broad- and narrow-sense heritability estimates provide support for the existence of additive effects in root weight, surface area and length in SS and RSA and the disease severity index $\bar{X}_{LLFR}$ in NS. However, dominance and/or epistatic effects also seem to be associated with biomass and LH in inoculated populations. Previously, Crosby (2000) demonstrated the importance of additive genetic control of traits related to the length and surface of the root system in melons in response to vine decline pathogens. Such genetic control has been reported for different root parameters in other crops (Clarke and McCaig 1993, Johson et al. 1996). The heritabilities obtained by Crosby (2000) for the surface area and length of the root system in melon, both before and after infection with *M. cannonballus*, were higher than those obtained in the current study (some exceeded 1). The authors attributed these high values to the method used, which often inflates heritability estimates, and to the dramatic heterotic effect on certain traits in the progeny of several of the crosses examined.

One or a few genes seem to be involved in the control of most root traits, but one must take into account the limitations in the experimental methodology which underestimates the number of least effective factors. A monogenic or oligogenic control will facilitate the selection of these traits in breeding programmes.

These data agree with the genetic model for resistance/tolerance to vine decline previously derived from Pat 81, as assayed by the percentage of collapse in field-grown plants (Iglesias et al. 2000a). This also supports the idea that the occurrence of mild lesions in a vigorous root system can lead to a reduction in the incidence of collapse. This relationship implies that selection for resistant plants in segregating populations may be accomplished by assaying solely the infection on the roots to avoid the difficulties associated with reproducing the vine collapse syndrome under field conditions. It may also be possible to carry out selection of plants with improved root structure in pathogen-free soil (i.e. larger, longer and more branched root systems) in order to increase plant tolerance to vine collapse.

A preliminary selection experiment has been conducted and described. Selection based on the $\bar{X}_{LLFR}$ index enabled identification of BC_1 individual plants with mild root lesions and vigorous, widely branched roots after infection at the end of the crop cycle. These plants were then used to continue the introgression process for resistance to vine decline. The characterization of the second backcross to 'Piñonet', 'Piel de sapo' showed that selection was effective for most disease-associated traits. These early generation results are suggestive of possible gain from selection in more advanced cycles of introgression when 'Piñonet', 'Piel de Sapo' is used as the recurrent parent. Because of moderate heritability, it is suggested that a combination of selection methods (individual and family) be used to improve selection effectiveness. For example, the characterization of selfed progeny derived from selected plants may provide information less affected by environment, thereby allowing for more precise single-plant selection. Moreover, in this case where traits are controlled primarily by few genes contributing additive effects and where moderate heritabilities occur, the identification of target trait-linked molecular markers may be useful for enhancing breeding efficiency.

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