VARIATIONS IN FRUIT PLOIDY LEVEL AND CELL SIZE BETWEEN SMALL- AND LARGE-FRUITED OLIVE CULTIVARS DURING FRUIT ONTOGENY

GOMEZ-JIMENEZ MC*, CAMARERO MC, BRIEGAS B, VASQUEZ P, CORBACHO J

Laboratory of Plant Physiology, Universidad de Extremadura, 06006 Badajoz, Spain

Correspondence

Maria-Carmen Gomez-Jimenez, Email: mcgomez@unex.es

Objectives

Olive (*Olea europaea* L.) is one of the major oil fruit tree crops worldwide. However, the mechanisms underlying olive fruit growth remain poorly understood. In the present study, we consider the variability of olive fruit size in order to address the question of its dependence on fruit ploidy and cell size during fruit ontogeny. For this, we made a quantitatively comparative analysis of fruit development in three olive cultivars that differed in the final fruit size. This included cytology and ploidy analyses associated with fruit development in an olive small-fruited cultivar, 'Arbequina', and two large-fruited cultivars, 'Picual' and 'Manzanilla Sevillana'. Specifically, we examined mitotic activity, using flow-cytometric analysis, and mesocarp cell area of the developing olive fruits to determine whether cell division and/or cell expansion might be reduced in the small fruit, or conversely increased in the case of large fruit. In addition, to demonstrate the role of endoreduplication in olive fruit growth control, we investigated the cell ploidy profile during early and late fruit development in pericarps of the three olive cultivars.

Materials and Methods

Plant Material Three cultivars of olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) differing markedly in fruit size, i.e., 'Arbequina', 'Picual', and 'Manzanilla Sevillana', were used. From these trees (10 trees/cultivar), flowers were collected at the anthesis stage (0 days post-anthesis, DPA) and fruits were sampled at 7, 14, 21, 28, 35, 42, and 49 DPA, spanning a time from the fruit set to the time of endocarp lignification Flowers at the anthesis stage and whole fruits of 'Arbequina', 'Picual', and 'Manzanilla Sevillana' olive cultivars were weighed, and the longitudinal and transverse diameters were measured at different developmental stages.

Cytological Analysis The cytological study was performed as described by Parra and Gomez-Jimenez (2020). At least three biological replicates were made at each stage. The cell size (cell area) and pericarp thickness (cross-section) were determined using a CellProfiler image analysis system (Camarero et al., 2023).

Flow Cytometry Analysis The cell division period was precisely determined using flow cytometry with nucleus ploidy profiles taken from ovaries and fruits (Camarero et al., 2023). The DNA content (C value) of the olive fruit cells was assessed using flow cytometry with the use of internal calibration standards (Camarero et al., 2023). All the experiments were carried out in triplicate, and all data are presented as mean ± standard deviation

Results

1. Morphological changes during fruit development in olive cultivars

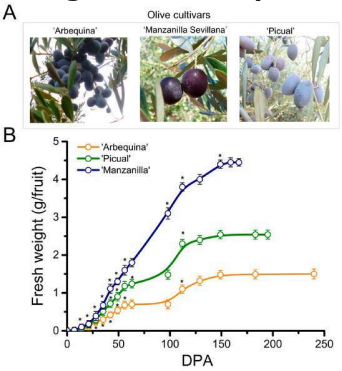

Figure 1. Fruit development of 'Arbequina', 'Manzanilla Sevillana', and 'Picual' olive cultivars with contrasting fruit size and shape. (**A**) Images of olive fruit morphology at the fully ripe stage in 'Arbequina', 'Manzanilla Sevillana', and 'Picual' cultivars at 260, 169, and 189 DPA, respectively. (**B**) Changes in fresh weight (FW) (g fruit^{-1}) of olive fruit during fruit growth and ripening in the three cultivars: 'Arbequina' with small and round fruit, 'Manzanilla Sevillana' with large and round fruit, and 'Picual' with large and elongated fruit. Each point is the average of 20 fruits. The values were estimated as means ± SD. Asterisks indicate statistically significant changes with respect to the preceding point according to Tukey's test (p < 0.05). DPA: days post-anthesis.

2. Early fruit development in olive cultivars

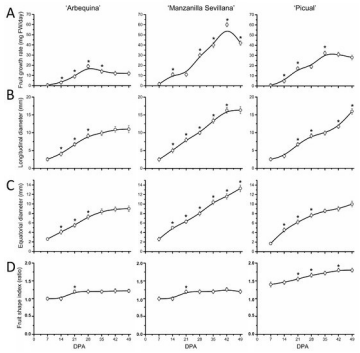

Figure 2. Morphological changes of olive fruit of 'Arbequina', 'Manzanilla Sevillana', and 'Picual' cultivars during early fruit development. (**A**) Changes in growth rate (mg FW day^{-1}), (**B**) longitudinal diameter (mm), (**C**) transverse diameter (mm), and (**D**) fruit shape index of developing olive fruit at 0, 7, 14, 21, 28, 35, 42, and 49 DPA from the three olive cultivars. Fruit shape index is length-to-width ratio of the fruit. Each point is the average of 5 fruits. The values were estimated as means ± SD. Asterisks indicate statistically significant changes with respect to the preceding point according to Tukey's test (p < 0.05).

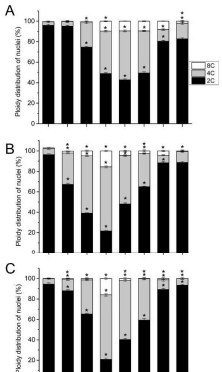

Figure 3. Nuclear ploidy levels from olive fruits during early development in (**A**) the 'Arbequina', (**B**) 'Manzanilla Sevillana', and (**C**) 'Picual' olive cultivars. The percentage of nuclei in 2C, 4C, and 8C are shown from 0 to 42 DPA in the developing fruits. Each point is the average of four samples. Asterisks denote significant differences based on Tukey's test (p < 0.05) from the preceding point, and bars are ± SD.

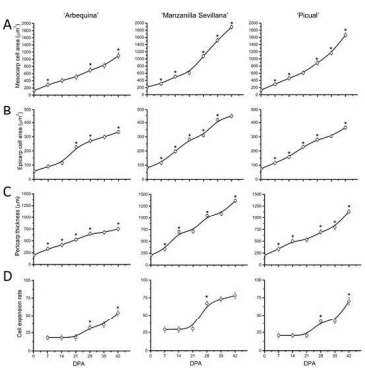

Figure 4. Cell parameters of olive fruit pericarp of 'Arbequina', 'Manzanilla Sevillana', and 'Picual' cultivars during early fruit development. (**A**) Mesocarp cell area (μm^2), (**B**) epicarp cell area (μm^2), (**C**) pericarp thickness (μm), and (**D**) cell expansion rate (mesocarp cell area/day) of developing olive fruit at 0, 7, 14, 21, 28, 35, and 42 DPA from the three olive cultivars. The cell area of fruit mesocarp and epicarp cells was measured during early fruit development (staining was with Calcofluor White) using confocal microscopy. Each point is the average of 5 fruits. The values were estimated as means ± SD. Asterisks indicate statistically significant changes based on Tukey's test (p < 0.05) from the preceding point.

3. Late fruit development in olive cultivars

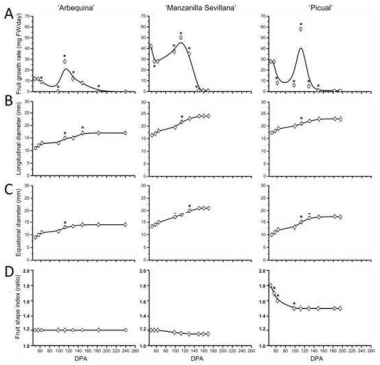

Figure 5. Morphological changes of olive fruit of 'Arbequina', 'Manzanilla Sevillana', and 'Picual' cultivars during fruit growth and ripening. (**A**) Changes in growth rate (mg FW day^{-1}), (**B**) longitudinal diameter (mm), (**C**) transverse diameter (mm), and (**D**) fruit shape index of developing olive fruits from the three olive cultivars. Fruit shape index is length-to-width ratio of the fruit. Each point is the average of 5 fruits. The values were estimated as means ± SD. Asterisks indicate statistically significant changes with respect to the preceding point according to Tukey's test (p < 0.05).

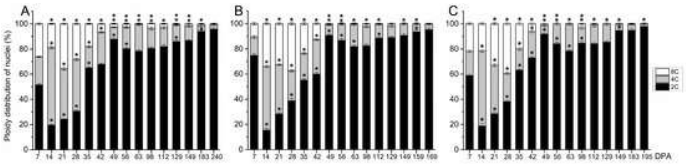

Figure 6. Nuclear ploidy levels from olive fruit pericarps (epicarp and mesocarp) during growth and ripening in (**A**) the 'Arbequina', (**B**) 'Manzanilla Sevillana', and (**C**) 'Picual' olive cultivars. The percentage of nuclei in 2C, 4C, and 8C are shown in the developing fruit pericarp (epicarp and mesocarp). Each point is the average of four samples. Asterisks denote significant differences based on Tukey's test (p < 0.05) from the preceding point, and bars are ±SD.

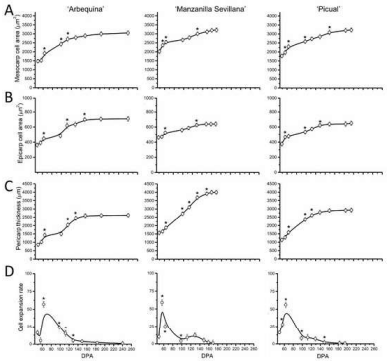

Figure 7. Cell parameters of olive fruit pericarp of 'Arbequina', 'Manzanilla Sevillana', and 'Picual' cultivars during fruit growth and ripening. (**A**) Mesocarp cell area (μm^2), (**B**) epicarp cell area (μm^2), and (**C**) pericarp thickness (μm) and (**D**) cell expansion rate (mesocarp cell area/day) of developing olive fruits from the three olive cultivars. Each point is the average of 5 fruits. The values were estimated as means ± SD. Asterisks indicate statistically significant changes based on Tukey's test (p < 0.05) from the preceding point.

Conclusions

We conclude that the basis for fruit size differences between olive cultivars is determined mainly in the early fruit growth phase. Although fruit pericarp cells increased in ploidy level during early fruit development, the cells displayed a low degree of endopolyploidization, and no association between ploidy level and size in cells was found in the pericarps. Thus, endoreduplication does not appear to be relevant to olive fruit size or to eventual fruit development. The present study indicates that the olive fruit of large-fruited cultivars must have acquired more active cell division and expansion, emphasizing the value of early growth events in determining the final fruit size in olive.

These data provide new findings on the contribution of fruit ploidy and cell size to fruit size in olive and ultimately on the control of olive fruit development.

This work was supported by grant to M.C. G-J (PID2022-138573OB-I00 funded by MCIN/AEI/10.13039/501100011033 and, by "ERDF A way of making Europe") from the Spanish Ministry of Science, Innovation and Universities.