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Short note

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Determining the main agronomic traits of snake melon (*Cucumis melo* var. *flexuosus* L.) fruits as affected by genotypic differences

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: The variability affecting the main agronomic traits of 10 snake melon genotypes (*Cucumis melo* var. *flexuosus* L.) (three F1 hybrids, six breeding lines and the widely grown and consumed traditional cultivar Mornagui), grown under greenhouse conditions during 2014 and 2015 seasons, was examined and compared. Their resistance to powdery mildew was also checked. The main production-driving agronomic traits varied significantly ($P<0.05$) depending on the genotype. The major skin colour determined visually was different among the considered genotypes. The hybrids (H5 and H12) and the breeding lines (L1, L2, L5, L6 and L9) had dark green skin. The fruits of the hybrid H7 were dark and light green and the breeding line L4 has intense and dark green fruits whereas cv. Mornagui was characterized by medium light green fruits. Regarding fruit shape, the hybrids H7 and H5 produced straight fruits whereas the hybrid H12 and the breeding lines; L2, L4, L5, and L9 and the cv. Mornagui had elongate fruits. However both breeding lines L1 and L6 were characterized by straight elongate fruits. The F1 hybrid H5 was the most productive (3.6 Kg/plant and ≈ 21 fruits/plant for total yield and number of fruit/plant respectively). These findings are useful for further breeding programs aiming to develop new powdery mildew resistant snake melon cultivars with satisfying agronomic traits.

1. Introduction

Snake melons (*Cucumis melo* var. *flexuosus* L.), belong to the Cucurbitaceae family largely distributed and consumed since the antiquity across large geographical area (Pandey *et al.*, 2010; Paris, 2012). The *flexuosus* distinguishes from other *C. melo* varieties by the twisted long to very long fruits, typically exceeding the 4:1 length-to-width ratio, characterized by a slightly hairy white or light green exocarp (rind) often furrowed by more or less deep grooves running lining the fruit surface. The rind covers a creamy white or pale green flesh (mesocarp and endocarp) containing numerous whitish edible seeds, deliquescent at maturity. Because the stem is rather thin, the plant is usually supported on trellises, where the snake-like fruits grow on vines attaining up to 120 cm lengths (Burger *et al.*, 2010).

Snake melon fruits are known all around the world with different trivial names: "alficoz" in Spain, "tortarello" or "cetrangolo" in Italy, "fakous" or "fegous" in Arabic Maghreb countries, "agoor" in Soudan, "acur", "hıtta" or "hıtı" in Turquie, "kakri" in India, "uri" in Japan and Armenian cucumber, "yard-long melon", "serpent-melon" or "Gutah" elsewhere (Solmaz *et al.*, 2016). Snake melon fruits are generally consumed raw at the immature stage of ripening with a preference for straight long and thick green fruits in the Mediterranean region. Here, this crop has been appreciated since antiquity for their crisp texture and refreshing, slightly acidic and non-sweet flavour, as revealed by their recurrent depiction in wall painting, sculptures and mosaics dating back to ancient Egypt and Roman Empire, as well as by the presence of references in the Islamic and Jewish literature (Paris *et al.*, 2011; Paris, 2012). The fruits are stomachic and seeds are also traditionally utilized as antitussive, digestive, febrifuge and vermifuge (Duke and Ayensu, 1985).

Besides the differences in skin colour and in the longitudinal furrowing of the fruits, snake melons genotypes differ also in various productivity-driving and quality traits including early marketable and total yield, numbers of fruits per vine and resistance to main pest diseases (eg. fusarium and powdery mildew) (Pandey *et al.*, 2010). Powdery mildew is the main fungal disease affecting similarly greenhouse and open-field grown cucurbits and particularly snake melon. This disease is easily, recognizable by the whitish powdery fungal growth developing on many parts of the plant and fruits (Sitterly 1978; Zitter *et al.*, 1996) . The disease is primarily caused by two fungal species around the world: *Golovinomyces orontii* and *Podosphaera xanthii* and are considered as an important limiting factor for snake melon production (Fernández-Ortuño *et al.*, 2006; Bellón-Gómez *et al.*, 2015). Therefore, growers and breeders are increasingly looking for resistant genotypes in order to overcome this problem although these fungal species are developing also new strains more virulent.

Previous research on snake melons mainly focused on the effect of salinity, sowing period and harvesting intervals on fruit yield, the phenological phases and morphological traits of the plants, the influence of different rootstocks on production in soilless cultivation, the *in vitro* plant regeneration, seed priming, the variability among genetic, morphological, vegetative, fruit and yield parameters of germplasm, the impact of breeding hermaphrodite lines on yield and, least but not last, the identification of fruit morphological and quality traits by QTL mapping (Reviewed in Ilahy *et al.*, 2019).

Genotypic differences strongly influence the microbial community composition in the rhizosphere (Aydi-Ben-Abdallah *et al.,* 2019). Pre- and post-harvest manipulations also significantly impact quality traits of horticultural crops (Siddiqui *et al.*, 2015, Siddiqui and Singh, 2015; Ilahy *et al.*, 2018). Furthermore, in snake melon genotypes carrying resistance to powdery mildew, the quality traits might be affected with respect to ordinary and susceptible genotypes. Thus, this study investigates the genotypic variability affecting the main agricultural traits of different snake melon genotypes (three F1 hybrids, six breeding lines and a traditional reference cv. Mornagui) grown under greenhouse condition. The overall aim is to select the most promising genotypes (in terms of agronomic traits and mildew resistance), to be used in further snake melon breeding programs

2. Materials and Methods

Plant material

Ten powdery mildew resistant (PMR) snake melon genotypes were used in our study (Three hybrids; H7, H5 and H12), six breeding lines; L1, L2, L4, L5, L6 and L9) and the largely grown and consumed snake melon cultivar Mornagui. The breeding lines were heirlooms landraces previously selected for their higher productivity and powdery mildew resistance levels as well as desirable agricultural traits generally preferred by the consumer (length and skin colour).

The (F1) hybrids were developed in a line x line mating design, in which parents characterized by high levels of powdery mildew resistance (L2 and Mornagui), were crossed with the selected breeding lines (L1, L5 and L6) characterized by good powdery mildew resistance coupled with high productivity and desirable phenotypic traits. The breeding lines and the obtained hybrids were exchanged with different laboratory working on the same topics in Italy, Hungary and India.

Plug trays were used to grow snake melon seedlings at the beginning of December 2013 and 2014. Four weeks later seedlings were transferred into a sandy soil (Fig. 1) under unheated water-impermeable plastic screens greenhouse, recommended for growing vegetable crops particularly cucurbits during winter cycles, using 125 and 150 cm as in- and between-row separations respectively at the region of Teboulba (35°63'N, 10°95'E) in the coastal zone of Tunisia. The experimental design was a randomized complete block design (RCBD) with three blocks (replication). Each replication consisted of 10 plants per genotype. They were subjected to agricultural practices commonly adopted by high-yielding farmers in this region. The production methods were in accordance with those reported recently in Ilahy *et al.* (2019).

Fig. 1 - Overview of the considered snake melon trial.

Determination of the agronomic characteristics

Powdery mildew resistance was determined on the basis of visual evaluation of the developed symptoms. Fruits were harvested weekly at the immature stage of ripening. Yield was determined following counting and considering all fruits on each plant. Early yield (agricultural output discriminating cultivars with precocious production with respect to others) and total yield [Kg of fresh weight (FW)/plant], number of fruit per plant (N. fruits/plant), average fruit weight (g) were determined on all the trials plants. In addition, fruit length and diameter were also recorded for all fruits. Fruit length and diameter (cm) were determined using a Vernier caliper.

Experimental design and statistical analysis

The effect of genotypic differences on the agronomic traits of snake melon was assessed by analysis of variance (ANOVA). When a significant difference was detected, means were compared using the least significant difference (LSD) test ($p<0.05$). All statistical analysis was performed using SAS Version 6.1 software (SAS Institute, Cary, NC, USA). Agglomerative Hierarchical Cluster analysis was used to determine differences and similarities among the genotypes, and the distance measure used was Euclidean distance computed between each population by the Ward method.

3. Results and Discussion

The main agronomic traits of the snake melon hybrids and breeding lines grown under greenhouse during the productive seasons 2014 and 2015 are reported in Table 1. Pooled data of the two years were analyzed. Plants of the different genotypes exhibited visually an important vigour with exceptional foliage cover (Figs. 1, 2).

The major skin colour was visually different among the evaluated genotypes. The hybrids H5, and H12 and the breeding lines L1, L2, L5, L6 and L9 had dark green skin (Fig. 2). The hybrid H7 was dark and light green and the breeding line L4 has intense and dark green fruits whereas cv. Mornagui was characterized by medium light green fruits. Regarding fruit form, the hybrids H7 and H5 produced straight fruits and H12, L2, L4, L5, 'Mornagui' and L9 had elongate fruits. However both breeding lines L1 and L6 were characterized by straight elongate fruits.

The early and total yields (expressed as Kg /plant), the number of fruits per vine, as well as the average

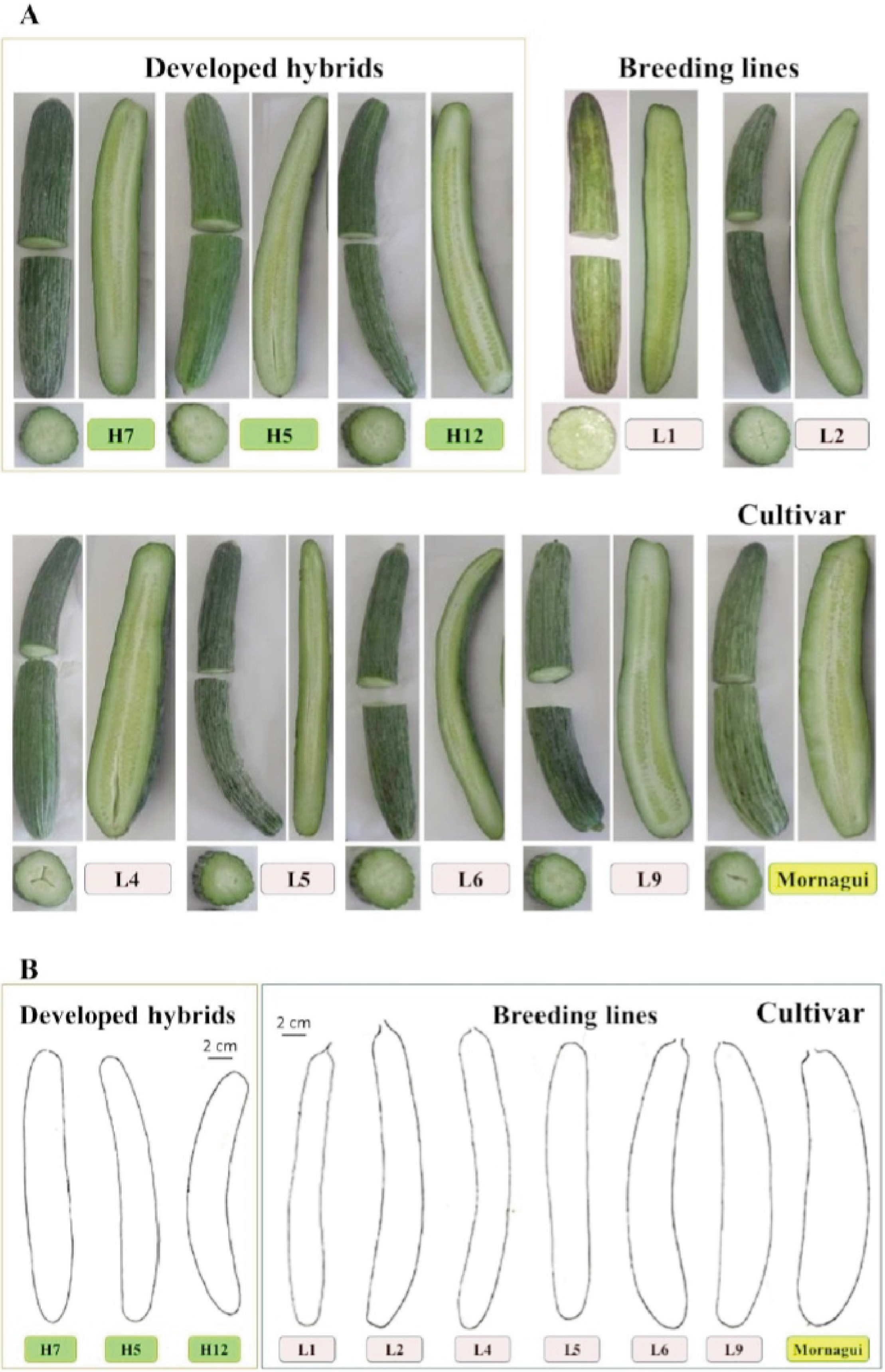

Fig. 2 - External appearance, longitudinal and cross sections (A), and schematic representation of the morphology (B) of the different snake melon genotypes peponides under analysis (hybrids, breeding lines and the traditional cultivar Mornagui).

fruit weight, length, diameter and length/width ratio varied significantly (P<0.05) between genotypes (Table 1). Early yield ranged from 0.9 Kg per plant for the breeding line L5 and the cultivar Mornagui to 1.5 and 1.7 Kg per plant for the hybrids H5 and H7, respectively. The breeding line L9 had the lowest total yield (1.9 Kg per plant) and 10.7 fruits per plant, while the highest values were recorded for the hybrid H5 (3.6 Kg per plant and 20.7 fruits per plant). The average fruit weight ranged between 151.0 g to 184.3 g for the breeding line L6 and Mornagui cultivar, respectively. In agreement to the low weight, the breeding line L6 produced short fruits (19 cm length as average) with the lowest length/width ratio (5.14), while the fruits of the hybrid H5 and of the breeding line L1 were the longest (32.0 and 32.6 cm, respectively) and showed the highest length to width ratio (8.36 and 8.65, respectively). Fruit diameter is an important agricultural trait for snake melon as consumers generally prefer long fruits with low diameter (width). The lowest fruit diameter (3.3 cm) was measured for the breeding line L5 and the highest value (4.2 cm) was obtained for the hybrid H7 and cv. Mornagui. To our knowledge, these presented data are among the first reports on snake melon horticultural traits. Nevertheless, with respect to total yield, our data exceed the range of values (0.5-3.5 kg/plant) reported by Aydi-Ben-Abdallah *et al.* (2019) and (0.3-2.0 Kg per plant) reported by Abdelmohsin *et al.* (2015) for open field and greenhouse grown monoecious cultivars of the *flexuosus* group (Alimin, PI222187 and Snake melon) and for the hermaphroditic breeding lines generated by crossing the *flexuosus* parents with the melon accession Paul, while they fell within the range reported by the same authors for number of fruits per vine (2-25). No correspondence was found, instead, between our findings and the number of fruits per plant (5.8-7.2) and fruit length (33-90) measured in five stable inbred lines of cultivated landraces of snake melons grown in open field at Wad Medani (Sudan) by Yousif *et al.* (2010); while they were in accordance with those of Ali-Shtayeh *et al.* (2017) for fruit weight (53-201 g) and length/width ratio (3-9) in a study comprising 50 accessions of snake melons grown in different open fields across the West Bank, and were slightly lower than the length (48.3 cm) and width (10.7 cm) values reported by Ramamurthy and Waters (2015) for a pale-green fleshed, non-sweet accession (USDA PI435288) grown it the fields of the Lincoln Agronomy Research Farm of the University of Nebraska.

Powdery mildew resistance visual evaluation showed that the snake melon line L9 exhibited similar resistance level to the ordinary cultivar Mornagui and also to the hybrid H5 (L2 x L1).

Table 1 - Agronomic characteristics evaluated in snake melon hybrids, breeding lines and cultivar Mornagui grown under greenhouse conditions during the seasons 2014 and 2015 (pooled data of the two years were analyzed)

Genotypes	Major skin colour	Fruit form	Early yield (Kg/plant)	Total yield (Kg/plant).	Number of fruits/plant	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	PMR*
Developed hybrids (Line x Line)									
H7 (Mornagui X L5)	Dark/light green	Straight	1.7±0.1a	3.4±0.5 ab	20.3±2.9 ab	164.0±2.3	29.8±1.2 b	4.2±0.2 a	+
H5 (L2 X L1)	Dark green	Straight	1.5±0.1 a	3.6±0.3 a	20.7±2.0 a	173.6±1.2	32.0±1.0 a	3.7±0.1 bcd	+ +
H12 (Mornagui X L6)	Dark green	Elongate	1.4±0.2 b	3.0±0.2 abc	18.4±0.3 abc	169.0±16.7	22.0±0.6 e	4.1±0.2 ab	+
Breeding lines									
L1	Dark green	Straight elongate	1.4±0.3 b	3.3±0.6 ab	19.4±3.8 abc	172.7±6.1	32.6±0.1 a	3.9±0.3 abc	+
L2	Dark green	Elongate	1.1±0.1 b	2.7±0.1 abcd	16.0±0.6	166.3±3.7	31.2±0.1 ab	3.8±0.1 abcd	+
L4	Intense dark green	Elongate	1.3±0.1 ab	2.7±0.2 bcd	17.0±1.5 abc	153.6±2.7	27.5±0.3 c	3.8±0.4 abc	+
L5	Dark green	Elongate	0.9±0.2 b	2.1±0.2 cd	13.7±1.8 cd	154.6±5.2	19.2±0.1 f	3.3±0.1 d	+
L6	Dark green	Straight elongate	1.0±0.1 b	2.2±0.1 cd	14.7±0.7 bcd	151.0±2.6	19.0±0.6 f	3.7±0.1 bcd	+
L9	Dark green	Elongate	1.0±0.2 b	1.9±0.5 d	10.7±1.8 d	174.0±12.1	22.5±0.1 e	3.6±0.3 cd	+ +
Cultivar Mornagui	Medium/light	Elongate	0.94±0.1 b	2.5±0.2 bcd	13.6±1.2 cd	184.3±12.9	25.2±0.6 d	4.2±0.3 a	+ + +

The data refer to visually assessed snake melon fruits and are the average of at least 10 independent replicates.
Mean ± SD followed by the same letters do not differ significantly (LSD test, P<0.05).
* PMR is the evaluation of the Powdery mildew resistance, based on visual evaluation of the symptoms developed.

The dendogram from the hierarchical ascending classification (Fig. 3) has discriminated snake melon genotypes into 3 main clusters (level of trencature 5). The first cluster comprised 4 genotypes (the hybrid H5 and the breeding line L1 in a sub-cluster and the hybrid H7 and the breeding line L2 in the other sub-cluster) characterized by the most producing yield and the most elongated fruits. The second main clusters comprised 3 genotypes (the hybrid H12, the breeding line L9 and the traditional cv. Mornagui) mainly characterized by intermediate producing yields and intermediate fresh weights. The third cluster comprised the breeding lines L5, L6 and L4 characterized by the small fruit weight and the small fruit length.

4. Conclusions

The variability detected, for the main agronomic traits, in different snake melon genotypes (F1 hybrids and breeding lines) can be useful for further conventional snake melon breeding programs aiming to improve their agronomic traits and could contribute to the breeding of powdery mildew-resistant elite cultivars suitable for greenhouse and open field conditions.

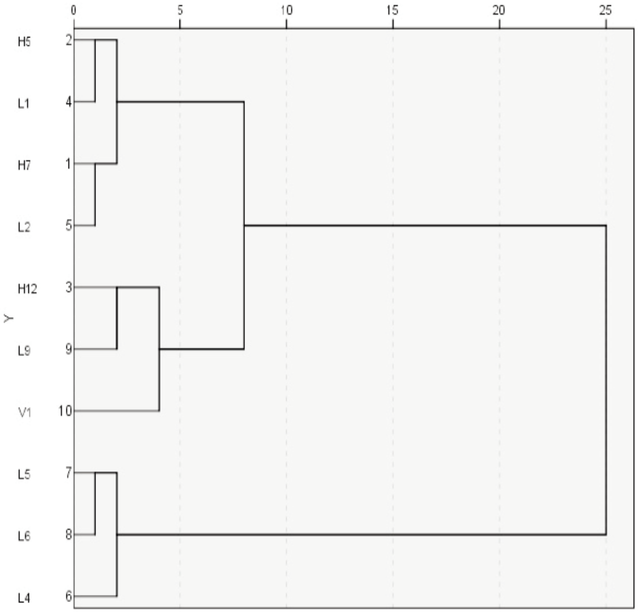

Fig. 3 - The hierarchical ascending classification oh the studied snake melon genotypes. L1, L2, L4, L5, L6 and L9 are the breeding lines H5 (L2 x L1), H7 (Mornagui x L5) and H12 (Mornagui x L6) are the hybrids, V1 is the traditional cv. Mornagui.

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