Study of Natural Variation in Root Structure within *Cucumis melo* L. using in vitro culture.

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Natural variation in root systems is being exploited in many crops, such as maize (6), rice (5), lettuce (2), etc. In these cases, wild taxa have been useful sources of variation for root architecture, since their roots usually exploit more unpredictable and stressful soil environments than the cultivated taxa (4).

The species *C. melo* includes two subspecies, *melo* and *agrestis* (3). Most cultivated melons are included in the subspecies *melo*. The subspecies *agrestis* includes wild, semi-wild and weedy germplasm that can be used as the donor of valuable genes for breeding cultivated melons, as both taxa are fully interfertile. In previous studies performed in fields and greenhouses, we found that the accessions belonging to the two subspecies display different root structures (1). However, the study of root systems recovered from soil substrate is not an easy task. *In vitro* culture techniques facilitate *in vivo* studies of root development.

In this study we have used *in vitro* culture to evaluate the root development of three accessions, *C. melo* subsp. *melo* cv Piel de sapo (PS), which is the most cultivated type in Spain, *C. melo* subsp. *agrestis* PI 161375 (PI), both PS and PI being used as parents for the Spanish map of melon, and *C. melo* subsp. *agrestis* Pat 81, which has been reported as being resistant to *Monosporascus* root rot (1)

Fifteen seeds of the three accessions (PS, PI 161375, and Pat 81) were surface sterilized for 20 min in 50% bleach with Tween-20, and rinsed 3 times with sterile water. Tubes with standard MS medium (pH 5.7), including 30 g/l of sucrose, vitamins, and 200mg/l of cefotaxime, were prepared and used to sow the sterilized seeds. After growing in the dark for two days, the germinated seeds were transferred into transparent plates (23x19x1cm) filled with 300 ml of the same medium. The plates were grown in a growth chamber (25ºC, 16/8 h light/dark). Once a day for 15 days a digital image was taken of each root with a scanner. The digital images were analyzed with the specific software for roots WinRhizo-Pro 2003b (Regent Instruments Inc. Canada). The evaluated traits were: the total root length (the sum of the lengths of all of the roots), L (cm); the root projected area, PA (cm^2); the average root diameter, D (mm); the length of the primary root (cm), the number of laterals emerging from the primary root, NL; and the root width (the maximum horizontal distance between the root tips of the furthest lateral roots), W (cm).

PS developed roots with greater projected area (PA) and greater total length (L) than PI 161375 or Pat 81 (Fig 1). However, a higher PA or L does not necessarily imply an enhanced capacity for soil exploration and water/nutrient absorption. Another parameter such as L/PA could provide more information seeing as it measures the root investment in exploring more soil volume. L/PA was higher in PI 161375 and Pat 81 than in PS (Fig1). This parameter was negatively correlated to the root diameter (D). PS developed thicker roots than PI 161375 and Pat 81 (Fig 1). Generally, the absorptive roots are those which are thinner and of a higher order than the structural roots. Therefore, our results indicate that the accessions of the subspecies *agrestis* are more efficient at producing absorptive length per unit of projected area.

The number of lateral roots (NL) was highest in PS, followed by Pat 81 and PI (Fig. 1). In general, the number of lateral roots is restricted by the length of the primary root. In our study, PS showed a higher density of lateral roots (4.8 lateral roots/cm of primary root at 15 days after sowing) in comparison with Pat 81 or PI (3.8 roots/cm and 2.0 roots/cm respectively). In PS the third order laterals appear quickly (at 4-5 days after sowing) and continuously. On the contrary, the few laterals of Pat 81 and PI 161375 do not branch till the 8^{th} day after sowing on average, and the tertiary roots appear scattered on the secondary laterals. This *in vitro* culture assay also provided the opportunity of following the spatial distribution of the roots in the medium. The lateral roots of PS grew more horizontally (W of 14 cm), while Pat 81 and PI tended to grow more vertically (W of 8.5 and 6.8 cm respectively). Our results indicate that these wild accessions have priority in penetrating the soil with the minimum carbon investment (few and thin roots), while PS used a larger resource input to explore the topsoil layer rapidly.

Additionally, when we studied the root systems of these accessions in adult plants grown in different soil environments, we observed a higher length and branching level (number of laterals and root orders) in the wild taxa in comparison with PS (1). This discrepancy seems to suggest that wild taxa save resources in less stressful conditions, such as the *in vitro* culture in an artificial medium. However, they have a higher plasticity and can react dramatically by modifying their root architecture in stressful soil environments (6).

The methodology used has allowed the study of the main differences between the root systems of cultivated melons *vs* wild melons. Further studies could help to select accessions for their improved root systems according to our needs.

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Figure 1. Evolution of the different parameters measured in the roots of *C. melo* subsp. *Melo* cv Piel de sapo (PS), *C. melo* subsp. *Agrestis* Pat 81, and *C. melo* subsp. *agrestis* PI 161375 during 15 days after sowing (DAS). A) Root projected area (PA, cm^2), B) root length per unit of projected area (L/PA) (cm^{-1}), C) number of laterals derived from the primary root (NL), D) average diameter of the root (D, mm).