New Cucumis Rootstocks for Melon: ‘UPV-FA’ and ‘UPV-FMy’

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Grafting plants onto resistant rootstocks is a cultural practice spread worldwide to cope with biotic and abiotic stresses, such as fungal diseases, nematodes, drought, salinity, and extreme temperatures. For Cucurbitaceae crops, the most common rootstocks are interspecific hybrids between Cucurbita maxima Duchesne and Cucurbita moschata Duchesne (Davis et al., 2008). Besides their tolerance to biotic and abiotic stresses, hybrid Cucurbita rootstocks are preferred because they show good emergence performance and develop long and thick hypocotyls that facilitate grafting. These hybrids have however some important shortcomings. Their excess of vigor can cause a delay in the flowering and ripening processes in grafted plants (Soteriou et al., 2016), they are not resistant to nematodes (Cohen et al., 2014; Özarslan and Özarslan, 2011), and often they have negative impacts on fruit quality (Guan et al., 2015; Roupael et al., 2010; Soteriou et al., 2014). These effects on quality are dependent on the rootstock–scion interactions. In melon (Cucumis melo L.), for example, an increase of fruit size and seed cavity (Verzera et al., 2014) or modifications of flesh firmness (Colla et al., 2006; Zhao et al., 2011) as a result of grafting are common. Flesh color variations (Colla et al., 2006), vitrescence (Jang et al., 2014), and changes in pH and soluble solids content (Colla et al., 2006; Verzera et al., 2014) have also been reported.

In part because of these quality problems, grafting is less common in melon than in watermelon [Citrullus lanatus (Thunb.) Mansf.] and cucumber (Cucumis sativus L.). However, in recent years, the withdrawal of methyl bromide (a broad spectrum pesticide highly efficient against soilborne pathogens), and the increment of global temperature, have been favoring the spread of nematodes and fungi highly damaging for melons, such as Fusarium spp., Monosporascus spp., Macrophomina phaseolina (Tassi) Goidanich and Podosphaera xanthii (Castagne) Braun & Shishkoff. The global warming has also a negative impact on soil salinization and drought. Consequently, the use of grafted melon plants has gained increased attention. The use of rootstocks belonging to the same species/genus as the scions could minimize the quality problems observed when using Cucurbita hybrids as rootstocks. Even though some promising melon accessions have been selected and used to develop Fusarium oxysporum Schlechtend. Fr. f. sp. melonis (Leach & Currence) Snyder & Hans spp. and Monosporascus cannonballus Pollack & Uecker resistant rootstocks (Condurso et al., 2012; Fredes et al., 2016; Verzera et al., 2014), the assessment of a new germplasm that may provide a wider range of resistances without decreasing the fruit quality is a new challenge for melon grafting.

Apart from intraspecific variation, intragenic variation can also be exploited in melon grafting. The genus Cucumis includes a large number of African, Asian, and Australian wild species (Den Nijs and Visser, 1985; Renner et al., 2007; Sebastian et al., 2010; Singh and Yadava, 1984). Strong reproductive barriers prevent the use of these resources for melon breeding (Chen and Adelberg, 2000), but the genus is potentially a good source of new rootstocks. Resistance to nematodes and to soilborne and aerial fungi has been reported in some Cucumis species. To date, two species have been assayed as rootstocks: Cucumis metuliferus Naudin (Gisbert et al., 2017; Guan et al., 2014; Kokalis-Burell and Rosskopf, 2011; Nisini et al., 2002; Sigüenza et al., 2005) and Cucumis pulsatulus Naudin ex Hook.f. (Liu et al., 2015). Despite their effectiveness in the management of soil pathogens, lower soluble solids content and softer flesh have been observed in fruits from plants grafted onto C. metuliferus (Guan et al., 2014; Nisini et al., 2002). Other Cucumis species useful as sources of resistance to nematodes, Fusarium spp., or both are C. ficifolius A.Rich., C. zeyheri Sond., C. africanus L.f., C. anguria L., and C. myriocarpus Naudin (Den Nijs and Custers, 1990; Nisini et al., 2002). One of the main difficulties when using these wild species is the reduced size and vigor of the seedlings. Differences in hypocotyl diameters between the rootstock and the scion at the grafting union, either at the time of grafting or later during the development of the grafted plants (Pofu and Mashele, 2011; Pofu et al., 2013), can result in physiological collapse of melon plants under field conditions. Vigor of wild rootstocks can be increased by using interspecific hybrids, which have the additional advantage of combining resistances or other suitable traits from both parental lines. Although hybridization between different Cucumis species is extremely difficult, some successful crosses are possible (Kho et al., 1980; Matsumoto et al., 2012; Walters and Wehner, 2002), especially between some wild related African species, which are phylogenetically relatively close (Den Nijs and Visser, 1985; Sebastian et al., 2010).

The research groups of “Cucurbitis” and “Grafting Breeding” at COMAV-UPV (Centro de Conservación y Mejora de la Agrodiversidad Valenciana-Universitat Politècnica de València) are working together in the development and characterization of new experimental rootstocks for cucurbit crops (Fredes et al., 2017; Gisbert et al., 2016). Germplasm characterization and crossability studies have been performed within the Cucumis genus to generate vigorous rootstocks suitable for melon. Two new interspecific Cucumis hybrids with good characteristics, to be used as melon rootstocks have been developed: ‘UPV-FA’ (C. ficifolius × C. anguria) and ‘UPV-FMy’ (C. ficifolius × C. myriocarpus). Both hybrids have good compatibility with cultivars of the two main melon market classes (muskmelon and Piel de Sapo), are resistant to some of the main fungi that are pathogenic to melon, and do not negatively modify melon fruit quality.

Origin

The two new interspecific hybrids ‘UPV-FA’ and ‘UPV-FMy’ were obtained by crossing three wild African species: Cucumis ficifolius (F) (accession BGV12786) as female parent and the two other wild species as male parents, Cucumis anguria var. longipes (A) (accession BGV12795) and Cucumis myriocarpus (My) (accession BGV008535). The three accessions were held at the COMAV’s Genebank. Hybriddizations were
conducted in a greenhouse at the UPV, under controlled conditions during the spring-summer season of 2015. Direct and reciprocal crosses were performed in all cross combinations, but fruit set was only obtained using C. ficifolius as female parent. Results are in accordance with previous studies, reporting that these three species are in the same compatibility group (Singh and Yadava, 1984), and with the occurrence of some form of unilateral incongruity between C. ficifolius and other African species such as C. anguria, C. myriocarpus, C. zeyheri, C. dipscus Ehrenb. ex Spach., and C. figarei A.Rich. (Den Nijs and Visser, 1985). The fruit set percentage was around 70% in both hybrids, quite similar to the value of 80% found in the self-pollinations of the parentals. Also, the average number (± se) of viable seeds per fruit found in the hybrid fruits (227.3 ± 9.9 and 132.7 ± 17.1 for ‘UPV-FMy’ and ‘UPV-FA’, respectively) was similar or even higher than the number found in fruits derived from selfings (164.3 ± 32.8, 64.3 ± 14.5, and 129.3 ± 5.2 for F, My, and A, respectively). The germination capacity of the hybrid seeds was tested under different experimental conditions, reaching 100% in treatments with cold stratification (Cáceres et al., 2016a). The hybrid seeds germinated earlier in comparison with their respective parentals (Cáceres et al., 2016a). Hypocotyl diameters were measured in the germinated seedlings at 21 d after sowing. The diameters of ‘UPV-FMy’ (0.28 ± 0.0062 cm) and ‘UPV-FA’ (0.25 ± 0.0075 cm) were higher than those of the wild parents (0.22 ± 0.0068, 0.24 ± 0.0033, 0.24 ± 0.0061 cm for F, My, and A, respectively). Seedlings were transplanted to the greenhouse, and the hybrid nature was confirmed using discriminant taxonomic traits between the parentals, such as the presence of aculei in stems and petioles, the ratio between the hyaline and opaque parts of aculei of ovaries, and the shape of ovaries and fruits (Kirkbride, 1993). Intermediate phenotypes between those of the parentals were observed for these characters, which support their hybrid nature (Fig. 1; Cáceres et al., 2016b).

The good cross compatibility of these two interspecific crosses, along with the higher germination rates and wider hypocotyls found in the hybrids compared with their parents, confirms the advantage of using interspecific hybrids instead of single wild Cucumis genotypes for grafting purposes.

Responses to Biotic and Abiotic Stress

Wild Cucumis species have been reported to possess resistance to several fungal diseases (Matsumoto et al., 2011; Thomas and More, 1990). Among these diseases, fusarium wilt, caused by F. oxysporum f. sp. melonis, is one of the most severe for melons worldwide. Both hybrids and their respective parents were confirmed as resistant to fusarium wilt by artificial inoculation of F. oxysporum f. sp. melonis race 1.2 that was performed in a growth chamber following the protocol described by Perchepied and Pitrat (2004). At 20 d after...
Table 1. Plant fresh and dry weights and root parameters of ‘UPV-FA’ (C. ficifolius × C. anguria), ‘UPV-My’ (C. ficifolius × C. myriocarpus), and C. metuliferus after 45 d of culture on BM medium with sorbitol (0, 0.05, 0.1, and 0.2 M).

| Rootstock | Sorbitol (M) | Plant fresh wt (g) | Plant dry wt (g) | Root length (mm) | Tip number | Root diam (mm) |
|-----------|-------------|-------------------|-----------------|-----------------|------------|---------------|
| ‘UPV-FA’  | 0           | 1.05 a            | 0.069 a         | 68.43 ab        | 169.86 ab  | 0.36 b        |
|           | 0.05        | 0.94 ab (–10.3%)  | 0.061 ab (–11.9%) | 91.10 a (+33.1%) | 220.28 a (+29.7%) | 0.36 b (+0.8%) |
|           | 0.1         | 0.50 d (–52.2%)   | 0.04 c (+41.8%)  | 58.32 bc (–14.8%) | 168.43 ab (–0.8%) | 0.37 ab (+3.1%) |
|           | 0.2         | 0.04 f (–96.0%)   | 0.008 c (–88.3%) | —               | —          | —             |
| ‘UPV-My’  | 0           | 0.73 c (–41.0%)   | 0.071 a          | 58.91 bc         | 131.86 bc  | 0.34 b        |
|           | 0.05        | 0.74 c (–17.2%)   | 0.055 b (–22.2%) | 73.11 ab (+24.1%) | 195.07 ab (+47.9%) | 0.36 ab (+8.0%) |
|           | 0.1         | 0.30 c (–64.2%)   | 0.027 d (–62.1%) | 34.62 d (–41.2%) | 78.26 cd (–40.7%) | 0.33 b (+2.5%) |
|           | 0.2         | 0.04 f (–95.1%)   | 0.006 c (–92.2%) | —               | —          | —             |
| C. metuliferus | 0         | 0.80 bc           | 0.059 ab         | 41.34 cd         | 97.92 cd   | 0.41 a        |
|           | 0.05        | 0.29 c (–63.9%)   | 0.023 d (–60.6%) | 19.20 de (+53.6%) | 41.75 de (+57.4%) | 0.38 ab (+9.0%) |
|           | 0.1         | 0.06 f (–92.9%)   | 0.007 c (+88.6%) | 3.35 c (+91.9%)  | 11.00 (–88.6%) | 0.32 b (+21.6%) |
|           | 0.2         | 0.02 f (–97.7%)   | 0.003 c (–95.0%) | —               | —          | —             |

| Number of fruit/plant | NG | Grafted onto ‘UPV-FA’ | Grafted onto ‘UPV-My’ | Grafted onto ‘UPV-My’ |
|-----------------------|----|-----------------------|-----------------------|-----------------------|
| Fruit weight (g)      | 727.08 a | 686.64 a              | 659.90 a              | 659.90 a              |
| Fruit length FL (cm)  | 10.56 a  | 10.29 a               | 10.39 a               | 10.39 a               |
| Fruit width FW (cm)   | 11.28 a  | 11.23 a               | 10.97 a               | 10.97 a               |
| Fruit shape (FL/FW)   | 0.94 b   | 0.92 a                | 0.95 b                | 0.95 b                |
| Cavity width (cm)     | 12.30 a  | 12.76 a               | 12.72 a               | 12.72 a               |
| Rind firmness (kg/cm²) | 2.77 a  | 2.77 a                | 2.78 a                | 2.78 a                |
| Total soluble solids (°Brix) | 12.33 a | 13.32 b               | 12.34 a               | 12.34 a               |
| pH                    | 6.14 a   | 6.29 a                | 6.21 a                | 6.21 a                |
| Hunter L              | 53.95 a  | 53.83 a               | 53.38 a               | 53.38 a               |
| Hunter a              | 11.36 a  | 12.16 b               | 11.24 a               | 11.24 a               |
| Hunter b              | 22.10 a  | 22.92 a               | 22.15 a               | 22.15 a               |

Table 2. Characteristics of muskmelon fruits harvested from nongrafted plants (NG) and plants grafted onto the experimental hybrids ‘UPV-FA’ (C. ficifolius × C. myriocarpus) and ‘UPV-My’ (C. ficifolius × C. anguria).

The two new putative rootstocks were also evaluated against osmotic stress using clones from plants maintained in vitro. These clones were grown on basal medium (4.4 mg L−1 Murashige and Skoog salts, including vitamins, 2% sucrose, and 0.75% plant agar), containing sorbitol at four different concentrations: 0, 0.05, 0.1, and 0.2 M. The addition of compounds such as sorbitol to culture medium reduces the water potential, so it is harder for plants to uptake water, simulating water-deficient conditions in soil (Verslues et al., 2006). The Piel de Sapo Pinonèt cultivar showed great growth inhibition in a previous assay in medium with sorbitol after 45 d of culture (the fresh weight of plants was reduced 49% and 92% in media with sorbitol at 0.05 and 0.1 M, respectively). Cucumis metuliferus, which is another wild species used as putative rootstock for melon (Gisbert et al., 2017; Guan et al., 2014; Kokalis-Burelle and Roskopf, 2011; Nisini et al., 2002; Sigüenza et al., 2005), was also included in the assay conducted to evaluate ‘UPV-FA’ and ‘UPV-My’. Differences in vine growth and rooting inhibition were observed after 30 and 45 d (Table 1). After these periods, plant fresh and dry weights were noted. Root characteristics, number of tips, and root diameter were measured using the WinRhizo’s software. At the lowest sorbitol concentration (0.05 M), vine growth and root development were inhibited in C. metuliferus after 30 d of culture, whereas both hybrids ‘UPV-FA’ and ‘UPV-My’ only showed a mild vine growth inhibition. This behavior was maintained after 45 d in culture, even at the intermediate sorbitol concentration (0.1 M) (Table 1). Rooting was not observed in any genotype in culture medium with 0.2 M sorbitol. This result was expected as no growth was also observed in other works using this very high concentration (Claeys et al., 2014). Of the two hybrids, ‘UPV-FA’ was more tolerant than ‘UPV-My’ (Table 1), with sorbitol causing less root length and branching reduction. A good root development is an important trait for putative rootstocks.

Performance of Rootstocks

The performance of ‘UPV-FA’ and ‘UPV-My’ as rootstocks was evaluated with two different melon scions. A first evaluation was performed in a greenhouse under hydroponic conditions, using a muskmelon Charentais as scion. Plants were grafted using the cleft procedure (Lee et al., 2010), and high rates of success were obtained (~90%). The responses of muskmelon plants grafted onto...
Table 3. Marketable yield and quality traits of melon fruits cv. Finura (Piel de Sapo) harvested from nongrafted plants (NG), self-grafted plants (SG), and plants grafted onto the experimental Cucurbita (C. maxima × C. moschata) hybrid F1, the commercial Cucurbita hybrid ‘Cobalt’, the commercial Cucumis melo rootstock ‘64-376RZ’, and two new experimental Cucumis F1: ‘UPV-FMy’ (C. ficifolius × C. myriocarpus) and ‘UPV-FA’ (C. ficifolius × C. anguria).

|                      | NG          | SG          | F1 ‘Cobalt’ | ‘64-376RZ’ | ‘UPV-FMy’ | ‘UPV-FA’ |
|----------------------|-------------|-------------|-------------|------------|-----------|----------|
| Marketable fruit yield/plot (kg) | 32.33 a     | 27.78 a     | 26.95 a     | 26.86 a    | 24.33 a   | 22.91 a  |
| Fruit weight (g)     | 2.502.27 ab | 2.578.25 ab | 2.960.15 c  | 2.806.50 bc| 2.351.94 a| 2.513.56 ab|
| Fruit length FL (cm) | 22.74 a     | 22.82 a     | 23.71 ab    | 24.14 b    | 22.45 a   | 23.39 ab  |
| Fruit width FW (cm)  | 15.31 a     | 15.32 a     | 16.28 c     | 15.71 bc   | 14.63 a   | 15.18 ab  |
| Fruit shape (FL/FW)  | 1.49 ab     | 1.49 ab     | 1.46 a      | 1.54 b     | 1.53 b    | 1.54 b    |
| Rind thickness (mm)  | 5.13 ab     | 5.44 b      | 4.62 a      | 5.42 b     | 5.46 b    | 4.68 ab   |
| Flesh thickness (cm) | 37.13 ab    | 38.92 ab    | 35.61 a     | 41.17 b    | 36.58 ab  | 36.59 ab  |
| Cavity width (cm)    | 5.76 a      | 5.77 a      | 7.22 c      | 6.03 ab    | 5.57 a    | 6.47 b    |
| Rind firmness (kg-cm⁻²) | 13          | 13          | 13          | 13         | 13        | 13        |
| Flesh firmness (kg-cm⁻²) | 2.15 a     | 2.41 a     | 2.24 a      | 2.60 a     | 2.48 a    | 3.26 b    |
| Total soluble solids (°Brix) | 11.93 ab   | 12.13 ab    | 13.59 c     | 12.56 b    | 12.41 b   | 11.23 a   |
| pH                   | 5.75 ab     | 5.78 ab     | 5.97 bc     | 6.09 c     | 5.50 a    | 5.59 a    |
| Hunter L             | 63.65 a     | 63.30 a     | 64.99 ab    | 68.30 b    | 66.11 ab  | 66.81 ab  |
| Hunter a             | -2.63 a     | -2.48 a     | -1.86 b     | -2.88 a    | -2.67 a   | -2.57 a   |

Different letters in the same row indicate significant differences according to the Duncan’s multiple range test ($P \leq 0.05$).

Average weight for the 16 fruit per treatment (four fruit/plot) used for quality characterization.
hybrids do not cause fruit size increase and only ‘UPV-FA’ slightly increased candy thickness, both effects commonly observed with Cucurbita hybrids rootstocks. An additional value of these rootstocks is their good tolerance to osmotic stress, especially in ‘UPV-FA’. Also, their tolerance to powdery mildew can facilitate their management during rootstock seed production, and as it has been reported in other crops (Guán et al., 2012), it can contribute to improve the scion response to this disease.

Availability

Small trial seed samples of all the breeding lines are available for research purposes (please contact authors).

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