

Uncommon disorders and decay in near-isogenic lines of melon and reference cultivars

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ABSTRACT

Postharvest disorders and rots can produce important economic losses in fruits stored for long time for exportation. The genetic and physiological basis of some disorders in melon (*Cucumis melo* L.) are unknown and particularly the possible relation with climacteric behavior. A collection of melon near-isogenic lines (NILs) (SC3-5 and seven more showing climacteric and two non-climacteric ripening pattern) were analyzed to study genetic and physiological aspects of fruit disorders and rots. Two non-climacteric (Nicolás; Inodorus Group; and Shongwan Charmi PI161375, Conomon Group) and two climacteric cultivars (Fado, Reticulatus Group; Védrañtais, Cantaloupensis Group) were used as reference. The field was divided in eight blocks containing one three-plant replication for each NIL, two for the parental cultivar Piel de Sapo and one or two for the reference cultivars. Replications evaluated were more than six in the cultivars studied. Plant problems included aphids, powdery mildew, and leaf wind injury. Preharvest fruit disorders included whole fruit cracking in cultivar Védrañtais and NIL 5M2, and styler-end cracking in cultivar Fado. Climacteric NILs with yellow skin were particularly affected by over-ripening, styler-end cracking, and sunburn during cultivation. At harvest, two NILs showed slight placental tissue necrosis which was inherited from SC and were also detected after storage. Other uncommon disorders seen at harvest or 30 days after storage at 8°C included warted skin (scarring), flesh discoloration (light brown or translucent areas), hollow flesh disorder, and deep furrow netting inherited from SC. Less common rots included grey mould, bacterial soft rot, *Penicillium* rot, cottony leak and internal *Cladosporium* rot. Styler-end hardness below 20 N·mm⁻¹ was associated with cracking and softening. The incidence of the disorders and rots was too low to confirm that the genetic component played a role in their development.

Keywords: *Cucumis melo*, decay, fruit quality, physiological disorders, postharvest, preharvest.

RESUMO

Acidentes fisiológicos e podridões atípicas em linhas quase-isogênicas de melão e em cultivares de referência

Analisaram-se frutos de melão (*Cucumis melo* L.) provenientes de uma coleção de linhas quase-isogênicas (NIL), SC3-5 e outras sete climatéricas e duas não-climatéricas, para avaliar a ocorrência de acidentes fisiológicos e a suscetibilidade a podridões. Como termo de comparação utilizou-se duas cultivares não-climatéricas (Nicolás, grupo Inodorus, e Shongwan Charmi ou acesso PI161375, grupo Conomon) e duas cultivares climatéricas (Fado, grupo Reticulatus, e Védrañtais, grupo Cantaloupensis). O experimento foi dividido em oito blocos contendo três plantas por repetição para NIL, dois para a cultivar parental Piel de Sapo (PS), e um ou dois para a cultivar testemunha. As repetições avaliadas foram mais de seis para as cultivares em estudo. Durante a produção, as folhas foram afetadas por pulgão, oídio e acidentes meteorológicos. Os acidentes fisiológicos que ocorreram nos frutos durante a produção incluíram fendilhamento na cultivar Védrañtais e na NIL 5M2 e fendilhamento da zona pistilar na cultivar Fado. As NIL climatéricas com casca amarela revelaram-se muito suscetíveis à sobre-maturação, fendilhamento pistilar e queimaduras solares durante a produção. Duas NIL exibiram uma ligeira necrose do tecido placentar, caráter herdado da linha PI161375. Outros acidentes fisiológicos atípicos observados na colheita ou após 30 dias a 8°C incluíram casca verrugosa, descolorações castanho-claras na polpa, translucência da polpa, cavidades na polpa e ornamentação profundamente sulcada na casca, herdada do PI161375. Observou-se a ocorrência de podridões pouco usuais em melão, nomeadamente podridão cinzenta, podridão bacteriana, *Penicillium* sp., *Phytophthora* sp. e cladosporiose interna. Uma dureza da região pistilar inferior a 20 N mm⁻¹ estava associada ao fendilhamento e ao amolecimento excessivo. A ocorrência destes acidentes fisiológicos ou podridões foi baixa para assegurar o envolvimento de fatores genéticos no seu desenvolvimento.

Palavras-chave: *Cucumis melo*, qualidade do fruto, acidentes fisiológicos, podridão, pós-colheita, pré-colheita.

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During recent years, melon export from Brazil has increased dramatically in terms of quantity and cultivar diversity (Sales *et al.*, 2004; Souza *et al.*, 1994; Nunes *et*

al., 2005). Although most of common occurring pests during plantation and fruit growth and development have been characterized (Zitter, 1986; Zitter *et al.*, 2004), and most disorders associated

with muskmelon development and shipment are well-known (Ceponis *et al.*, 1986; Snowdon, 1991; Cantwell & Portella, 1998;), still many questions concerning less common disorders

and their possible genetic component (whether they act alone or interact with decay) remains.

A collection of near isogenic lines (NILs) derived from a cross between the Spanish Piel de Sapo control genotype (PS, *Inodorus* Group) and the exotic Korean accession Shongwan Charmi (SC), PI161375 has recently been developed (Eduardo *et al.*, 2005). It is assumed that the parental lines of the mentioned cross (SC and PS) can be useful for studying melon preharvest and postharvest disorders (Périn *et al.*, 2002; Eduardo *et al.*, 2007; Fernández-Trujillo *et al.*, 2007), as well as possible disease resistance (Morales *et al.*, 2005).

The NIL collection including NIL SC3-5 and other NILs with shorter introgressions in linkage group (LG) III have revealed to be very useful in identifying QTLs responsible for several major postharvest disorders, including the link between susceptibility to disorders and climacteric behavior (Fernández-Trujillo *et al.*, 2007, 2008). These NILs also contain QTLs responsible for fruit quality and aroma traits (Eduardo *et al.*, 2007; Obando *et al.*, 2008; Obando-Ulloa *et al.*, 2008) that are being used for melon quality-oriented breeding programs. Previous reports indicated that shelf-life and ethylene production are inversely correlated (Zheng & Wolf, 2000). In fact, negative genetic correlation has been reported between major skin injuries or rots and climacteric behavior (Fernández-Trujillo *et al.*, 2008).

The aim of this paper was to describe some uncommon preharvest and postharvest disorders of a set of near-isogenic melon lines and reference cultivars. (Périn *et al.*, 2002; Obando *et al.*, 2007; Fernández-Trujillo *et al.*, 2008; Moreno *et al.*, 2008; Obando-Ulloa *et al.*, 2008;). In this paper, possible link between differences in cultivars, climacteric behavior and the occurrence of disorders and decay is especially emphasized.

MATERIAL AND METHODS

Plant material - the original parental lines for the near-isogenic lines

population were the *Cucumis melo* L. *Inodorus* Group Piel de Sapo (PS) and the exotic Korean accession Shongwan Charmi of the Conomon Group (SC). The development scheme of the NILs, through repeated backcrossing and molecular markers assisted selection, has been previously reported (Eduardo *et al.*, 2005). The NIL SC3-5ab was used to generate derived NILs with fewer introgressions by backcrossing with PS and molecular marker assisted selection (Eduardo *et al.*, 2007). Line SC3-5 was a NIL similar to SC3-5ab (Eduardo *et al.*, 2007). Nine NILs with fewer introgressions than SC3-5 were obtained (5Mx, x = 2 to 10). These NILs were also climacteric, except for 5M2 (not measured, but without the signs of skin color turning from green to yellow, which is typical of climacteric NILs) and the non-climacteric 5M7.

Commercial cultivars used as reference were cultivar Fado F1, *Reticulatus* Group (a *Galia* type from Semillas Fitó); an F1 hybrid of cultivar Nicolás (Syngenta Seeds, Torre Pacheco, Murcia); and the parental cultivar Védrañtais, *Cantaloupensis* Group, (Vilmorin Clause & Cie, La Menitre, France).

Experimental design - seeds were sown in seed plugs (plug trays) on March 9, 2005, and seedlings were hand-transplanted on April 12 to an experimental field located at Torre Pacheco (Murcia, Spain). The whole plot had 1,566 m², divided into 18 single rows, all covered with black plastic mulch 1.1 m wide and 22.9 µm thick. Planting distances were 2 m between rows and 1.5 m within plants from the same row.

A space of 1.5 m between blocks of each replication (three plants) was used to avoid fruit harvest confusions. Soil preparation, fertigation, plant protection, and other growing practices were those commonly used for melon cultivation in Mediterranean conditions (Fernández-Trujillo *et al.*, 2005, 2007; Eduardo *et al.*, 2007). Rows were drip-irrigated using two emitters per plant. The plot was surrounded by a border line of cultivar Nicolás.

The field was divided in eight blocks containing at least one three-plant

replication for each NIL (separated by 1.5 m between them or in relation to the border line), two for cultivar PS, and one or two for the reference cultivars (SC, Fado or Védrañtais) in a completely randomized design within the block.

The last block contained only two replications of three NILs (5M2, 5M9, 5M10) and six replications of cultivar PS. For cultivars SC and Fado, replications consisted of one single plant, though, in total, eleven or eight replicates, respectively, were planted in the field. The experimental design consisted of three plants per replication, n = 9 (for NILs 5M2, 5M9, and 5M10), n = 20 (for the parental line PS), and n = 7 for eight NILs - SC3-5 and 5M3 to 5M8 - and cultivar Nicolás).

The different number of replications for PS and the NILs was mandatory to use Dunnett contrast in order to establish differences between PS and the NILs. The nine replicates in some NILs were required for other parallel experiments and also to avoid possible plants death, taking into account their high susceptibility to decay due to the climacteric behavior (Eduardo *et al.*, 2007). Other details of the experiment have been previously reported (Fernández-Trujillo *et al.*, 2005).

The harvest period started on June 20 and ended on July 19, 2005, with fourteen harvests in total. The harvest indicators, which varied depending on the physiological behavior and cultivar, were: growth period, fruit size, skin waxing aspect, loss of skin trichomes, development of abscission layer, yellow ring or scar at the peduncle (1/2 to full slip), death of first leaf beside peduncle, fruit compactness, ground spot skin color, netting aspect, characteristic skin color or aroma for the climacteric NILs or reference cultivars, and soluble solids accumulation of samples obtained during the two weeks that preceded harvest (levels should be around 12 to 14° Brix for PS and cultivar Nicolás, and a minimum of 10° Brix).

To avoid mechanical damages to the very delicate skin, fruits of cultivar SC were covered for transportation to the laboratory with soft freshly harvested weeds.

Disorders inspection and evaluation - in order to study storage disorders and decay, at least four fruits per replicate were weighed and randomly stored in boxes at $8 \pm 0.6^\circ\text{C}$ and $87 \pm 2\%$ RH for 30 days (except for cultivars Fado and Védrañtais, which were only stored for 20 and 25 days, respectively). Fruits were covered with plastic liner (Plásticos del Segura, Murcia, Spain) one day after harvest to avoid water condensation.

Preharvest losses were examined in 153 fruits that were discarded during the three-week harvest of the NILs and reference cultivars. Digital pictures for every single fruit were obtained and the disorders and rots were identified in the laboratory, according to conventional methodology (Barnett & Hunter, 1999; Martínez & Fernández-Trujillo, 2007). Postharvest disorders were examined according to Fernández-Trujillo *et al.* (2007).

The uncommon disorders searched for were necrosis of the placental tissue, warted skin, net or stylar-end cracking, hollow flesh disorder (HFD), and slight sunburn symptoms. Healed stylar-end growth cracks (stylar-end cracking or healed split, basal healed cracking) were visually evaluated on a 0 to 4 visual scale, corresponding to none to very slight, slight, moderate, and severe symptoms (the latter two were usually associated with decay, although some hyphae of *Cladosporium* sp. and *Fusarium* sp. were only found under light microscopy, including all the very slight scales).

Data were transformed into a cracking index on a 0 to 100 scale. In order to discern the limits of stylar-end hardness that indicated over-ripening symptoms, this trait was measured in a separate experiment using five fruits per line at 21°C . For hardness measurement, a 2 kN cell setup with a double scale (only the 0 to 200 N scale was used using a 1/10 of the total graphic scale), was mounted on a textural machine (Ibertest Daganzo de Arriba, Madrid, Spain) with two deformation plates (one static). Hardness was calculated in N mm^{-1} by dividing the maximum force to obtain 2 mm deformation in whole melons, using 15 mm min^{-1} compression

speeds and 1 N preload (Fernández-Trujillo *et al.*, 2005). Symptoms of fruit stylar-end softening in a four-range scale (severe, moderate, slight and very slight) were evaluated by hand and categories were preliminarily accommodated to instrumental measurements.

Statistical analysis - when needed, data were transformed into their respective *arcsin* to fit normal distribution before being subjected to analysis of variance using JMP 5.1.2 (SAS Institute Inc., Cary, NC, USA). To study the effect of the cultivar SC introgressions, mean NIL values were compared with the control genotype PS by the Dunnett contrast at $p \leq 0.05$ (Eduardo *et al.*, 2007). If the PS was completely free from the disorder, no statistical analysis was performed. To study differences between cultigens SC3-5, PS, SC, Fado, Nicolás, and Védrañtais, data were subjected to analysis of variance and the significance among cultigen differences were assessed using the Tukey test, at $p \leq 0.05$.

RESULTS AND DISCUSSION

Preharvest damaged fruit evaluation was conducted with fruit discarded in the field (70%) or in the laboratory (30%). The main preharvest losses (no additives) were due to slight to severe fruit cracking (seen in 71% of lost fruits), over-ripening (seen in 18%), skin sunburn (seen in 11%), bacterial soft rot (seen in 16%), and birds and rodent attacks, natural dehiscence in the field (climacteric NILs were dehiscent) and fruit deformation (seen in 2%).

Regarding parental lines preharvest losses, Piel de Sapo (PS) fruits were free from over-ripening symptoms, cracking, and less than 5% fruit showed slight sunburn at harvest (data not shown), while Shongwan Charmi (SC) fruits did not crack but showed deep netting that usually formed skin scar while still attached to the plant. SC was also very sensitive to sunburn as its susceptibility to different diseases, fruit deformation, skin bruises, and damage frequently resulted in unmarketable fruit. To avoid this problem, well developed SC fruits were partly covered with fresh weeds

in the field.

Preharvest losses in reference cultivars were caused by moderate to severe cracking. This problem was particularly noticeable in cultivar Védrañtais melons (Figure 1A), representing 21% of the total losses in the field, 3.6 times more than what was observed in PS, and 6.4 fold the average losses of climacteric NILs.

The initial cracking in cultivar Védrañtais developed sometimes already 1-2 hours after harvesting and transporting to laboratory (about 3% of the fruits of this cultivar). Cracking was also a problem in 14% of fruits of cultivar Fado. In the field, 5M2 showed a similar cracking level as PS, particularly at the beginning of the season (Figure 1B). Melons from cultivar Védrañtais, whose skin is more sensitive to environmental factors or insect attacks compared to NILs or PS, frequently developed a warted skin that resembled the scarring commercially defined in netted melons (Figure 1C).

In the F1 hybrid non-climacteric cultivar Nicolás, some melons showed a slight aroma, typical of climacteric melon NILs, as observed by human nose (Figure 1D). This aroma was not detectable in the typical non-climacteric fruits obtained from cultivar Nicolás and was concomitant with partial skin degreening, leading to fruit with shorter shelf-life.

Preharvest losses in the NILs included moderate to severe sunburn symptoms in the fruit discarded in the field, particularly in climacteric NILs 5M4, 5M5, 5M9, 5M10 (Figure 1D; data not shown). Preharvest sunburn led to internal rind necrosis and the flesh close to the sunburn area was more prone to suffer cavities (data not shown).

Sunburn was the consequence of fruit exposure to the sun due to reduced foliar coverage provoked by aphid attack (*Aphis gossypii* Glover) and powdery mildew [*Sphaerotheca fuliginea* (Schlecht ex Fr.) Poll.], which affected mainly NILs and not the PS. The slight reduction of foliar coverage as a result of leaf injuries in most of plants before fruit set (Figure 2A & 2B) also contributed to sunburn. Leaf injury was

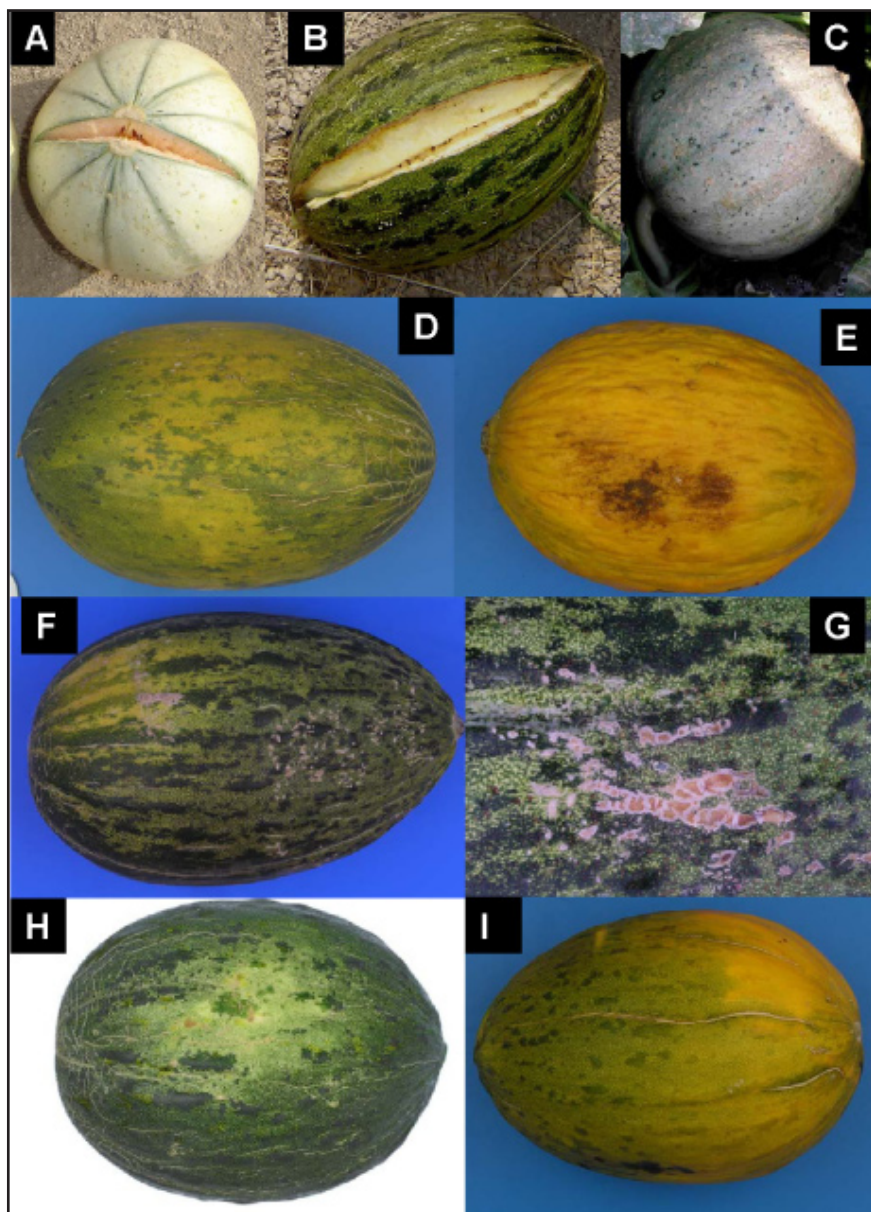


Figure 1. Skin and flesh disorders and decay at harvest in fruits of melon near-isogenic lines (NILs) and reference cultivars (alterações fisiológicas e podridões em frutos de melão de linhas quase-isogênicas e cultivares testemunhas); A: Whole fruit cracking Cantaloupensis Group, Naud, cultivar Védrañtais (rachadura completa em frutos do grupo Cantaloupensis, Naud e cultivar Védrañtais); B: Whole fruit cracking in the susceptible NIL 5M2 (rachadura completa em frutos da linha quase-isogênica suscetível 5M2); C: Warty skin (similar to scarring) in *C. melo* var. *cantalupensis*, Naud, cultivar Védrañtais (casca verrugosa, semelhante a cicatrizes, in *C. melo* var. *cantalupensis*, Naud, cultivar Védrañtais); D: Sunburn after harvest in NIL 5M5 (queima do sol após colheita em NIL 5M5); E: Abnormal skin yellowing in the cultivar Nicolás at harvest (amarelecimento anormal da casca na cultivar Nicolás na colheita); F, G: Warty skin in the parental Piel de Sapo (PS) line and detail (casca verrugosa e detalhe na linha parental Piel de Sapo (PS)); H: Typical blue-green color in the ground skin area of NIL 5M7, with a typical late harvest (coloração típica azul-esverdeada na área da casca sobre o solo de NIL 5M7, com colheita tipicamente atrasada); I: Deep furrow in netting of NIL 5M8 (ornamentação profundamente sulcada no NIL 5M8). Cartagena, UPCT, 2008.

probably due to wind injury causing leaf bruising. Other environmental factors alone or in combination may also cause leaf bruising, such as rain, temperature

fluctuations, dew magnifying glass effect, and even phytotoxic effects caused by pest treatment, because all these factors reduce plant vigor

(Blancard *et al.*, 1996).

Bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* (Jones) was found in the peduncle of 16% of field discarded over-ripe fruit (about 1 to 4 fruits per NIL or cultivar, except in NILs 5M6, 5M7, and 5M9, in which the bacteria did not develop). Fusarium rot, caused by *Fusarium* sp. Link, was negligible in the field (only one fruit in 5M4).

Fruit deformation and pear shaped fruit, which had small size, were more frequent in NILs 5M4, 5M8, 5M9, and 5M10 (data not shown). Fruit deformation or internal cavities in pear-shaped fruits have been attributed to poor pollination at certain moments of the season (Blancard *et al.*, 1996). Other uncommon skin and flesh disorders at harvest or after storage at 8°C were observed.

The scarring or warted skin was recorded at harvest but particularly after storage at 8°C, and also in some cultivar Nicolás fruits after subsequent storage trials at 21°C (data not shown). The warted skin disorders slightly spoil the skin appearance. The disorder was uncommon and not critical for PS or the nonclimacteric NIL 5M7 (Figures 1F and G), or in other NILs such as 5M3.

The warted skin disorder resembled cucurbit scab (Zitter, 1986). In previous studies, fruit warted skin has been associated with insects or environmental factors such as the sudden RH fluctuations in the field during the autumn or high irrigation levels (San Bautista *et al.*, 2004). Therefore, development of warted skin in refrigerated rooms is very most likely.

The warted skin disorder resembles scab but is not provoked by *Cladosporium cucumerinum* Ellis and Arth., which is responsible for scab (Zitter, 1986; Vegh1-Inra, 2005). As we did not observe the typical development symptoms of the fungus (such as its special leaf or stem symptoms, typical protuberant spots, or favorable low temperatures and high RH conditions in the field) we assume that the *Cladosporium* sp. observed in our experiment should be *C. herbarum* which only grows during storage or senescence leaves.

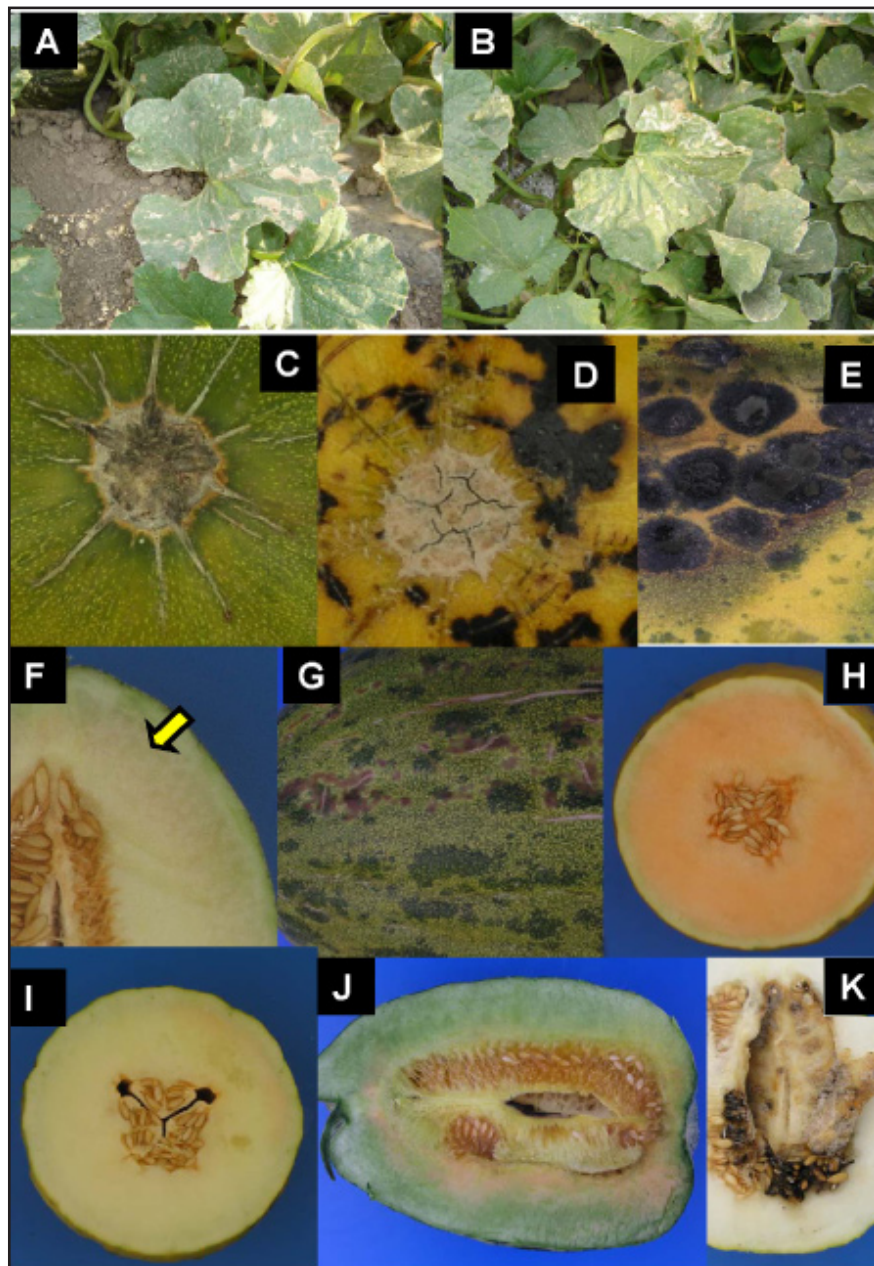


Figure 2. Preharvest leaf injuries and skin and flesh disorders and decay after 30 days at 8°C in fruit of several melon near-isogenic lines (NILs) and reference cultivars (injúrias foliares e alterações e podridão na casca e polpa de frutos armazenados por 30 dias a 8°C em linhas quase-isogênicas (LQI) e cultivares referências de melão); A: Wind injury in leaves of melon parental line (injúrias causadas pelo vento em folhas da linha parental de melão); B: Wind injury in leaves of NIL SC3-5 (injúrias causadas pelo vento na LQI SC3-5); C: Moderate stylar-end cracking in NIL 5M4 surrounded by scald (rachadura moderada na região pistilar na LQI 5M4, circundada por escaldadura); D: Stylar-end cracking in NIL 5M6 affected by Cladosporium rot (rachadura da região pistilar na LQI 5M6 afetada pela podridão de Cladospório); E: Cladosporium rot developing on a sunburn area plus skin scald in NIL 5M5 (podridão de Cladospório desenvolvida em área com queimadura de sol e escaldadura da casca na LQI 5M5); F: Very slight flesh browning of unknown origin in NIL 5M3 (leve escurecimento da polpa, de origem desconhecida, na LQI 5M3); G: External skin symptoms associated with slight internal flesh browning in NIL 5M3 (sintomas externos na casca associados ao leve escurecimento da polpa na LQI 5M3); H: Orange flesh in partly over-mature fruit of NIL 5M4 (polpa alaranjada em frutos parcialmente passados da LQI 5M4); I: Yellow flesh in partly over-mature fruits of NIL 5M10 (polpa amarela em frutos parcialmente passados da LQI 5M10). J: Typical incidence of necrosis of the placental tissue in the exotic Korean accession Shongwan Charmi, PI 161375, or SC, Conomon Group, also detected at harvest (incidência típica de necrose no tecido placentário no acesso coreano exótico Shongwan Charmi, PI 161375, ou SC, grupo Conomon, também detectado na colheita); K: Necrosis of the placental tissue colonized by *Cladosporium* rot in NIL 5M10 (necrose do tecido placentário colonizado por podridão de Cladospório na LQI 5M10). Cartagena, UPCT, 2008.

The NIL 5M7 showed difficulty in completing the ripening process, which was delayed by 7-10 days compared to the climacteric NILs. This was revealed by the presence of blue greenish areas in the area of the skin which was in contact with the soil (in less than 4% fruit), indicating partial immaturity on this side of the fruit and particularly in the area contacted with the soil (Figure 1H). These symptoms were probably associated with a poor leaf status, and the difficulty experienced by the plant to obtain resources for the fruit.

Deep furrow netting inherited from SC in some NILs was also detected (Figure 1I), and also symptoms of healed split and stylar-end cracking (alone or with visually Cladosporium rot) in the climacteric NILs (Figures 2C and D). If fruit with moderate sunburn were stored, they usually developed Cladosporium rot (Figure 2E).

Some fruits also developed a diffuse light brown pulp (Figure 2F), rarely accompanied by external symptoms (Figure 2G), while other fruits developed a typical orange flesh color, characteristic of senescence and inherited from SC (particularly in NIL 5M4, Figure 2H), or yellow flesh typical from PS in 5M10 (Figure 2I).

Placental necrosis was an internal disorder detected at harvest and also after storage at 8°C. Necrosis was detected in NIL 5M3 (7% fruit with slight necrosis after storage) and 5M10 (6% fruit at harvest and 2% after storage) (Tables 1 and 2; Figures 2J and K), which we assume was inherited from SC (17% fruit affected at harvest and 5% after storage; Tables 1 and 2). This necrosis was previously reported in SC and in other NILs with a possible association with Cladosporium rot (Eduardo *et al.*, 2007; Fernández-Trujillo *et al.*, 2007), though this association was not always detected in recent seasons (data not shown).

It could be hypothesized that preharvest conditions can be associated with necrosis, and refrigerated storage promotes the colonization of the mentioned disorder by providing conditions suitable for saprophytic fungi. However, no symptoms of fungal growth or bacteria were detected in

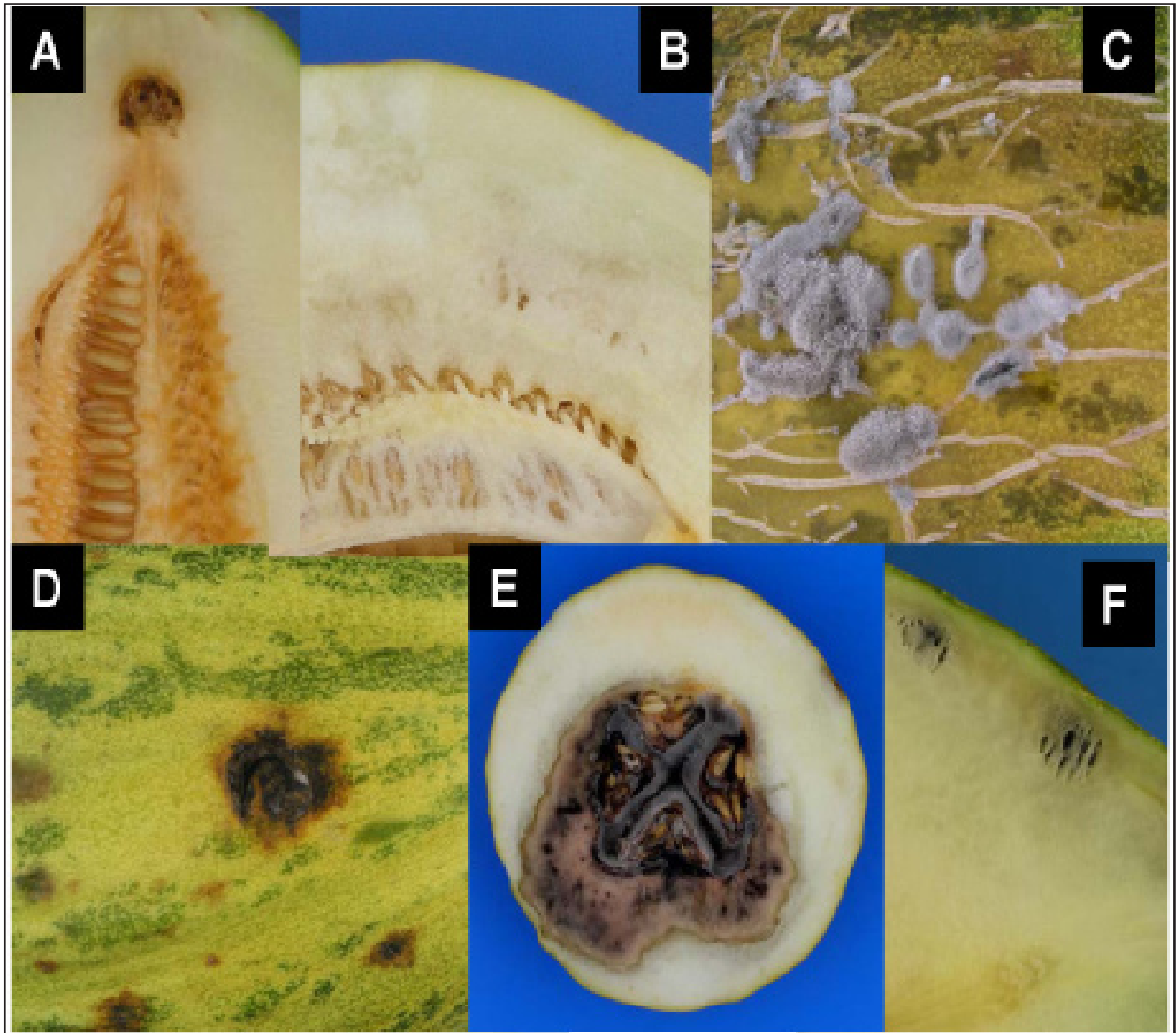


Figure 3. Skin and flesh disorders and decay in fruits of several near-isogenic lines (NIL) of melon and reference cultivars, after a 30-day storage period at 8°C (desordens e podridões da casca e polpa de frutos de várias linhagens quase isogênicas (LQI) e cultivares testemunhas de melão, após um período de 30 dias de armazenamento a 8°C); A: Detail of internal corky flesh in NIL 5M4, probably as a result of Alternaria rot (detalhe da polpa com textura de cortiça na LQI 5M4, provavelmente como resultado de podridão de Alternária); B: Hollow flesh disorder (HFD) in NIL 5M9 (polpa esponjosa na LQI 5M9); C: Grey mould rot in F1 hybrid of melon (Inodorus Group), cultivar Nicolás (mofo-cinza no híbrido F1 de melão (Grupo Inodoro), cultivar Nicolás); D: *Phytophthora phytophthora* on pitting in NIL SC3-5 (*Phytophthora phytophthora* causando lesões na LQI SC3-5); E: Internal *Cladosporium* rot in NIL 5M8 (podridão de Cladospório interna na LQI 5M8). F: Holes provoked by internal *Cladosporium* rot in NIL 5M5 (podridão de Cladospório interna na LQI 5M5). Cartagena, UPCT, 2008.

brown cells of the placental tissue and tissues surrounding (data not shown). The presence of the disorder in NIL 5M10 may indicate the presence of a QTL for necrosis not previously reported for SC3-5ab (Fernández-Trujillo *et al.*, 2007).

Other flesh disorders rarely found at harvest (Table 1) were corky dry texture with brown areas (Figure 3A) or the hollow flesh disorder in NILs 5M8,

5M9 and 5M10 (Figure 3B). The exact location of these disorders affecting small areas depended on the fruit section excised. The incidence of hollow flesh disorder in other NILs such as SC3-5ab at harvest or after storage (less than 10% fruits affected) suggests a higher presence of HFD in advanced stages of maturity or senescent fruit because it was only present in climacteric NILs (Fernández-Trujillo *et al.*, 2007). In cucumber, a similar disorder to HFD

is pillowy fruit disorder (PFD). PFD increases as a result of water stress under inadequate postharvest procedures (absence of hydrocooling after harvest or abuse of storage at high temperatures) that promote ripening and softening (Staub & Navazio, 1993; Navazio & Staub, 1994; Serce *et al.*, 1999;).

Stylar-end cracking after storage did not affect PS, but it mostly affected cultivar Fado (85% fruit) and 1-3%

Table 1. Fruit internal disorders at harvest in near-isogenic lines (SC3-5, 5Mx, x= 2 to 10) of melon, their parental lines, and reference cultivars (desordens internas em frutos na colheita em linhagens quase-isogênicas (SC3-5, 5Mx, x= 2 a 10) de melão, suas linhagens parentais e cultivares testemunhas). Cartagena, UPCT, 2008.

Pedigree	Replication	Total number of fruits	Internal flesh disorders (%)
SC3-5 ¹	7	18	0
5M2	9	16	0
5M3	7	14	14
5M4	7	15	7
5M5	7	14	21
5M6	7	4	0
5M7	7	14	0
5M8	7	14	0
5M9	9	21	6
5M10	9	18	6
PS ²	20	41	3±0.1 ⁶
ANOVA PS vs. NILs (Dunnett, $p<0.05$)			ns
SC ¹	11	23	17
Fado ³	7	13	0
Nicolás ⁴	6	13	0
Védrantais ⁵	8	17	2
ANOVA SC3-5, PS and cultivars (Tukey, $p<0.05$)			ns

¹Korean accession Shongwan Charmi (PI 161375), Conomon Group (acesso coreano Shongwan Charmi (PI 161375), grupo Conomon); ²PS= Cultivar Piel de Sapo parental line (linha parental da cultivar Piel de Sapo); ³*Cucumis reticulatus* cultivar Fado, Galia type (*Cucumis reticulatus* cultivar Fado, tipo Galia); ⁴*Cucumis melo* cultivar Nicolas, commercial F1 hybrid, Piel de Sapo type (*Cucumis melo* cultivar Nicolas, híbrido F1 comercial, Piel de Sapo); ⁵*C. melo* var. *cantalupensis* Naud, cultivar Védrantais (*C. melo* var. *cantalupensis* Naud, cultivar Védrantais); ⁶Means and standard deviation (média e desvio-padrão).

fruit of 5M2 or other climacteric NILs (Table 2). This level of cracking was in agreement with other results in Galia melons stored at ripening temperatures (Lima *et al.*, 2004).

Védrantais melons were dehiscent, leading to a rapid styler-end cracking when ripen in the field. Due to the softening at the fruit styler-end, the hardness of this area was measured. Fruit hardness limits for severe, moderate, slight, and very slight symptoms of fruit styler-end softening (in N·mm⁻¹) were <7, 7-12, 12-20, and 20-35, respectively. Fruit with fruit styler-end hardness values above 35 N·mm⁻¹ never showed fruit styler-end cracking, nor showed over-ripening symptoms. Fruit styler-end cracking developed at hardness values below 20 N·mm⁻¹ (data not shown).

Styler-end cracking was only

associated with the presence of climacteric behavior (Table 2), but whole fruit cracking was apparently the result of a genotype × irrigation interaction, as occurs in watermelon (Abdel-Al, 1975). The cracking in cultivar Védrantais was probably associated with the sudden onset of ethylene production in the beginning of climacteric (Flores *et al.*, 2007), which was impossible to control during cultivation practices, such as fertigation management, in an uniform plot containing NILs and reference cultivars.

Styler-end cracking later resulted in *Fusarium* sp. or bacterial soft rot when NILs were stored at 10°C or 21°C (data not shown). The fruit styler-end cracking (healed split in the first steps of the cracking) or water-soaking disorder are common commercial defects in melon (Fernández-Trujillo *et al.*, 2007), but only in 5M2 this vitreous texture was

observed (data not shown).

Uncommon rots detected after 30 days of storage at 8°C (absent or ranging from 2 to 5% in the NILs) sometimes showed higher levels in reference cultivars, but not in PS, and were as follows (Table 2; data not shown): grey mould rot caused by *Botrytis* sp. Pers. (detected after storage in NIL 5M10 and cultivars SC and Nicolás; Figure 3C); cottony leak, caused by *Phytium* sp. (on small pitted areas after storage in NIL SC3-5 and in PS; Figure 3D); bacterial soft rot located in NILs SC3-5, 5M4, and 5M9; *Penicillium* sp. Link. (in NIL 5M5 and cultivar SC, as a secondary fungi); *Fusarium* sp. (particularly on cultivar SC after three weeks of storage or more); and *Acremonium* sp. affecting the placental tissue (in NIL 5M10 and cultivar SC).

Internal Cladosporium rot covering the placental tissue (Figure 3E) or forming small internal cavities close to the skin (Figure 3F) was rarely detected. Internal Cladosporium rot affected the pulp tissue as a small cavity close to the epidermis (in a few fruit of NILs 5M8 or 5M10), and was usually concomitant with external observations of brown and soft skin. Sometimes it caused a slight decay mostly detected at the peduncle or in netted areas. This internal Cladosporium rot, associated or not with placental necrosis (Figure 3E), was externally undetectable except in advanced stage of development when it was revealed by a typical smell to soil.

Internal cavities with Cladosporium rot (Figure 3F) were probably associated with skin or peduncle lesions (due to sun radiation, sunburn, etc.) caused by a micro cracked epidermis during fruit development and storage. From this point of view, peduncle dehiscence, skin susceptibility to sunburn, etc., would modulate the onset of Cladosporium rot. In fact, our hypothesis is that *Cladosporium* sp. is probably unable to cause skin damages in melon because cannot metabolize it and so only would proliferate with difficulty in the pulp under microaerobic conditions.

Aerial hyphae can only proliferate when skin damages are evident. This is necessary to form the conidiospores bearing conidia to spread the fungus.

Table 2. Losses and physiological disorders in fruits of near-isogenic lines and other cultivars of melon, after a 30-day storage period at 8°C, except for fruits of cultivars Fado and Vêdrantais, stored for 20 and 25 days, respectively (perdas e desordens fisiológicas em frutos de linhagens quase-isogênicas e outras cultivares de melão após um período de armazenamento de 30 dias a 8°C, exceto para frutos das cultivares Fado e Vêdrantais, armazenados por 20 e 25 dias, respectivamente). Cartagena, UPCT, 2008.

NIL or cultivar	SC3-5	5M2	5M3	5M4	5M5	5M6	5M7	5M8	5M9	5M10	PS ¹	P ²	SC ³	Fado ⁴	Nic ⁵	Ved ⁶
Replicates	7	9	7	7	7	7	7	7	9	9	20	-	8	5	7	8
<i>Fusarium</i> rot (%)	2a	0	0	0	1	2	0	0	0	0	0a	ND	8a	13a	0a	0a
<i>Penicillium</i> rot (%)	0a	0	0	0	2	0	0	0	2	4	0a	ND	5a	0a	14a	0a
<i>Botrytis</i> rot (%)	0a	0	0	0	0	0	0	0	1	3	0a	ND	9a	0a	0a	2a
Bacterial soft rot (%)	2a	0	0	1	0	0	0	0	1	0	0a	ND	0a	0a	0a	0a
Net cracking (%)	1.6a	8.2	3.9	2.1	0.0	0.0	2.0	6.7	0.0	1.9	4±4a ⁷	NS	0b	0b	0b	0b
Skin wartness (%)	0.0a	2.3	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	1±1a ⁷	NS	0a	0a	5a	0a
Stylar-end crack-ing (%)	3.4a	2.5	0	2.1	0	0	0	0	3.4	1.1	0a	ND	0a	85b	0a	4a
Necrosis of the placental tissue index (%) ^u	0a	0	0.9	0	0	0	0	0	0	0.4	0a	ND	5b	0a	0a	0a

Means followed by the same letter in the column did not differ significantly from each other, Tukey Test, $p < 0.05$ (médias seguidas de mesma letra na coluna não diferem significativamente entre si, teste de Tukey, $p < 0.05$); ND= not determined because PS was free from the disorder or rot (não determinado porque PS não apresentou a desordem ou podridão); ¹PS= cultivar Piel de Sapo parental line (linhagem parental da cultivar Piel de Sapo); ²P= probability for the Dunnett test (probabilidade de acordo com o teste de Dunnett); ³SC= Korean accession Shongwan Charmi (PI 161375), Conomon Group (acesso coreano Shongwan Charmi (PI 161375), grupo Conomon); ⁴Cucumis *reticulatus* cultivar Fado, Galia type (*Cucumis reticulatus* cultivar Fado, tipo Galia); ⁵Cucumis *melo* cultivar Nicolas, commercial F1 hybrid, Piel de Sapo type (*Cucumis melo* cultivar Nicolas, híbrido F1 comercial); ⁶C. *melo* var. *cantalupensis* Naud, cultivar Vêdrantais (C. *melo* var. *cantalupensis* Naud, cultivar Vêdrantais); ⁷Means and standard deviation (média e desvio-padrão).

Most of the microorganisms reported in postharvest are common in Cucurbitaceae or other fruit species around the world, and cause grey mould rot or Cladosporium rot (Martínez & Fernández-Trujillo, 2007; Snowdon, 1991). *Alternaria* sp., *Rhizopus* sp., *Fusarium* sp., *Stemphylium* sp., *Trichothecium* sp., *Penicillium* sp., *Aspergillus* sp., *Epicoccum* sp., *Erwinia*, and *Pseudomonas* bacteria damages have also been reported in previous studies (Ceponis *et al.*, 1986; Snowdon, 1991; Chen *et al.*, 2001; Morris *et al.*, 2001; Albuquerque *et al.*, 2005). This is while, internal Cladosporium rot is not common and only *Alternaria* and Cladosporium rot are observed in cold stored Piel de Sapo type and *Fusarium* rot and Mucor rot in other C. *melo* Inodorus Group (Ceponis *et al.*, 1986; Snowdon, 1991; Yang *et al.*, 2003; Albuquerque *et al.*, 2005).

The importance of rare microorganisms in melons such as *Penicillium expansum*, in spite of being restricted to the fruit surface,

resides in potentially increasing fruit susceptibility to later infection by pathogenic microorganisms (Richards & Beuchat, 2005).

The uncommon physiological disorders and melon defects reported herein may also be attributed partially to the differences in genetic background and the different behavior of the progenitors and/or the ethylene insensitivity conferred by the recessive allelic forms of SC (PÉRIN *et al.*, 2002). NILs were efficient at revealing dissect complex traits, including disorders and decay (Eduardo *et al.*, 2007; Fernández-Trujillo *et al.*, 2007). However, some of them such as hollow flesh were rarely found in the main NIL collection and were not detected in PS. Many of the defects reported herein may be of importance in some degree from a processing and appearance point of view, but clearly introduced greater variability in fruit internal quality. However, some of the defects may be an indication of a deteriorated fruit quality that renders the fruit useless

for further processing (e.g. fresh-cut fruit, cremogenates, etc.) (Cantwell & Portella, 1998). These minor disorders can be incorporated in future “expert systems” for the automatic diagnosis of melon disorders (Latin *et al.*, 1990). New NILs for fine mapping are required in order to confirm the results of every disorder.

In summary, preharvest losses were mainly due to fruit cracking and, to a lesser extent, bacterial soft rot, over-ripening, and skin sunburn. The NILs with climacteric behaviour were more susceptible to over-ripening, skin sunburn, hollow flesh disorder and stylar-end cracking, accompanied by softening as measured by hardness values below 20 N·mm⁻¹ in this zone. Other uncommon disorders such as warted skin, flesh discoloration to light brown or translucent areas were not associated with the climacteric pattern.

Necrosis of the placental tissue in two NILs and deep furrow netting were inherited from the Korean accession SC but were unrelated with climacteric

behavior. Some uncommon decays such as internal Cladosporium rot, seems to be associated with skin deterioration. The incidence of the uncommon disorders or the rots described was too low to confirm the influence of a possible genetic component in their development.

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