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Research Article

Elaboration of table olives: assessment of new olive genotypes†

Running title: New olive genotypes for table olive processing

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Abstract

Thirty-three new genotypes coming from a table olive cross-breeding program were evaluated to assess their adaptability to standard processes, namely Spanish-style and black ripe olives. Different physical and chemical parameters were evaluated in fresh fruits, processed olives and brines. Most of these traits exhibited large variability among all genotypes and within each cross. Seven new genotypes, 05-138, 05-166, 05-217, 05-322, 05-499, 04-1390 and 04-1396, were selected based on fruit weight, pulp-to-pit ratio, olive oil content and bruising parameters. In particular, two genotypes (04-1355 and 05-271) did not develop fermentation by lactic acid bacteria. Some genotypes had firmness problems in the final product, especially the 02-1000, and in both elaboration processes this cultivar was excessively soft. Other genotypes developed an unacceptable color when they were processed as Spanish-style olives. Nevertheless, all new genotypes could be elaborated as both Spanish-style and black ripe olives by adapting specific criteria in the processes for each genotype. Correlations between traits measured in fresh fruit and those measured in processed olives were explored.

Practical applications: Currently, the consumption of table olives is increasing and the table olive industry has a greater problem with the raw material that reaches its cooperatives. The relevance of this work is that almost all genotypes tested have produced a final product of high quality. In addition, they have agronomic advantages over traditional cultivars. It opens up the possibility to employ theses new cultivars to obtain table olives, both Spanish-style and black ripe olives, with less post-harvest and processing problems.

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1 Introduction

The olive species (*Olea europaea* L.) is an emblematic crop in the Mediterranean basin and one of the most important fruit trees, with more than 10 million ha grown worldwide according to the International Olive Council [1]. Two main products are derived from olive fruits: table olives and olive oil being both considered staple foods of the Mediterranean Diet. The consumption of table olives has increased by 182% in the last 26 years [2] and its global production reached around 2,563,700 tons in the last five seasons [1].

In the last decades the olive industry has dramatically changed and new olive growing techniques and systems are being adopted by olive farmers: irrigation, fertilization, mechanization of pruning and harvesting, and super high density hedgerow planting, among others. Many of the traditional olive cultivars do not meet the requirements for this new olive growing era [3]. Consequently, the demand for new cultivars has driven the development of different olive breeding programs [1]. Most of these programs were focused on developing new olive cultivars for high-quality olive oil production [5], with higher concentration of phenolic compounds [6] or rich in omega-3 content [7] and sterol content [8], among others. The resistance level to external development of Verticillium symptoms is also an important selection criteria [9]. Just a few programs have been oriented towards obtaining new table olive cultivars [10-12]. In Spain, a cross-breeding program specifically focused on table olives was initiated in 2003.

The evaluation of progenies in olive breeding programs is a long-term commitment due partly to the long juvenile period of the species. Thus, much effort has been done to shorten olive juvenility in breeding programs with good results [13].

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The breeder should select the traits to be used as selection criteria according to the goal of the program. In this sense, another fact hampers table olive breeders' work: the olive fruit may not be consumed directly but need to be processed to eliminate its distinct bitterness. There are two main commercial table olives, Spanish-style and black ripe olives, whose processing consists of treating the fruits with a dilute NaOH solution to remove their bitterness [14]. There are other trade preparations of fermented green and black olives that involve the direct brining of olives without any alkaline treatment, which are known as natural olives [12, 15].

New table olive cultivars should be ready to use for any table olive process and, thus, a complete evaluation of cross-breeding progenies would need to include processed olives. Nonetheless, first evaluation of genotypes is usually based solely on fresh fruit quality traits such as fruit size, flesh to stone ratio, color or firmness, among others [11]. In more advanced stages of breeding, once the outstanding individuals are selected, the suitability of those genotypes to be processed for table olives must be evaluated [12] along with other more complex traits such as the content of healthy compounds like phenolics and triterpenic acids [16]. Furthermore, the possibility of correlating traits of unprocessed fruits with quality traits of the final processed olives is especially interesting since it would give the chance to breeders for indirect selection based on fresh fruit traits. Unfortunately, there is almost no information regarding this matter.

The aim of this paper was to evaluate the suitability for green (Spanish-style) and black-ripe table olive processing of 33 new preselected genotypes coming from cross-breeding and four olive cultivars previously used as genitors. Additionally,

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possible correlations between quality traits in fresh and processed olives were explored.

2 Materials and methods

2.1 Plant material

Thirty-three new table olive genotypes, as well as four traditional cultivars, were analyzed in this study. All the new genotypes were preselections of the University of Seville (US) table olive breeding program and come from four different crosses ('Manzanilla de Sevilla' x 'Gordal Sevillana', 'Changlot Real' x 'Manzanilla de Sevilla' , 'Gordal Sevillana' x 'Santa Caterina' , 'Manzanilla de Sevilla' x 'Santa Caterina') and one open-pollination population from 'Manzanilla de Sevilla'. Table 1 shows the identification code of the genotypes and the crosses. The parental cultivars (genitors) involved in the crosses ('Changlot Real', 'Gordal Sevillana', 'Manzanilla de Sevilla' and 'Santa Caterina') were also analyzed.

Crosses were performed in spring 2002, 2004 and 2005. Trees were established in an experimental field at IFAPA Las Torres-Tomejil, Alcalá del Río (Sevilla, Spain). They were planted in ridges with a 5 x 3 m or 5 x 1.5 m layout. The canopy height was established at 1.5 m by pruning lateral shoots. Fertigation and standard cultural practices were applied in the orchard to ensure tree growth. Parental cultivars were also planted and grown in the same experimental field and conditions.

The genotypes analyzed in this work were selected based on the earliness of first bearing and optimal fruit traits. Olive fruits were picked by hand at maturity index 1 and a representative sample of approximately 10 kg was collected for each genotype and cultivar.

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2.2 Spanish style table olives processing

Fruits were put into 3L PVC vessels and covered with 16 g/kg (w/w) NaOH solution for 5-13 hours until the lye had penetrated two-thirds of the way to the pit. Subsequently, a wash of the fruits was made with tap water for 9-17 hours according to the genotype treated and then covered with 115 g/kg (w/v) NaCl solution. Then, a spontaneous fermentation by lactic acid bacteria was carried out and, after six months, the olive quality was measured.

2.3 Black ripe olives processing

Fruits were placed into 3L PVC vessels and covered with a brine of 90 g/kg NaCl and 12 g/kg acetic acid. After six months of anaerobic fermentation, the olives were darkened as black ripe olives in twelve PVC cylindrical containers with conical bases. The process consisted of placing 1kg of fruits in 1L of 15 g/kg (w/w) NaOH solution for 4-5 hours, which was sufficient time for the lye to reach 50% of the flesh. The olives were then covered with tap water and air was bubbled through the mixture for 18 hours. Afterwards, the olives were put in a new NaOH solution (15 g/kg, w/w) for 2-4 hours, which was sufficient time for the lye to reach the pit. After draining, they were put in a new washing solution, and air was bubbled for 24 hours [17]. Finally, the liquid was poured off and the fruits were covered with a 1 g/kg of ferrous gluconate solution and aerated for another 24 hours. The olive quality was then measured without any sterilization stage to highlight differences among genotypes.

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2.4 Fruit morphology and bruising of fresh olives

The average weight of the fruit and the stone were measured from samples of 0.5 kg of fruits. Pulp-to-pit ratio was calculated as the difference in fresh weight between fruit and stone. Fruit shape was estimated as the average ratio between the maximum longitudinal and equatorial diameters (mm) measured in fifty fruits. Bruising incidence was determined after harvest as the percentage of bruised fruits in a sample of 100 fruits.

2.5 Oil content analysis

It was estimated on a 0.5 kg sample of fruits by a NMR analyzer Minispec NMS100 (Bruker Optik GmbH, Ettlingen, Germany).

2.6 Analysis of the phenolic and oleosidic compounds

The extraction of phenolic compounds from the olive pulp was based on the methodology proposed elsewhere [18] using dimethyl sulfoxide (DMSO). An aliquot of DMSO extraction solution (250 µL) was homogenized with 250 µL of internal standard (syringic acid 0.2 mmol/L in DMSO) and 500 µL of DMSO. A volume of this mixture (20 µL) was injected for HPLC analysis.

Also, a mixture of 250 µL of brine, 250 µL of internal standard (2 mmol/L syringic acid in H_2O) and 500 µL of deionized water was filtered through a 0.22 µm pore size nylon filter. An aliquot (20 µL) was injected into the chromatograph. The fermentation brines were analyzed after 15, 30 and 180 days.

The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600E pump, a Waters column heater module and a Waters 996 diode array detector

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(Waters Inc., Mildford, MA). A Spherisorb ODS-2 (5 lm, 25 9 4.6 mm i.d., Waters Inc.) column was used. Separation was achieved using an elution gradient with an initial composition of 90% water (pH 3.0) and 10% methanol. A flow rate of 1 mL/min and a temperature of 35°C were used in all experiments. The wavelengths selected for phenolic and oleosidic compounds were 280 and 240 nm, respectively. Commercial standards and isolated compounds by semi-preparative HPLC were used to quantify the phenolic and oleosidic compounds as described elsewhere [19].

2.7 Sugar compounds analysis

The extraction of sugar compounds from the olive pulp was based on the methodology proposed elsewhere [20] using hot water as extraction solution. These compounds were analyzed by HPLC [20]. The system consisted of a Waters 2695 Alliance with a pump and autosampler included, the detection being performed with a Waters 410 refractive index detector. A Rezex RCM-Monosaccharide Ca+ (8%) column (300 × 7.8 mm i.d., Phenomenex) held at 85°C and deionized water as eluent at 0.6 mL/min was used. Quantification of glucose was made by using a commercial standard.

2.8 pH, free acidity and NaCl content of brines

The pH was measured using a Crison model 2001 pH meter (Crison Instruments, Barcelona, Spain). The concentration of sodium chloride was analyzed by titration with 0.1 mol/L silver nitrate solution, using potassium chromate solution as indicator. Free acidity was measured by titration using a Metrohm 670 Titroprocessor (Herisau, Switzerland) up to pH 8.3 with 0.2 mol/L NaOH and expressed as g/kg (w/w) of lactic acid.

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2.9 Analysis of organic acids

Brines were filtered through a 0.22 µm pore size nylon filter and an aliquot of 20 μL was injected into the chromatograph. The HPLC system was described above (section sugar compounds analysis.). The separation was achieved by isocratic elution using water acidified (pH 2.5) as mobile phase and a Spherisorb ODS-2 (5 μm, 250 × 4.6 mm, Waters, Inc.) column held at (30ºC). The flow rate was 1.2 mL/min. Quantification of lactic and acetic acids was made by using a commercial standard.

2.10 Superficial color of processed olives

Colorimetric measurements on processed olives were performed using a BYK-Gardner Model 9000 Color-view spectrophotometer (Silver Spring, Md., U.S.A.), equipped with computer software to calculate the CIE *L** (lightness), *a** (redness), and *b** (yellowness) parameters by scanning the surface from 400 to 700nm. The data of each measurement was the average of 10 replicate measurements, each made on one olive. In particular, the surface color of Spanish table olives was expressed as a color index (i), as described elsewhere [21]. Olive color can be analytically classified as better (i > 33.6), excellent (30.2 < i < 33.6), good (26.8 < i < 30.2), acceptable (23.7 < i < 26.8), bad (21.0 < i < 23.7), and very bad (i < 21.0). In the case of surface color of black ripe olives, the color was expressed as reflectance at 700 nm (R_{700}); lower reflectance values indicate darker fruit.

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2.11 Firmness of fresh and processed olives

The firmness was measured both in fresh and processed fruits using a Kramer shear compression cell coupled to an Instron Universal Testing Machine (Canton, Mass., U.S.A.). The firmness was expressed as kN/kg pitted olives. The value was the mean of 10 measurements, each of which was performed on three pitted olives.

2.12 Statistical analysis

The results of each parameter were expressed as mean values with standard deviation. Data for each genotype and for each cross was subjected to analysis of variance (ANOVA) and Duncan's test ($P < 0.05$) was used to discriminate among the mean values. Pearson's correlation coefficients between all pairwise combinations of traits measured were calculated ($P \leq 0.001$, $P \leq 0.01$; $P \leq 0.05$). All data analyses were performed using the StatGraphics Plus 5.1. Software package (Manugistics Inc., USA).

3 Results and discussion

3. 1 Chemical and physical characteristics of unprocessed fresh fruits

Table 1 shows the results of the different parameters analyzed in the fresh fruits for all the new genotypes and the four genitors involved in the crosses. Most of the traits exhibited large inter and intra-crosses variability among the genotypes, which is a frequent pattern reported [11, 22]. Significant differences among mean values per crosses were only found for fruit weight.

An important parameter for processing table olives is the size of the fruit since in the majority of cases, once it has been processed, the fruit will be pitted before

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being packed; that is why a medium-large-sized fruit with a high pulp-to-pit ratio is of more interest.

Mean fruit weight values per crosses ranged between 2.6 g (ChRxM) and 5.1 g (M-OP) although among all analyzed genotypes the size range was larger. Most crosses were statistically similar in terms of the size of the fruit, with an average value of 5 g/fruit, which tends to be the average value of the 'Manzanilla de Sevilla' type [23], although the fruits of the latter evaluated in this work were smaller (3.5 g/fruit). All genotypes with a value above 4 g/fruit were considered of interest according to the above comments: 24 out of the 33 genotypes analyzed. The ChR x M cross showed a significant lower fruit weight (2.6 g/fruit). The low average size of one of the genitors, 'Changlot Real' (3.4 g, [23]) may be the cause of the reduced fruit size of their progeny. These genotypes were to be rejected according to the value considered for this parameter.

The values obtained for the pulp-to-pit ratio per cross were high and above those obtained for the genitors in the same growing conditions. The higher the pulp-to-pit ratio, the more flesh found in the fruit and the smaller the size of the pit, making the olives more appealing to the consumer. All genotypes with a value of ≥ 6 were considered of interest based on our experience and consumers preferences: 18 genotypes out of 33.

Fruit shape was not a discriminating trait since it was very similar among genotypes ranging from 1.1 to 1.5.

Regarding oil content, a high variability was observed ranging from 26 g/kg to 132 g/kg, being both values registered within the same cross (M-OP). From a healthy point of view, new genotypes with low oil content may be a goal in table olive

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breeding programs. In this sense, some of the analyzed genotypes exhibited very low oil contents, being particularly interesting the genotype 04-1396 with an oil content below 30 g/kg (Table 1).

Fruit appearance and, particularly, the absence of injuries and damage is an extremely important quality trait regarding table olives. Bruising is the most common type of mechanical damage in olives, generally associated with superficial browning but also with internal damage within the mesocarp [24]. Results showed a large variation among the new genotypes ranging from 4% (05-217) up to 74% (05-408) of bruised fruits, both within the same cross (M-OP). Nevertheless, many of them had a much lower percentage of bruising than the traditional Spanish table cultivars, 'Manzanilla de Sevilla' and 'Gordal Sevillana' under the same growing conditions, both showing over 60% of bruised fruits. However, 'Santa Caterina' had 41% of bruised fruits and showed a good appearance. To discriminate between new genotypes it was selected a bruising percentage ≤40% as cut-up value.

Having taken all these parameters into account, the study ascertained that four genotypes (12%) met the best values to be selected for use as table olives: 04-1396, 05-138, 05-166, 05-322. These genotypes showed a large size, a good pulp-to-pit ratio, a low percentage of oil content and a relatively low percentage of bruising. Other interesting genotypes that could be chosen were 04-1390, 05-217 and 05-499 since they exhibited high fruit weight and pulp-to pit ratio and a very low bruising incidence.

The presence of phenolic compounds, oleuropein in particular, in the fresh fruit was another area of interest. This compound is responsible for the bitterness of the fruit and should be eliminated during processing. The results showed that the major phenolic compound in the fresh fruit was oleuropein, as previously observed by other

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researchers [25]. Fig. 1a shows that most of the new genotypes were within an oleuropein range of 40 to 80 mmol/kg dry fruit; values of the genitor 'Manzanilla de Sevilla' are included within this range. The oleuropein data was in line with the results obtained by other researchers for cultivars such as 'Manzanilla de Sevilla' and 'Hojiblanca' [25].

In terms of processing, the objective is to find a sweet cultivar, such as 'Gordal Sevillana', whose level of oleuropein is below 9 mmol/kg dry fruit; in this sense, there were very few new genotypes with these characteristics, just 10.7% (5-322, 05-217, 05-166). On the other hand, from a nutritional point of view, it is interesting to find a genotype with a high concentration of this polyphenol, which on hydrolysis yields a high concentration of hydroxytyrosol, a substance well known for its beneficial effects on human health [26]. Once again, some few new genotypes (14%) showed a high concentration of oleuropein (04-1167, 05-271, 05-455, 04-1355).

However, the main objective of this research was to find a new genotype able to be processed as Spanish-style green olives and black ripe olives so that low polyphenols concentration was desirable.

Also, the percentage of available sugars, mainly glucose (Fig. 1b), must be considered in order to obtain an acceptable fermentation during processing. In general, 50% of genotypes contained 150–200 mmol/kg fresh pulp, the same as the 'Manzanilla de Sevilla' cultivar. Four genotypes (04-1355, 04-1390, 05-237, 05-326) had a concentration above 200 mmol/kg fresh pulp and only two genotypes lower than 100 mmol/kg fresh pulp: 02-1003 and 05-161. The cultivar 'Gordal Sevillana' was included within the latter range. Despite the great variability observed in terms of fermentable sugars, all new genotypes were suitable for use as table olives.

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3. 2 Chemical and physical characteristics of Spanish-style green olives

Table 2 contains the chemical values of brines after six months of fermentation. It was observed that, after this time, almost all brines of Spanish green olives reached a pH value below 4.2 units. Moreover, the value of free acidity was within a range of 2.4 to 11.7 g/kg, and this acidity was mainly due to the presence of lactic acid, which was detected by HPLC within a range of 1.2–12.2 g/kg. All these data corresponded clearly to a lactic acid fermentation, which is characteristic of this type of processing [27, 28]. Therefore, it could be argued that, from a fermentation point of view, the new genotypes are suitable for use as Spanish-style green olives. Only two exceptions were found: genotypes 04-1355 and 05-271, which had a pH value of 5.6 units, in whose brines only 1.2 g/kg of lactic acid was detected. Clearly, no lactic fermentation took place in these two tests, which may be the result of using a standard method of preparation, rather than a specific treatment for each genotype. After 15 days of fermentation, the analysis of the composition of their brines detected the presence of a high concentration of antimicrobial compounds (dialdehydic form of decarboxymethyl elenolic acid free or linked to hydroxytyrosol and Oleoside 11-methyl ester), which reached values of 2.69 and 1.16 mmol/L for 04-1355 and 05-271 genotypes respectively. After 30 days of fermentation, the concentration of these antimicrobial compounds in the brine had doubled. Other researchers had already pointed out that these compounds had a high level of antimicrobial activity with respect to lactic acid bacteria and that their combination was highly effective from a total value of 0.75 mmol/L onwards [19].

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In terms of concentration of phenolic compounds in these brines, which would be balanced by the concentration in the fruits, it should be noted that all genotypes had very similar values among themselves and also similar to the values compiled in the bibliography [27]. The lowest average value was shown by cross M x SC, which was also the cross with the highest variability for this parameter. However, this difference was not statistically significant. It is also important to mention that hydroxytyrosol was responsible for 90–95% of this composition.

One of the main quality parameters of Spanish-style green olives is the firmness of the final product. Results achieved by the different genotypes are shown in Table 3. Statistically significant differences were found among crosses, showing the cross M x G the lowest mean value of fresh fruit firmness and cross G x SC the highest. Processing caused a loss of firmness of at least 50% compared to the firmness observed in the fresh fruit. Most crosses had a firmness of around 30 kN/kg pitted olives, which is a similar result to that reported by other researchers [14]. The bibliography reveals a mixed picture for this parameter, data ranges from 16 kN/kg pitted olives for Spanish-style 'Manzanilla de Sevilla' olives [28] — which coincides with the values registered for the genitor 'Manzanilla de Sevilla' — to much higher values of around 55 kN/kg pitted olives for this same cultivar and processing [29]. For some genotypes it was not possible to measure firmness (02-1000, 04-1396) or the values obtained were especially low (05-138, 05-322); fruits of these genotypes had very soft skin and the pulp was stuck to the pit, which rendered the pitting phase impossible. This demonstrates that, for the final product, this parameter will be influenced to a great extent by the processing. Nevertheless, the initial firmness is also important and for most of the problematic genotypes, the value of the fresh fruit was low.

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Regarding Spanish-style green olives, the color index (Fig. 2a) and CIELAB parameters (Table 4) were assessed. The first measurement indicated that over 60% of the genotypes were classified as having an acceptable or good color—this corroborates the data compiled by other researchers for Spanish-style 'Manzanilla' green olives [14, 28]. There were very few cases of genotypes with excellent color (02-1003, 02-1038, 02-469, 05-326, 05-138) or above (04-1390) and fewer cases still of poor color (05-343, 05-271, 05-150, 05-54) or very bad (04-1355, 05-166). Some of these coincide with genotypes that had not undergone lactic acid fermentation (Table 2). No significant differences were found between crosses for this parameter. The analysis of lightness data (Table 4) confirmed again that the color developed by all genotypes was within the range found for genitors and for any olive processed as a Spanish-style green olives [28, 29]. In terms of *b** intensity, all genotypes presented an average value of approximately 34 units, which is common for this type of processing, although statistically significant differences were observed among crosses based on this parameter. Furthermore, *a** values indicated that all also showed a red intensity of 3.9 units, whereas two genotypes, 04-1355 and 05-271, stood out due to their low values. These are precisely the ones that did not develop lactic acid fermentation.

3. 3 Chemical and physical characteristics of Black ripe olives

All genotypes were preserved in acidic conditions by initially adding acetic acid (12 g/kg) to the brine before they were processed as black ripe olives. Table 2 contains the chemical parameters of the fermentation brines. After six months, the chemical properties of these brines were at expected levels [17, 30]; the majority had a pH value lower than 4 units and a very high level of free acidity. In this case, the concentration

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of acetic and lactic acids was responsible for that free acidity. Approximately 30% of the new genotypes did not develop fermentation by lactic acid bacteria. It must be noted that within 15 days of fermentation, around 0.58 to 3.44 mmol/L of total antimicrobial compounds was detected in the brines; while they were not observed in the fermentation brine of any fermenters where lactic acid was detected.

Regarding the concentration of phenolic compounds, there was a high variability, even within the same cross (M-OP), although there were no statistically significant differences among crosses. During this fermentation phase, oleuropein decreased, although a considerable concentration (0.2–3.93 mmol/L) was maintained after six months, which made the fruit even bitterer. However, a high concentration of hydroxytyrosol and other ortho-diphenol derivatives were detected, which is of utmost importance, as a higher concentration of these compounds favors the attainment of a darker final product [31].

Table 3 lists the firmness values of oxidized black olives after the color fixation step. The value of this parameter was largely homogenous for all of the genotypes assessed, as data deviation was low, although significant differences among crosses were observed. The average value obtained, around 35 kN/kg pitted olives, was higher than that reported by other researchers [32-34]. This may have resulted from a lack of sterilization in the studied genotypes, which is the final phase of the production process for black ripe olives. Only one genotype, 02-1000, showed a much lower value. In fact, it was complicated to measure its firmness, as the fruits were very soft and very difficult to pit.

The reflectance value at 700 nm (Fig. 2b) or CIELAB parameters (Table 4) were color parameters assessed for oxidized black olives. In general, none genotype

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achieved the appropriate intense black color required, which may also have been due to the fact that the sterilization phase required by this product was not carried out. Most commercial packaged olives develop R_{700} values below 5 units [33] but roughly 49% of genotypes and their genitors presented R_{700} value within a range of 5.0–7.0 units, and 15% of cultivars had a value higher than 9.0 units. The development of good color in this product depends on three essential factors: (i) raw material — a high concentration of ortho-diphenol derivatives is important [31, 34]; (ii) the preservation system [32]; and (iii) the blackening and sterilization steps [17, 34].

The common lightness value for this product is approximately 20 units [34], but the value found in our samples ranged from 21.9 units (04-1355) to 28.8 units (05-465). Values of *a** and *b** parameters were lower than the common values, which produces more bluishness.

3. 4 Significant correlations between all analyzed parameters

Table 5 shows the most significant Pearson correlation coefficients found between all pairwise combinations of parameters analyzed in this study. There were some very significant values among them, for example the high correlation between a lower pH value and an increase in free acidity and the concentration of lactic acid, both in green olives (–0.80 and –0.91) and black olives (–0.92 and –0.92). Similarly, significant correlations related to the color of the fruits were found, which confirmed that an increase of *L** in Spanish-style green olives corresponds with a decrease of parameter *b** (–0.67) and an increase of the color index (0.96), while an increase of *L**, *a** and *b** in black olives matches an increase of the R_{700} value (0.71, 0.77 and 0.68). Especially interesting from the point of view of breeders were the correlations between fresh

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fruit and processed fruit traits. This would allow indirect selection for important table olive traits through the analysis of fresh olives instead of processed ones as it has been reported for olive oil traits [35]. In this sense, the content of oleuropein in the fresh pulp was highly correlated with all chemical parameters of the Spanish-Style green olives: pH (0.82), free acidity (-0.76), lactic acid (-0.80) and the content of phenols (0.56). It was interesting to observe the significant positive correlation between the fresh fruit's firmness and the firmness of the Spanish-style green fruit produced (0.57).

4 Conclusion

In conclusion, the new genotypes selected to be elaborated as table olives have to satisfy agronomic requirements, high weight (≥ 4 g/fruit), good pulp-to-pit ratio (≥ 6), low oil content (≤ 70 g/kg) and low to moderate bruising percentage. Four genotypes out of 33 met those requirements: 04-1396, 05-138, 05-166 and 05-322. Three additional genotypes (04-1390, 05-217 and 05-499) were specially selected for their very low bruising percentage (≤ 16%), an important issue for mechanical harvesting. With regard to the elaboration of the studied genotypes as Spanish-style olives, it was concluded that all of them developed a good fermentation by lactic acid bacteria and an acceptable final product. There were two exceptions, 04-1355 and 05-271, in whose fermenters a high concentration of antimicrobial compounds was detected and, consequently, the final product was inappropriate.

All the fruits of the new genotypes were preserved correctly under anaerobic acid conditions and no problem was detected during processing as black ripe olives. The major problems detected were with the firmness of the final product, especially

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with the genotype 02-1000, which indicates that a specific process should be designed for each new genotype.

Correlations found between some fresh fruit traits and the Spanish-style olives open the possibility of indirect selection in table olive breeding programs.

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Table 1. Genotypes studied by crosses. Meaning of the abbreviation of each cross. Traits of unprocessed fruits. Different capital letters within the same column indicate significant differences among mean values per cross according to Duncan's test at P<0.05

Genotypes	Cultivar or Cross	Fruit weight (g)	Pulp-to-pit ratio	Fruit shape	Oil content (g/kg)	Bruising (%)
Genitors	'Manzanilla de Sevilla' (M)	3.5	4.4	1.3	79	66
	'Gordal Sevillana' (G)	10.8	5.9	1.2	73	60
	'Changlot Real' (ChR)	-	-	1.5	69	9
	'Santa Caterina' (SC)	9.1	5.6	1.3	69	41
02-1000	'Manzanilla de Sevilla' x 'Gordal Sevillana'	5.6	6.7	1.2	-	44
02-1003		3.4	5.7	1.5	86	54
02-1010		4.7	7.6	1.3	88	40
02-1030		7.4	9.6	1.1	94	31
02-1038		4.0	5.6	1.4	84	61
	Mean M x G	5.0 (1.6)[a] B	6.9 (1.7)	1.3 (0.2)	88 (4)	46.2 (11.7)
04-1144	'Changlot Real' x 'Manzanilla de Sevilla'	3.2	7.6	-	95	34
04-1159		2.6	5.5	1.4	78	30
04-1167		2.0	6.2	1.3	72	18
	Mean ChR x M	2.6 (0.6) A	6.4 (1.1)	1.4 (0.0)	85 (9)	27.3 (8.3)
02-469	'Manzanilla de Sevilla' open pollination	4.0	6.0	1.3	86	40
04-1355		2.7	4.7	1.3	124	38
04-1390		7.4	7.5	-	118	13
04-1396		7.2	6.0	1.3	26	38
04-1445		4.3	6.8	1.1	132	52
	Mean M-OP	5.1 (2.1) B	6.2 (1.0)	1.2 (0.1)	97 (44)	36.4 (14.3)
05-593	'Gordal Sevillana' x 'Santa Caterina'	4.4	4.9	1.4	74	42
05-653		5.2	8.8	1.2	74	48
	Mean G x SC	4.8 (0.6) AB	6.9 (2.7)	1.3 (0.1)	74 (00)	45 (4.3)
05-054	'Manzanilla de Sevilla' x 'Santa Caterina'	3.8	4.0	1.3	48	37
05-056		4.6	5.4	1.4	49	59
05-138		4.0	6.6	1.3	60	40
05-150		5.7	5.4	1.3	92	44
05-161		4.6	6.5	1.3	95	63
05-166		4.7	6.9	1.4	35	15
05-217		4.7	7.8	1.1	73	4
05-237		4.0	5.3	1.3	70	6
05-271		4.0	4.6	1.3	73	17
05-322		4.6	6.4	1.3	52	40
05-326		3.4	5.9	1.2	71	50
05-343		4.0	5.2	1.3	44	27
05-396		3.8	4.9	1.2	41	52
05-402		3.9	5.6	1.3	47	29
05-408		4.1	6.4	1.3	79	74
05-455		6.3	5.4	1.3	75	20
05-465		4.5	7.5	1.3	78	32
05-499		5.6	10.4	1.2	88	16
	Mean M x SC	4.4 (0.8) AB	6.2 (1.4)	1.3 (0.1)	65 (18)	34.8 (19.9)

[a]mean and standard deviation between parenthesis.

Selected because they have a weight ≥ 4g

Selected because they have a pulp-to-pit ratio ≥ 6

Selected because they have an oil content ≤ 70 g/kg

Selected because they have a bruising percentage ≤ 40%

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Table 2. Chemical parameters of brine after 6 months of storage

	Spanish green olives				Natural green olives				
Genotypes	pH	Free acidity (g/kg)	Lactic acid (g/kg)	Phenols[a] (mmol/L)	pH	Free acidity (g/kg)	Lactic acid (g/kg)	Acetic acid (g/kg)	Phenols (mmol/L)
M	3.8	8.0	9.4	8.3	3.6	22.3	9.7	11.0	13.2
G	3.8	8.4	10.9	3.9	3.5	20.1	9.8	8.2	5.1
ChR	4.0	9.9	10.5	12.0	4.1	11.3	ND	8.0	13.9
SC	4.2	3.7	8.4	5.2	3.9	12.3	1.2	8.2	5.9
02-1000	3.7	8.7	10.2	5.2	3.4	20.4	10.0	8.0	8.9
02-1003	3.8	8.9	10.2	7.0	3.5	21.5	9.8	8.1	13.1
02-1010	4.1	5.4	6.6	7.7	3.5	18.3	8.6	8.3	12.1
02-1030	3.8	8.1	9.8	8.0	3.5	19.0	9.2	8.5	11.0
02-1038	3.8	8.3	9.8	5.8	3.6	18.0	11.1	8.8	7.3
Mean M x G	3.8 (0.2)[b]	7.9 (1.4)	9.3 (1.5)	6.8 (1.2)	3.5 (0.1)	19.4 (1.5)	9.7 (0.9)	8.3 (0.3)	10.5 (2.4)
04-1144	3.8	8.9	11.3	8.3	3.4	18.9	9.1	6.7	13.8
04-1159	4.2	7.8	8.4	9.0	3.6	19.9	9.4	8.9	8.8
04-1167	3.8	10.1	10.0	12.4	4.0	11.0	ND	7.4	15.3
Mean ChRxM	3.9 (0.2)	8.9 (1.2)	9.9 (1.5)	9.9 (2.2)	3.7 (0.3)	16.6 (4.9)	6.2 (5.3)	7.7 (1.1)	12.6 (3.4)
02-469	3.8	7.7	9.7	6.5	3.6	16.0	6.3	8.3	8.2
04-1355	5.6	2.5	1.2	12.8	3.9	11.9	ND	7.7	22.3
04-1390	3.7	8.2	10.6	5.5	3.3	24.2	14.0	9.1	15.5
04-1396	3.8	8.0	9.9	6.6	3.7	17.1	5.0	8.9	9.0
04-1445	3.9	7.8	9.2	11.1	3.6	19.6	7.3	10.6	18.4
M-OP	4.1 (0.8)	6.8 (2.4)	8.1 (3.9)	8.5 (3.2)	3.6 (0.2)	17.8 (4.5)	6.5 (5.0)	8.9 (1.1)	14.7 (6.1)
05-593	3.7	9.0	10.0	10.3	3.9	11.2	ND	7.7	10.5
05-653	3.8	8.7	13.4	8.8	3.4	21.5	10.6	7.7	12.5
Mean G x SC	3.7 (0.0)	8.9 (0.2)	11.7 (2.4)	9.5 (1.0)	3.7 (0.4)	16.4 (7.3)	5.3 (7.5)	7.7 (0.0)	11.5 (1.4)
05-054	3.7	8.0	10.8	4.7	4.0	12.0	ND	7.9	6.4
05-056	3.8	9.7	12.2	5.4	3.5	19.8	5.9	8.4	5.7
05-138	3.7	9.3	10.5	6.9	3.4	18.7	9.8	7.7	6.8
05-150	3.7	8.8	10.0	9.6	4.1	10.3	ND	8.1	13.4
05-161	3.8	9.8	10.2	5.1	3.5	18.4	9.6	7.6	8.9
05-166	3.9	10.0	8.5	4.9	3.4	19.2	11.0	7.3	5.0
05-217	4.0	7.4	8.7	5.2	3.7	18.3	ND	7.3	9.2
05-237	3.9	10.5	10.6	0.6	3.5	19.1	10.1	7.6	14.1
05-271	5.6	2.4	1.3	8.1	3.9	12.8	ND	8.8	16.3
05-322	3.8	11.7	10.6	3.9	3.5	21.0	10.6	7.5	5.6
05-326	3.8	10.4	10.1	6.5	3.5	17.6	6.8	8.6	8.5
05-343	3.9	9.0	8.7	7.4	4.0	11.4	ND	7.5	10.0
05-396	3.8	9.6	10.2	8.8	3.4	19.9	10.0	8.1	12.6
05-402	3.8	8.1	11.1	8.0	4.0	11.3	ND	7.8	9.0
05-408	3.7	8.7	11.2	8.6	3.5	19.5	6.7	8.2	12.2
05-455	4.1	6.5	5.5	9.4	3.5	17.1	6.3	8.5	16.9
05-465	3.8	10.5	10.5	5.8	3.5	17.9	8.0	8.0	7.7
05-499	3.8	9.8	10.3	9.1	3.5	19.0	8.4	8.1	13.0
Mean M x SC	3.9 (0.4)	8.9 (2.0)	9.5 (2.5)	6.6 (2.3)	3.6 (0.2)	16.9 (3.5)	5.7 (4.4)	7.9 (0.4)	10.1 (3.7)

[a]Sum of hydroxytyrosol, hydroxytyrosol-1-glucoside, hydroxytyrosol-4-glucoside, salidroside, tyrosol, *p*-cumaric acid, verbascoside, dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol, oleuropein, ester caffeic acid with secologanoside and comselogoside. [b]mean and standard deviation between parenthesis.

Genitors: M='Manzanilla de Sevilla', G='Gordal Sevillana', SC= 'Santa Caterina', ChR= 'Changlot Real'

OP=Open Pollination; ND: not detected

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Table 3. Firmness values (kN/kg pitted olives) in fresh and processed olives. Analyses were carried out in fresh and processed olives, after 6 months of fermentation in Spanish green olives and after color fixation step in Black ripe olives. Different capital letters within the same column indicate significant differences among mean values per cross according to Duncan's test at P<0.05

Genotypes	Fresh fruit	Spanish green olives	Black ripe olives
M	61.1	14.2	35.9
G	51.9	28.6	20.4
ChR	100.3	47.3	44.2
SC	51.8	34.3	31.8
02-1000	47.6	-	18.6
02-1003	46.8	36.3	35.2
02-1010	71.7	33.2	35.6
02-1030	39.7	28.2	43.4
02-1038	62.0	28.6	36.2
Mean M x G	53.6 (13.0)[a]A	31.6 (4.0)AB	33.8 (9.0)A
04-1144	66.2	24.0	40.8
04-1159	56.3	34.3	30.4
04-1167	61.0	48.9	39.8
Mean ChRxM	61.2 (5.0)B	35.8 (13.0)B	37.0 (6.0)B
02-469	68.7	17.7	31.2
04-1355	84.7	38.1	41.3
04-1390	39.1	22.2	24.2
04-1396	65.2	-	34.4
04-1445	59.5	39.4	38.1
Mean M-OP	63.4 (17.0)B	29.4 (11.0)A	33.8 (7.0)A
05-593	84.8	59.4	39.5
05-653	65.3	27.3	30.5
Mean G x SC	75.1 (14.0)C	43.3 (23.0)C	35.0 (6.0)AB
05-054	66.8	29.8	33.0
05-056	68.9	30.1	35.0
05-138	47.0	9.7	26.4
05-150	67.3	29.9	22.7
05-161	58.0	27.0	28.4
05-166	65.3	35.8	36.7
05-217	69.5	23.0	32.3
05-237	56.8	26.0	31.8
05-271	55.7	41.1	36.0
05-322	34.5	11.1	27.8
05-326	55.0	32.9	35.7
05-343	67.6	32.2	31.4
05-396	81.6	47.9	36.2
05-402	68.9	36.2	35.2
05-408	69.7	32.3	29.1
05-455	63.1	33.7	33.7
05-465	-	31.9	35.2
05-499	75.2	33.5	31.9
Mean M x SC	63.0 (11.0)B	30.2 (9.0)A	32.1 (4.0)A

[a]mean and standard deviation between parenthesis.

Genitors: M='Manzanilla de Sevilla', G='Gordal Sevillana', SC= 'Santa Caterina', ChR= 'Changlot Real'

OP=Open Pollination

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Table 4. Color parameters (*L**, *a** and *b**) of processed olives. Analyses were carried out after 6 months of fermentation in Spanish green olives and after color fixation step in Black ripe olives. Different capital letters within the same column indicate significant differences among mean values per cross according to Duncan's test at P<0.05

	Spanish green olives			Black ripe olives		
Genotypes	*L**	*a**	*b**	*L**	*a**	*b**
M	53.9 (0.4)[a]	4.2 (0.1)	34.6 (0.4)	25.1 (1.1)	0.7 (0.0)	-0.8 (0.1)
G	53.0 (0.0)	3.5 (0.2)	36.7 (0.2)	22.0 (0.4)	1.8 (0.3)	0.0 (0.3)
ChR	50.0 (1.6)	4.9 (0.3)	33.7 (1.2)	26.1 (0.9)	2.2 (0.1)	0.7 (0.1)
SC	49.0 (0.6)	4.1 (0.0)	32.5 (0.8)	23.4 (0.2)	1.3 (0.0)	-0.4 (0.0)
02-1000	51.3 (0.6)	3.9 (0.3)	38.8 (0.5)	23.3 (1.8)	2.9 (0.2)	2.2 (0.5)
02-1003	55.7 (0.4)	4.2 (0.0)	32.7 (0.2)	26.0 (1.5)	1.8 (0.4)	-0.1 (0.9)
02-1010	50.4 (0.3)	4.7 (0.1)	31.6 (0.6)	23.4 (1.4)	1.5 (0.1)	0.3 (0.2)
02-1030	53.8 (0.9)	4.1 (0.1)	33.4 (1.2)	23.1 (0.0)	1.3 (0.5)	-1.0 (0.6)
02-1038	56.3 (0.9)	4.2 (0.1)	36.8 (0.3)	27.9 (1.1)	1.5 (0.6)	0.4 (0.8)
Mean M x G	53.5 (2.5)	4.2 (0.3)	34.6 (2.9)AB	24.7 (2.2)	1.8 (0.7)	0.4 (1.2)
04-1144	53.4 (1.3)	3.3 (0.4)	34.5 (1.0)	22.9 (0.0)	1.1 (0.2)	0.0 (0.2)
04-1159	50.0 (1.6)	4.5 (0.2)	32.4 (1.3)	26.6 (0.6)	1.3 (0.0)	-0.4 (0.3)
04-1167	51.5 (1.5)	3.9 (0.1)	36.1 (1.8)	22.3 (0.1)	1.2 (0.3)	0.4 (0.3)
Mean ChR x M	51.7 (1.9)	3.9 (0.6)	34.3 (2.0)AB	23.9 (2.1)	1.2 (0.2)	0.0 (0.4)
02-469	54.9 (1.0)	4.7 (0.2)	37.8 (0.4)	22.9 (0.6)	2.4 (0.4)	1.5 (0.3)
04-1355	49.4 (2.2)	1.1 (0.1)	33.5 (1.9)	21.9 (0.5)	2.2 (0.2)	1.3 (0.2)
04-1390	61.7 (0.5)	4.7 (0.2)	43.1 (1.0)	23.7 (0.0)	1.6 (0.3)	0.0 (0.3)
04-1396	50.7 (0.3)	4.7 (0.0)	33.5 (0.7)	23.9 (1.6)	0.7 (0.3)	-0.9 (0.3)
04-1445	53.3 (1.6)	4.4 (0.1)	33.7 (1.8)	23.5 (0.3)	0.9 (0.1)	-0.7 (0.2)
M-OP	54.0 (4.6)	3.9 (1.5)	36.3 (4.1)B	23.2 (1.0)	1.6 (0.8)	0.2 (1.1)
05-593	52.5 (0.6)	3.2 (0.2)	28.1 (2.5)	22.2 (0.8)	0.9 (0.0)	-0.4 (0.1)
05-653	54.2 (1.1)	4.4 (0.2)	35.4 (0.5)	24.5 (1.3)	1.3 (0.2)	-0.6 (0.1)
Mean G x SC	53.3 (1.2)	3.8 (0.7)	31.7 (4.5)A	23.3 (1.6)	1.1 (0.2)	-0.5 (0.1)
05-054	49.3 (1.1)	4.0 (0.2)	32.6 (0.4)	25.5 (1.2)	2.9 (0.2)	2.4 (0.1)
05-056	55.6 (1.0)	3.5 (0.0)	37.4 (1.6)	24.8 (0.5)	2.7 (0.2)	1.3 (0.3)
05-138	56.3 (0.8)	3.3 (0.3)	38.2 (0.3)	24.2 (1.1)	2.2 (0.2)	1.0 (0.1)
05-150	47.8 (0.7)	4.7 (0.3)	32.1 (2.8)	22.3 (0.1)	1.1 (0.0)	0.4 (0.1)
05-161	52.6 (0.6)	4.2 (0.1)	30.6 (1.9)	24.2 (0.8)	0.9 (0.1)	-0.5 (0.1)
05-166	47.2 (1.0)	3.5 (0.2)	29.8 (0.2)	25.9 (1.3)	1.8 (0.4)	1.3 (0.6)
05-217	53.9 (1.1)	3.7 (0.7)	34.6 (1.1)	26.1 (1.0)	3.2 (0.2)	2.2 (0.4)
05-237	54.9 (1.2)	4.1 (0.2)	36.6 (1.0)	25.0 (0.1)	1.5 (0.0)	0.2 (0.1)
05-271	51.5 (0.1)	1.8 (0.4)	33.0 (0.6)	22.7 (0.2)	1.5 (0.4)	-0.1 (0.2)
05-322	52.6 (0.5)	4.0 (0.1)	37.4 (0.2)	25.7 (0.3)	2.2 (0.1)	1.8 (0.1)
05-326	57.0 (0.2)	4.1 (0.1)	40.7 (0.4)	23.7 (0.6)	2.0 (0.1)	0.8 (0.2)
05-343	49.2 (0.1)	3.9 (0.1)	29.0 (0.9)	22.1 (0.0)	1.0 (0.0)	-0.3 (0.0)
05-396	52.4 (1.0)	3.2 (0.2)	28.8 (0.5)	24.0 (0.3)	0.6 (0.1)	-1.3 (0.3)
05-402	51.4 (0.7)	3.9 (0.0)	34.8 (0.6)	23.7 (0.3)	2.2 (0.4)	0.9 (0.5)
05-408	54.4 (0.3)	4.7 (0.1)	37.5 (0.2)	24.5 (0.6)	0.9 (0.1)	-1.0 (0.2)
05-455	53.2 (0.6)	3.3 (0.0)	30.8 (1.6)	25.0 (0.3)	1.6 (0.2)	-0.2 (0.4)
05-465	52.8 (0.2)	4.1 (0.2)	30.2 (0.5)	28.8 (1.5)	0.8 (0.2)	-0.9 (0.0)
05-499	51.3 (0.6)	3.9 (0.1)	32.2 (0.5)	22.6 (0.2)	0.7 (0.0)	-0.3 (0.1)
Mean M x SC	52.4 (2.8)	3.8 (0.7)	33.7 (3.6)AB	24.5 (1.7)	1.7 (0.8)	0.4 (1.1)

[a]mean and standard deviation between parenthesis.

Genitors: M='Manzanilla de Sevilla', G='Gordal Sevillana', SC= 'Santa Caterina', ChR= 'Changlot Real'

OP=Open Pollination

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Table 5. Pearson's correlation coefficients between pairs of traits studied. Only coefficients with absolute values above 0.5 are included

	GrF	BlF	GrpH	GrFA	GrLA	GrP	NFA	NLA	NAA	NP	GrL*	Gra*	Grb*	Gri	BlR
OC										0.62***					
Ole			0.82***	-0.76***	-0.80***	0.56**				0.75***		-0.66***			
FF	0.57***					0.52***		-0.51**							
GrF	--	0.57***				0.54***	-0.51**						-0.51**		
BlF		--				0.50**									
GrpH			--	-0.80***	-0.91***							-0.72***			
GrFA				--	0.79***										
GrLA					--							0.65***			
GrP						--				0.64***					
NpH							-0.92***	-0.92***			-0.55***			-0.50**	
NFA							--		0.90***		0.58***			0.57***	
NLA								--			0.52**			0.52***	
NP										--					
GrL											--		-0.67***	0.96***	
Grb													--	0.70***	
BlL															0.71***
Bla															0.77***
Blb															0.68***

*Statistical significance at P ≤ 0.05;**Statistical significance at P ≤ 0.01; ***Statistical significance at P ≤ 0.001

OC: Oil Content in fresh fruit
Ole: Oleuropein in fresh pulp
FF: Fresh fruit firmness
GrF: Green olives firmness
BlF: Black olives firmness
GrpH: Green olives pH
GrFA: Green olives free acidity
GrLA: Green olives lactic acid
GrP: Green olives phenols
GrL: Green olives *L**
Gra: Green olives *a**
Grb: Green olives *b**
Gri: Green olives color index i
NpH: Natural green olives pH
NFA: Natural green free acidity
NLA: Natural green olives lactic acid
NAA: Natural green olives acetic acid
NP: Natural green olives phenols
BlL: Black olives *L**
Bla: Black olives *a**
Blb: Black olives *b**
BlR: Black olives color R_{700}

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Figure 1.

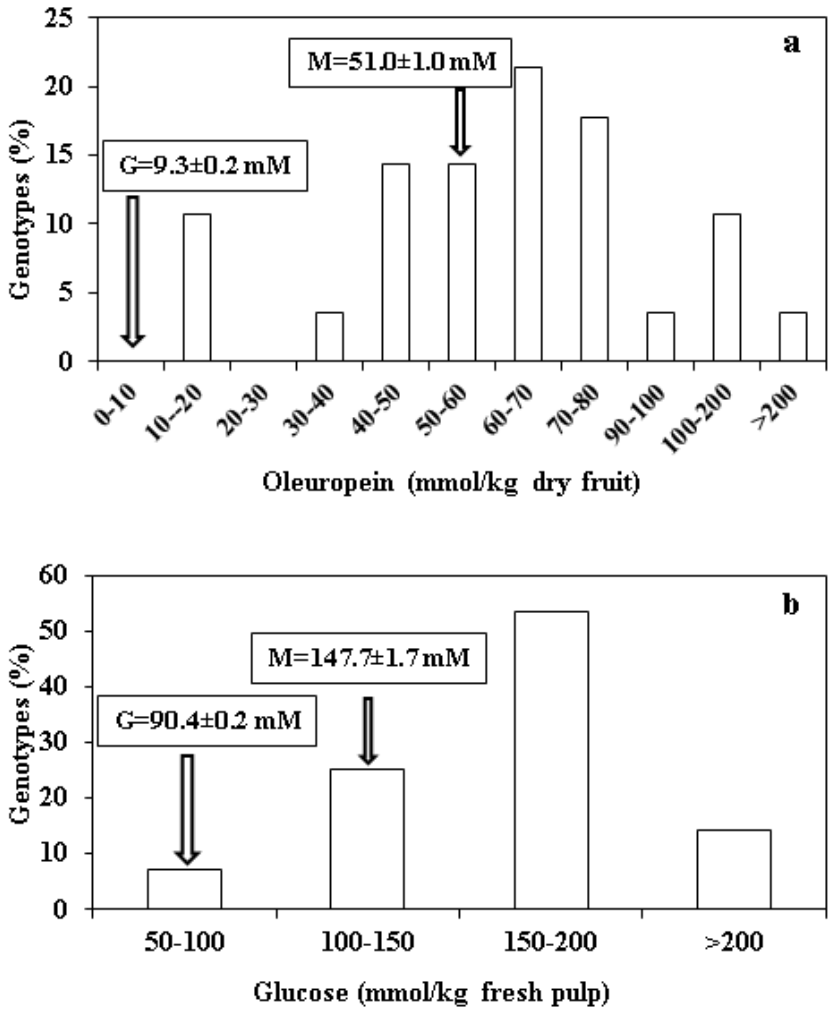

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Figure 2.

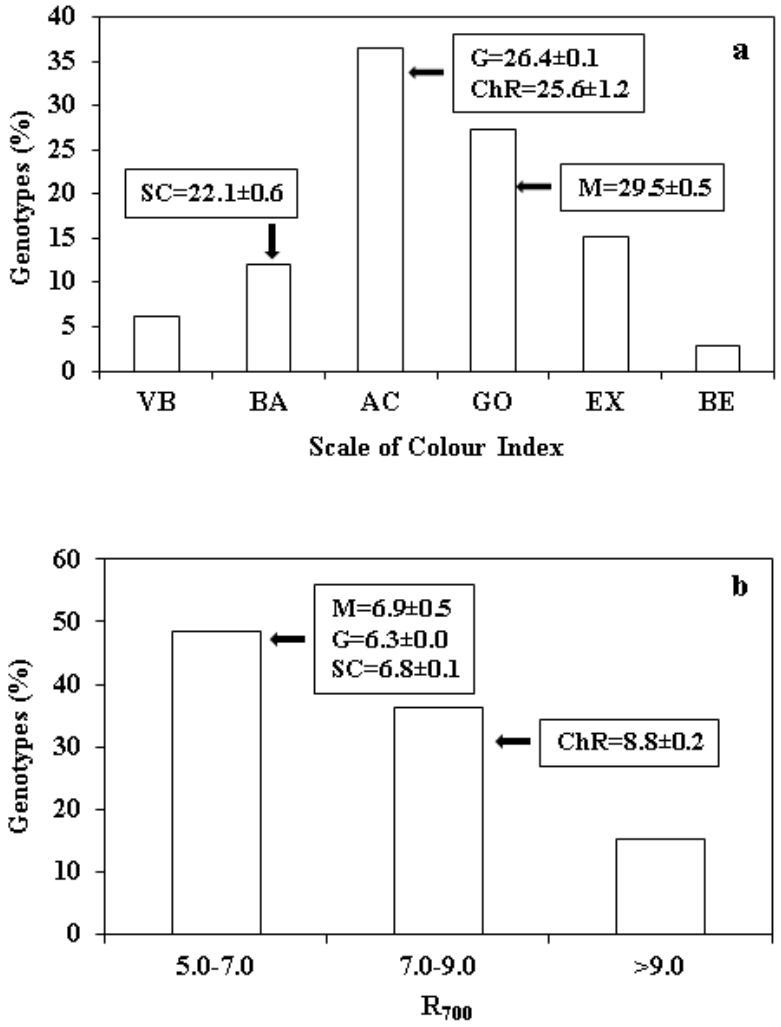

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Figure legends

Figure 1. Distribution of new genotypes based on oleuropein (a) and glucose (b) concentration in fresh olives. Values for genitors 'Manzanilla de Sevilla' (M) and 'Gordal Sevillana' (G) are indicated.

Figure 2. Color parameters of processed fruits. Distribution of new genotypes based on Color Index Scale in Spanish green olives (a) and based on R_{700} value in black ripe olives (b). Analyses were carried out after six months of fermentation in Spanish green olives and after the color fixation step in black ripe olives. Color Index Scale: VB (very bad), BA (bad), AC (acceptable), GO (good), EX (excellent), BE (better). Values for genitors 'Manzanilla de Sevilla' (M), 'Changlot Real' (ChR), 'Gordal Sevillana' (G) and 'Santa Caterina' (SC) are indicated.