Plant Genetic Resources: Characterization and Utilization

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Morphological characterization of the 1-year-old shoots in tetraploid vs. diploid leccino olive (*Olea Europaea* L.) cultivar

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Research Article

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Abstract

Polyploidization is known to cause changes in the ploidy levels of plant somatic cells that affect the morphological, physiological and chemical composition. The aim of this research was to investigate the effects of tetraploidization in olive. To do this, several characteristics of 1-year-old shoots of two olive genotypes were compared: the diploid cultivar Leccino (L), and its tetraploid mutant Leccino Compact (LC), considered a slow-growing genotype. LC differed significantly from L in the morphological characteristics, with higher values of diameter, dry mass and volume of the stem (46%, 103%, 102%, respectively), and higher area, mass and volume of the individual leaf (43%, 66%, 73%, respectively). LC also had thicker, longer and wider leaves (30%, 10%, 34%, respectively) and significantly lower leaf density (7%) and lower specific leaf area, leaf mass ratio and leaf area ratio (17%, 4%, 18%, respectively). Internode length and stem density were not significantly different. The results allowed us to thoroughly characterize the effects of tetraploidy on 1-year-old shoots in olive, and also suggest that the slow growth of LC is due to its lower leaf area per unit of total biomass, which reduces leaf area production and, consequently, light interception, resource availability and tree growth. These results will be useful for genetic improvement programmes and for planning further exploitation of tetraploidy in horticulture.

Introduction

Generally, the chromosome set of each somatic cell consists of two sets of chromosomes, and this normal condition is defined as diploid (indicated with 2n), while with polyploidy (or genome duplication) the number of chromosomes within the nucleus of a cell is greater than 2n (Dar and Rehman, 2017). Polyploidy is known to play an important role in plant evolution and ecology (Hegarty and Hiscock, 2008; Soltis *et al.*, 2014; Ruiz *et al.*, 2020), and it has been defined as a relevant phenomenon also for some genera belonging to the Oleaceae family (Taylor, 1945). Polyploidization is a process that can occur naturally, or it is induced (Islam *et al.*, 2022; Liu *et al.*, 2022). In plants, polyploidy is a pathway that allows to obtain new cultivars (Abdolinejad *et al.*, 2021). Artificial polyploidization is regularly used in plant breeding programmes, as genome doubling often results in improved functionality of some tissues and desired horticultural properties (De Baerdemaeker *et al.*, 2018).

At the phenotypic level, with the polyploidy important modifications of particular scientific interest are manifested. Variations in ploidy levels can induce changes in plant cells that affect morphological traits, anatomical structure, physiological responses and chemical composition (Doyle and Coate, 2019; Ruiz *et al.*, 2020; Trojak-Goluch *et al.*, 2021). The most frequently observed morphological and anatomical changes due to ploidy variation in plants are: size of stem, leaf, flower, stomata, stomatal density, leaf length/width ratio and shape, plant height, etc. (Trojak-Goluch *et al.*, 2021). Some studies report that polyploid plants are more resistant to environmental stresses (Ruiz *et al.*, 2020; Islam *et al.*, 2022) or to biotic stresses as found in the trifoliate orange (Wei *et al.*, 2020), in apple (Podwyszyńska *et al.*, 2021), and also in olive, with some tetraploid genotypes having greater resistance to the olive leaf spot, due to the fungal pathogen *Venturia oleaginea* (Pannelli *et al.*, 1992; Rugini *et al.*, 1996).

The cultivated olive (*Olea europaea* subsp. *europaea*) belongs to a genus that includes diploid species, with a chromosome number 2n = 46 (Rugini *et al.*, 1996; Besnard *et al.*, 2008). However, cases of tetraploidy (subsp. *cerasiformis*) and hexaploidy (subsp. *maroccana*) have been found in some subspecies (Besnard *et al.*, 2008). Polyploids are easily achieved with the use of chemical products (colchicine, oryzalin, etc.) or with physical means such as ionizing radiation (Rugini and Gutiérrez-Pesce, 2006). Polyploidy in olive has been studied with the aim to obtain compact genotypes suitable for high density systems, or to induce resistance to biotic and abiotic stress, and at the same time characterized by a high yield potential (Pannelli *et al.*, 1992). By irradiation

of self-rooted olive trees of the cultivars Frantoio and Leccino, two mutants with a compact habit were obtained, distinguished by the abbreviations FC and LC, respectively for Frantoio Compact and Leccino Compact (Pannelli *et al.*, 1990). These mutated olive trees have several morphological and physiological differences compared to the mother trees, such as shoots with larger diameters and shorter internodes, larger and thicker leaves, higher CO_2 assimilation rate and tolerance to drought (Pannelli *et al.*, 1990; Rugini *et al.*, 2016a). These tetraploid trees also differ anatomically, with larger cell size in leaf, stem and root tissues (Pannelli *et al.*, 1992). Furthermore, tetraploidy increased the size of flowers, ovaries and ovary cells, but not fruit size (Caporali *et al.*, 2014).

LC has been considered a low-vigour genotype (therefore characterized by slow growth and relatively small canopy), that could be used in crop intensification or as a rootstock (Pannelli *et al.*, 1992; Rugini *et al.*, 2020). So far, however, the mechanisms that explain the reduced size of LC have not been identified. This is important, because if the slow growth and compact habit depends on characteristics of the aerial part of the tree, using it as a rootstock would not reduce canopy growth and size in grafted trees (Paoletti *et al.*, 2023). The ideal genotype for intensive systems is one that grows slowly only as a consequence of early fruiting and thus greater biomass partitioning into fruit and lower partitioning into vegetative growth (Rosati *et al.*, 2017, 2018a, 2018b; Paoletti *et al.*, 2021). If a genotype grows slowly for other reasons, the slow growth may delay fruiting as well, making the genotype unsuitable for intensive systems. The tetraploid Leccino has had little success as a cultivar, because it associates the slower growth with reduced and delayed yield, compared to the diploid Leccino. We hypothesize that its slower growth and reduced size are related to changes in assimilate partitioning among vegetative components (i.e. leaves and stems), due to the tetraploidy, making this genotype not ideal for intensive systems, or indeed for any system. At present, there is some information on the morphological characteristics of the 1-year-old shoots in LC, but the data are still scarce and incomplete. However, there is evidence that its leaves and stems are thicker (Pannelli *et al.*, 1990, 1992). This might imply a reduced leaf area per unit of whole-shoot biomass. This could entail slower growth compared to the diploid form due to reduced light interception and photosynthesis per unit of shoot biomass. Therefore, the aim of this work was to have a complete evaluation of how tetraploidy affects the morphology of the shoots in tetraploid Leccino, compared to its diploid form, and investigate whether tetraploidy brings about differences in partitioning, especially in leaf area per unit of shoot biomass, which could explain the slower growth of the tetraploid.

Materials and methods

Experimental description

Two olive genotypes were selected and compared in this study: Leccino (L) the diploid control, and Leccino Compact (LC) a tetraploid mutant of L considered as a slow growing genotype. Three adult trees of similar size and age (about 30 years) of each olive genotype were chosen. The trees of this two genotypes were grown under the same management and were cultivated in the same orchard, located in the Umbria region, in central Italy (Lat. 42°46′22″ N, Long. 12°51′26″ E, Alt. 450 m a.s.l.). Trees were trained to a vase and they were spaced 10 by 7 m. Trees were cultivated according to traditional local standards, and no irrigation was applied. No chemical treatments against diseases were applied, but no visible signs of diseases were apparent at the time of sampling.

Plant material and data collection

On 30 January 2023, five 1-year-old shoots from each of three different olive trees per each genotype were collected (15 shoots per genotype). The shoots were collected from each tree, around the whole periphery of the canopy. Shoots were chosen of different length in order to represent the whole spectrum of shoot length present in the canopy. Length, number of nodes and number of leaves were evaluated for each shoot. For each shoot, two perpendicular diameters were measured with a digital calliper at the base of the stem. Stem diameter was the mean of these two measurements. Average internode length was calculated by dividing shoot length by the number of nodes per shoot. For each shoot, a scanned image of all leaves was taken to determine the total leaf area. The total leaf area was determined from the pixel-area calculation through pixel values using Photopea, an open-source programme for image processing. The individual leaf area was calculated by dividing the total leaf area by the number of leaves per shoot. For each shoot, the length (excluding petiole) and the maximal width of each leaf were measured. These two measurements were used to calculate the length-to-width leaf ratio. From three leaves selected from the basal, median and apical portion of each shoot, three disks of known area were cut, using a paper puncher, avoiding the midrib of the leaf. Immediately after cutting, leaf thickness was measured on each disk, using a digital calliper. Disks were dried in a ventilated oven at 35°C until constant weight, then weighed. Specific leaf area (SLA) was calculated by dividing the disc area by its dry weight after drying. The fresh stem volume of each shoot was measured by immersing the individual stems in a graduated cylinder. Stems were then dried with the same procedure as for the leaf disks. Stem dry mass was then determined, and the density of the dry stem was calculated from the dry mass and the fresh stem volume. The same method was used to determine leaf density, using only the three disks, instead of the whole leaf, to avoid including the midrib and the petiole, which would bias the measurements of the leaf lamina density and create noise in the data. The remaining leaves and leaf portions were dried as described for disks and stems. Leaf mass ratio (LMR) was calculated for each shoot, as the ratio of the leaf dry weight to total shoot dry weight (leaf mass + stem mass). Leaf area ratio (LAR) was calculated for each shoot, as the ratio of the total shoot leaf area to total shoot dry weight (leaf mass + stem mass). Stem-to-leaf dry mass ratio was obtained for each shoot, as the ratio of the stem dry weight to leaf dry weight.

Statistical analysis

The effects of the ploidy level on each parameter were statistically analysed by a one-way ANOVA or by covariance analysis (ANCOVA) in cases of covariation with stem length of the analysed parameter (e.g. diameter, dry mass, volume and node length). When the genotype effects were significant post hoc tests were performed and averages were compared using the Tukey HSD test ($P < 0.05$, $P < 0.01$ and $P < 0.001$). Relationships between parameters were evaluated by calculating the coefficients of determination (R^2) and the statistical significance of the fits.

Results

Effect of tetraploidy on stem parameters

Stem diameter increased linearly with its length and for both genotypes stem length ranged from about 7 to 32 cm (Fig. 1a). LC had significantly larger diameters than L (+46% on average) (Table 1).

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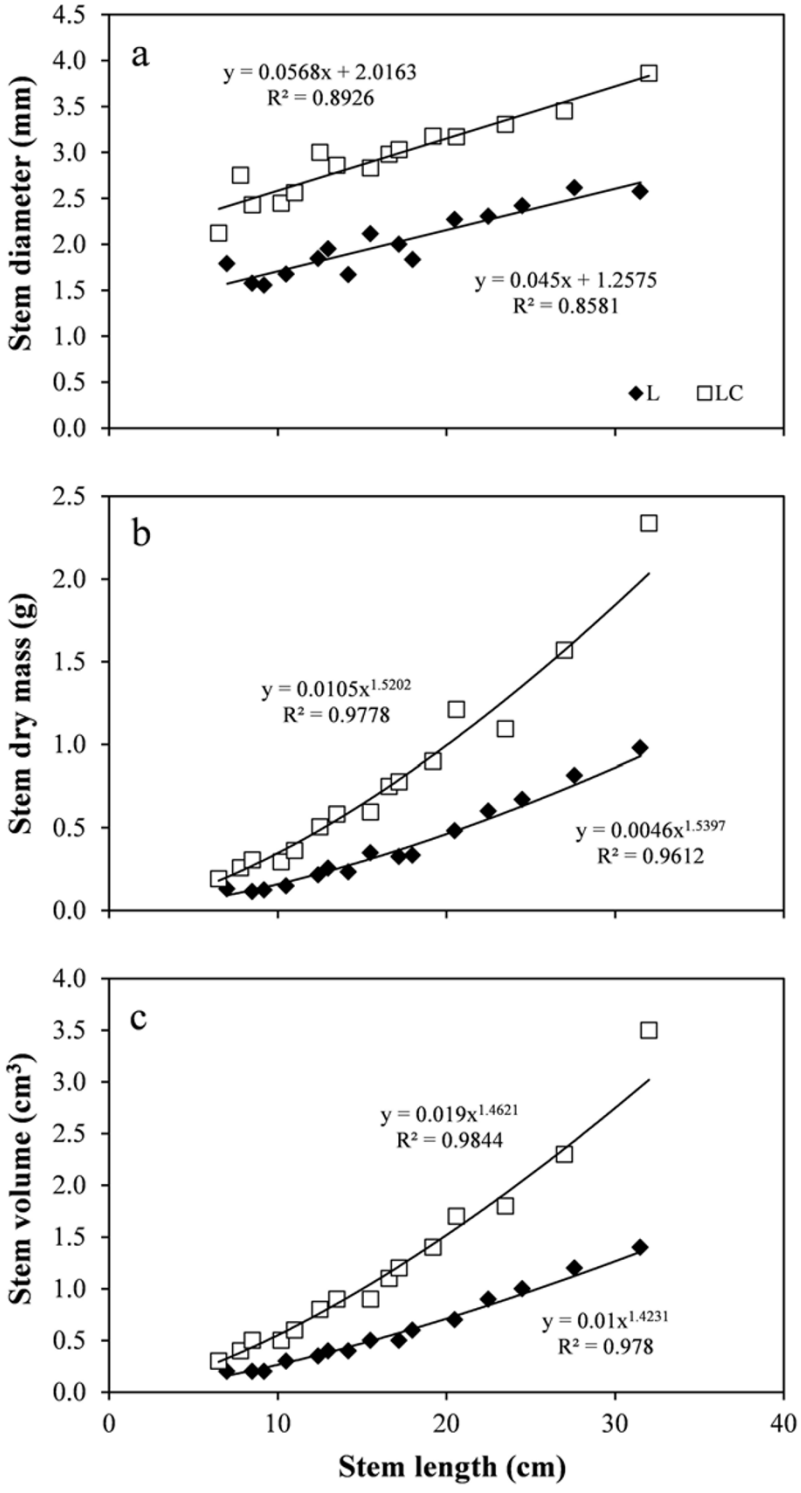

Figure 1. Relationship between stem diameter (a), stem dry mass (b), stem volume (c), and stem length for 1-year-old shoots of the two olive genotypes Leccino (L, diploid) and Leccino Compact (LC, tetraploid). Each point represents a single measured value. The genotype had a statistically significant effect as reported in Table 1.

Internode length also increased linearly with stem length (data not shown), but no significant difference was found between the two genotypes (Table 1). Stem dry mass increased exponentially with length, in both genotypes (Fig. 1b). At any stem length, LC had a significantly greater (about double) stem dry mass than L (Fig. 1b and Table 1). Stem volume also increased exponentially with length (Fig. 1c). Also in this case, LC had a volume significantly greater than L for the same length, about double on average (Table 1). There were no differences in stem density between the two genotypes (Table 1). Stem volume was linearly and positively correlated with stem dry mass with nearly identical regressions between genotypes (Fig. 2).

Effect of tetraploidy on leaf parameters

Total leaf area (i.e. all leaves of the shoot) was positively correlated with stem length (Fig. 3a). Total leaf area in LC was significantly

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Table 1. Comparison of the morphological characteristics between leccino (diploid control) and its tetraploid LC

1-year-old shoot parameters	Leccino (2n)	Leccino Compact (4n)	
Stem length (cm)	16.8 ± 1.88	16.1 ± 1.90	n.s. (1)
Stem diameter (mm)	2.01 ± 0.091	2.93 ± 0.114	*** (2)
Node number (*n*)	6.8 ± 0.60	6.9 ± 0.51	n.s. (2)
Internode length (cm)	2.41 ± 0.089	2.25 ± 0.119	n.s. (2)
Stem dry mass (g)	0.385 ± 0.0696	0.782 ± 0.1504	*** (2)
Stem volume (cm^3)	0.590 ± 0.0974	1.193 ± 0.2214	*** (2)
Stem density (g cm^{-3})	0.633 ± 0.0154	0.644 ± 0.0087	n.s. (1)
Total leaf area (cm^2)	51.2 ± 5.68	73.6 ± 6.87	*** (2)
Total leaf dry mass (g)	1.03 ± 0.122	1.71 ± 0.162	*** (2)
Total leaf volume (cm^3)	2.15 ± 0.240	3.78 ± 0.353	*** (2)
Leaf density (g cm^{-3})	0.545 ± 0.0064	0.508 ± 0.0069	*** (1)
Leaf length (cm)	4.25 ± 0.074	4.69 ± 0.068	*** (1)
Leaf width (cm)	1.16 ± 0.022	1.55 ± 0.026	*** (1)
Length-to-width leaf ratio (n)	3.71 ± 0.061	3.07 ± 0.034	*** (1)
Leaf thickness (mm)	0.366 ± 0.0083	0.475 ± 0.0071	*** (1)
Individual leaf area (cm^2)	3.69 ± 0.121	5.25 ± 0.150	*** (1)
Individual leaf dry mass (g)	0.074 ± 0.0030	0.122 ± 0.0034	*** (1)
Individual leaf volume (cm^3)	0.156 ± 0.0056	0.270 ± 0.0072	*** (1)
Specific leaf area SLA (cm^2 g^{-1})	50.5 ± 1.04	41.7 ± 1.00	*** (1)
Leaf area ratio LAR (cm^2 g^{-1})	37.7 ± 1.12	30.8 ± 0.95	*** (2)
Leaf mass ratio LMR (g g^{-1})	0.750 ± 0.0152	0.717 ± 0.0203	** (2)
Stem-to-leaf dry mass (g g^{-1})	0.342 ± 0.0289	0.411 ± 0.0421	** (2)

Data are averages ± S.E. Of 15 shoots, of varying length, per genotype. Within each row, statistically significant differences are indicated as: n.s. not significant; $^{**}P < 0.01$; $^{***}P < 0.001$. (1) denotes that the significance was determined by ANOVA, (2) denotes that the significance was determined by ANCOVA with stem length as the covariate.

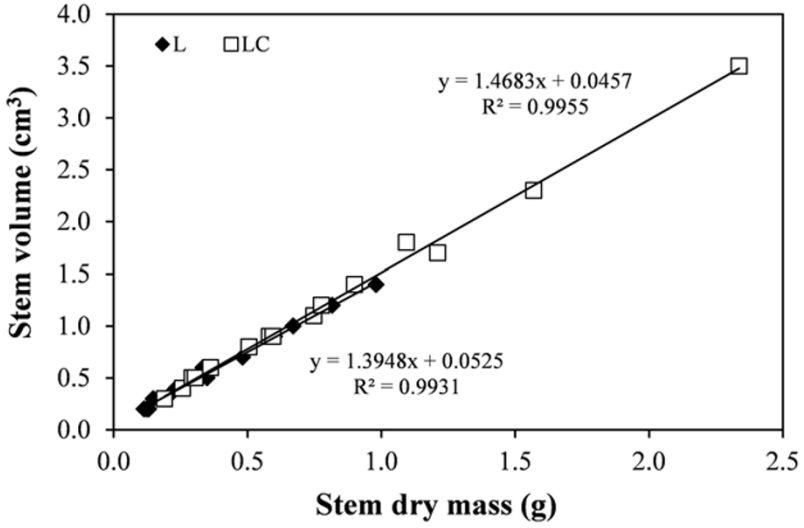

Figure 2. Relationship between stem dry mass and stem volume for 1-year-old shoots of the two olive genotypes Leccino (L, diploid) and Leccino Compact (LC, tetraploid). Each point represents a single measured value.

higher than in L (about 44% on average, Table 1). Total leaf dry mass also increased with stem length, and was significantly higher by almost 66% in LC (Fig. 3b and Table 1). The same was found for total leaf volume, which was nearly 76% higher in LC (Fig. 3c and Table 1).

LC had a significantly lower (about 7%) leaf density than L (Table 1). Total leaf volume was linearly and positively correlated with total leaf dry mass (Fig. 4).

Leaves in LC were significantly longer (about 10%) and wider (about 34%) than in L (Table 1). The length-to-width leaf

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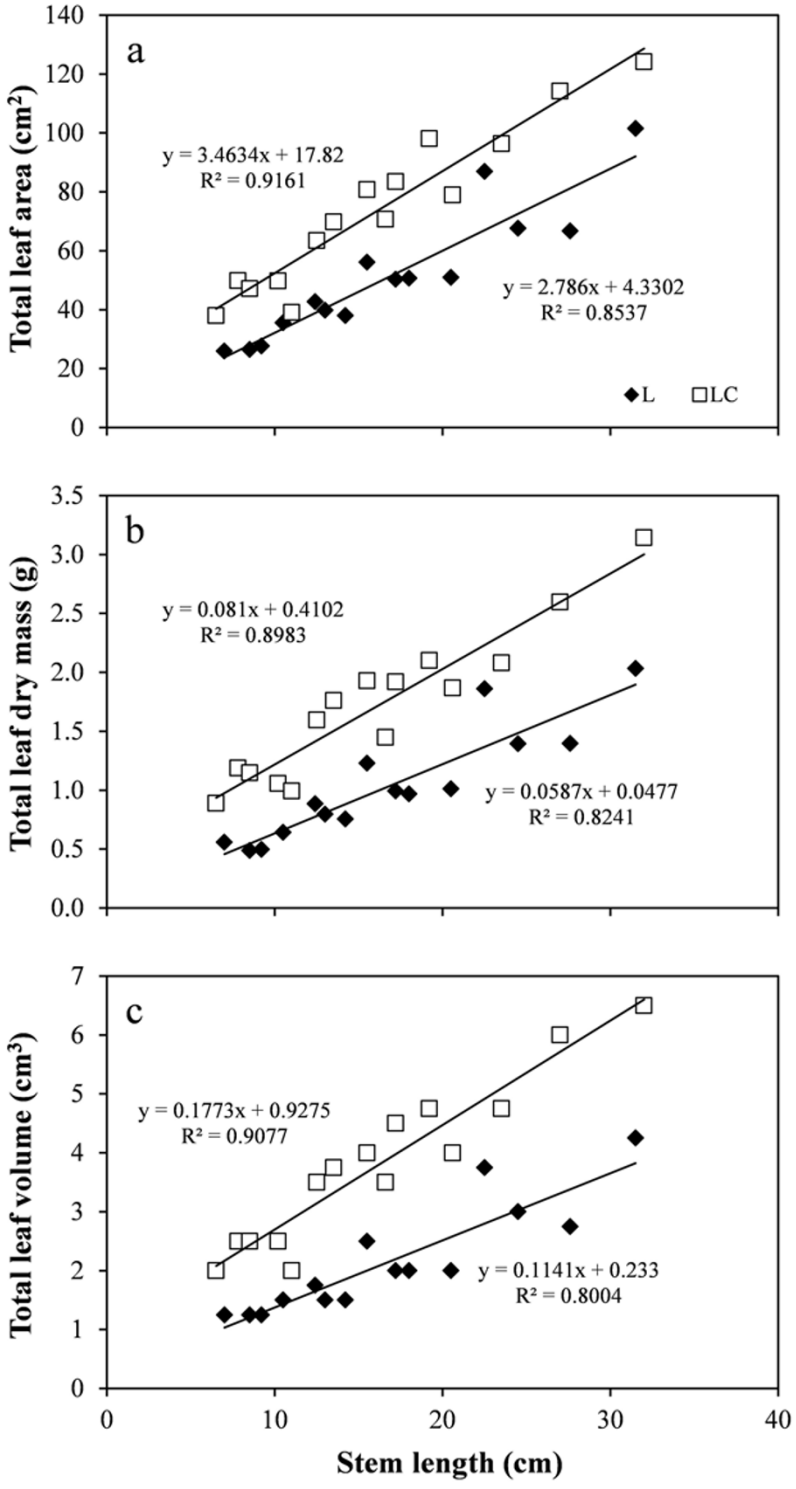

Figure 3. Relationship between total leaf area (i.e. all leaves of the shoot) (a), total leaf dry mass (b), total leaf volume (c), and stem length for 1-year-old shoots of the two olive genotypes Leccino (L, diploid) and Leccino Compact (LC, tetraploid). Each point represents a single measured value. The genotype had a statistically significant effect as reported in Table 1.

ratio was significantly lower in LC than in L by about 17% (Table 1). Furthermore, in LC the leaves were significantly thicker (+ 30%) than in L. LC had darker green leaf colour than L (Fig. 5a).

LC had significantly greater individual leaf area, individual leaf dry mass and individual leaf volume (+43%, +66% and +73% respectively, Table 1).

SLA was significantly lower (by about 17%) in LC than in L (Table 1).

Effect of tetraploidy on the wood-to-leaf biomass ratio

The leaf area ratio (LAR) decreased linearly with increasing stem length (Fig. 6a). LAR was significantly lower in LC, approximately

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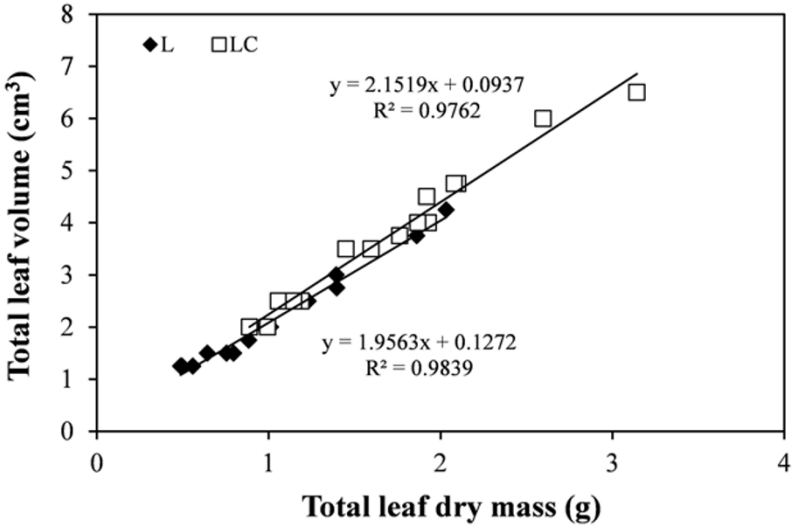

Figure 4. Relationship between total leaf dry mass and total leaf volume for 1-year-old shoots of the two olive genotypes Leccino (L, diploid) and Leccino Compact (LC, tetraploid). Each point represents a single measured value.

Figure 5. (a) Leaves of Leccino Compact (LC, tetraploid) (top) and Leccino (L, diploid) (bottom). LC leaves are evidently darker and of a different green colour than leaves of L. (b) 1-year-old shoot of L (left), and LC (right). The greater thickness of LC stem, at equal length, should be noted. The thicker stem in LC implies greater stem-to-leaf biomass ratio. This, added to thicker leaves, implies lower leaf area per unit of shoot (stem + leaves) biomass ratio.

18% less than in L (Table 1). Leaf mass ratio (LMR) also decreased with increasing stem length (Fig. 6b).

LMR was significantly lower in LC, although only by about 4% (Table 1). Stem-to-leaf dry mass increased with increasing stem length, was significantly higher in LC (by about 20% Table 1). The different thickness of the stem and the different level of stem-to-leaf ratio can also be appreciated in Fig. 5b.

Discussion

Although some data on LC already existed in the literature, this study provides a much more complete and detailed picture of the effects of tetraploidy on leaf and stem morphology. We analysed for the first time a set of 1-year-old shoots with a wide range of length, thus providing information on trait variability with shoot length. Furthermore, we considered a wide series of traits and their ratios, (such as the LMR, the LAR and the stem-to-leaf dry mass), which had been never considered before for the characterization of polyploid genotypes.

Pannelli *et al.* (1990) found that, for the same length of the 1-year-old shoot, LC had a larger diameter (about 38%) than L, in agreement with our results (Table 1). Also, in the common fig, a greater (+48 to 59%) basal diameter of the shoots has been observed for tetraploid plants compared to diploid ones (Abdolinejad *et al.*, 2021). An *in vitro* experiment with pear also indicated larger diameters for tetraploid shoots, compared to diploid ones (Sun *et al.*, 2009). Thicker stems for the same length, with tetraploidy, was also found in other plants such as *Plumbago auriculata* (Jiang *et al.*, 2020) and in two *Citrus* species (Jokari *et al.*, 2022).

In the present study, LC had slightly shorter (though not significantly) internodes (i.e. both the node number and shoot length were similar, Table 1), while Pannelli *et al.* (1990) found significantly shorter internodes in LC than in L.

Although it has been previously stated that LC has larger leaves than L (Pannelli *et al.*, 1992; Rugini and Gutiérrez-Pesce, 2006; Rugini *et al.*, 2020), no data were available. The present data confirm this statement and further show that LC leaves are both larger

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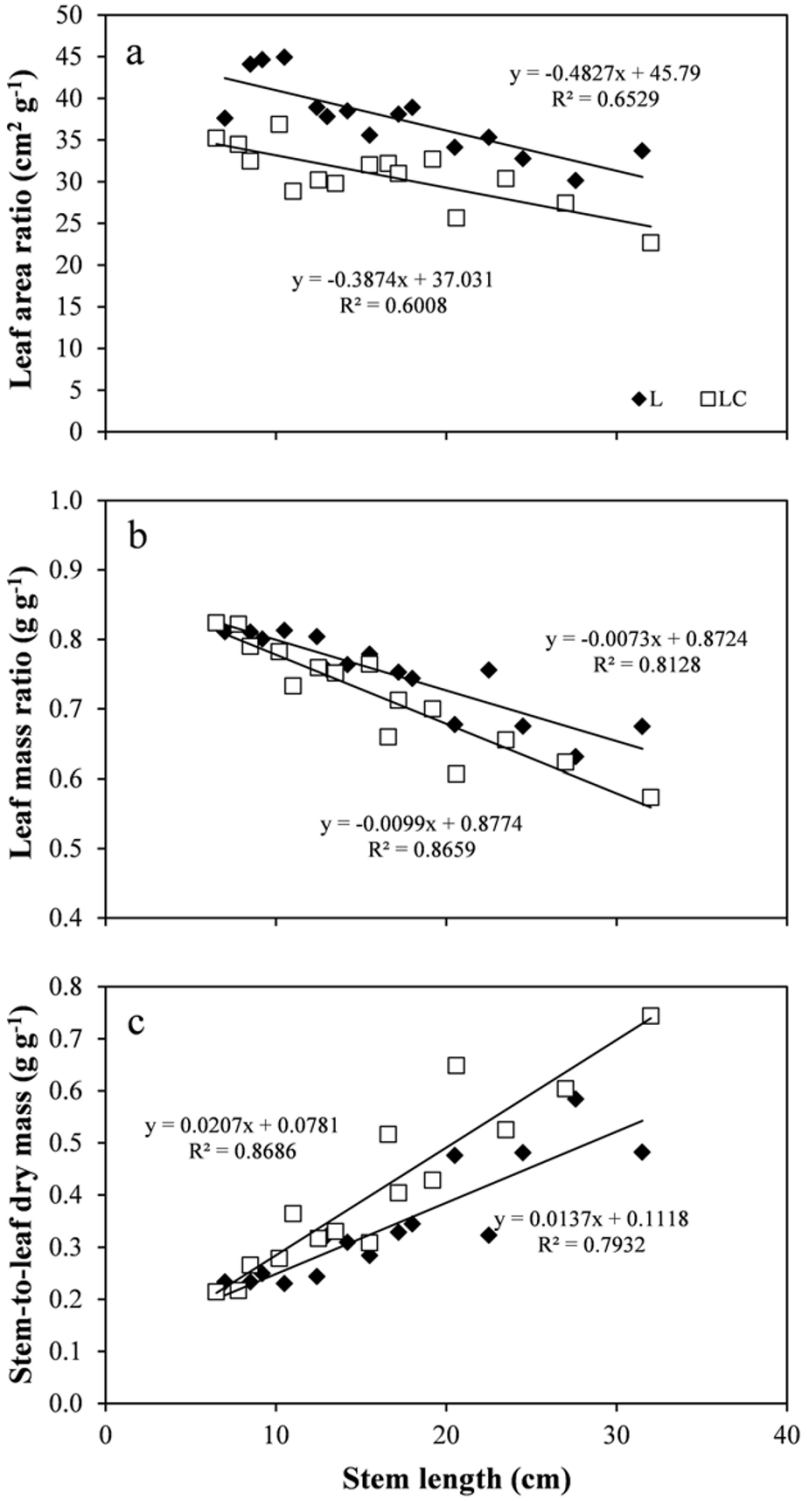

Figure 6. Relationship between leaf area ratio (a), leaf mass ratio (b), stem-to-leaf dry mass (c), and stem length for 1-year-old shoots of the two olive genotypes Leccino (L, diploid) and Leccino Compact (LC, tetraploid). Each point represents a single measured value. The genotype had a statistically significant effect as reported in Table 1.

and longer (Table 1). Larger leaves with tetraploidy have been reported also in several other species such as in *Ligustrum japonicum* (also in the Oleaceae family) (Fetouh *et al.*, 2016), in clones of *Pyrus communis* (Sun *et al.*, 2015), in *Salix viminalis* (Dudits *et al.*, 2016), *Morus multicaulis* (Xi-Ling *et al.*, 2011) and *Ziziphus jujuba* (Shi *et al.*, 2015; Wang *et al.*, 2019).

The leaf length-to-width ratio here found for L (3.71, Table 1) was consistent with what has been observed in a previous study with the same cultivar (Petruccelli *et al.*, 2020). The Leccino leaf is defined as elliptical (Saqib *et al.*, 2019), and since the length-to-width leaf ratio for both genotypes (L and LC) was lower than 4, their leaves can be considered elliptical (Mikhail, 2015;

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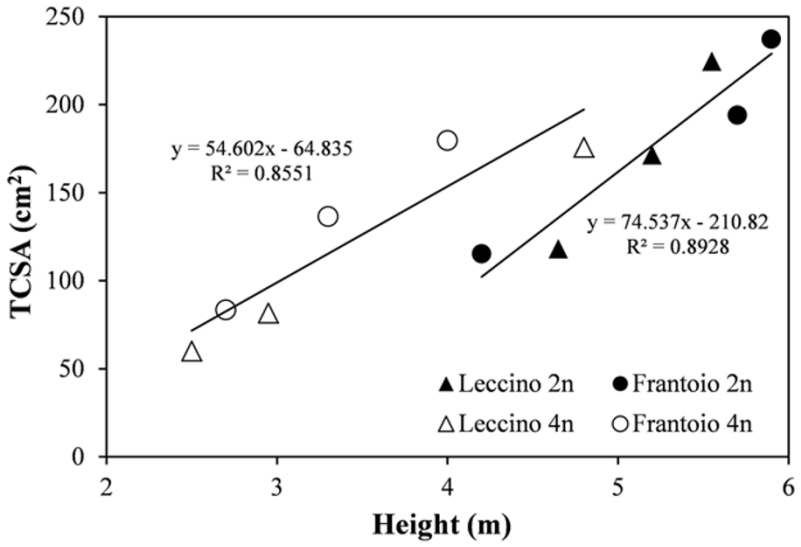

Figure 7. Relationship between plant height and trunk-cross-sectional area (TCSA) in four genotypes: Leccino (diploid and tetraploid) and Frantoio (diploid and tetraploid). Data graphically re-elaborating from Rugini et al. (2016b).

Touati *et al.*, 2022). However, LC had a markedly more elliptical leaf than L, in fact the length-to-width leaf ratio was significantly lower (3.07, Table 1). This ratio decreased significantly in tetraploids compared to the corresponding diploids, also in London plane (Liu *et al.*, 2007), black locust (Li *et al.*, 2021), Chinese jujube (Shi *et al.*, 2015; Cui *et al.*, 2017), mulberry (Xi-Ling *et al.*, 2011) and apple (Podwyszyńska *et al.*, 2021; Wójcik *et al.*, 2022).

Pannelli *et al.* (1990) found that LC had a thicker (about 30%) leaves than the diploid. The mean values obtained from Pannelli *et al.* (1990) were 0.517 mm for L and 0.670 for LC, therefore much higher than those found in this study (0.366 and 0.475 respectively for L and LC, Table 1), probably due to different sampling methodologies. In fact, in this study, thickness was measured on leaf disks, thus excluding the midrib. In Pannelli *et al.* (1992) on the other hand, the thickness was consistent with our observations for both genotypes. Increased leaf thickness with tetraploidy has been observed also in other species such as *Ligustrum japonicum* (Fetouh *et al.*, 2016), *Platanus acerifolia* (Liu *et al.*, 2007), *Robinia pseudoacacia* (Li *et al.*, 2021), *Morus multicaulis* (Xi-Ling *et al.*, 2011), *Malus domestica* (Hias *et al.*, 2017) and *Pyrus communis* (Sun *et al.*, 2015).

Another marked difference between the tetraploid leaves and the diploid ones was the colour, which was markedly darker green in LC (Fig. 5a). This characteristic had been also noted in other tetraploid plants such as London plane (Liu *et al.*, 2007), black locust (Li *et al.*, 2021), Rangpur lime (Allario *et al.*, 2011), Chinese jujube (Shi *et al.*, 2015; Cui *et al.*, 2017; Wang *et al.*, 2019), crape myrtle (Ye *et al.*, 2010), pear (Sun *et al.*, 2009) and apple (Hias *et al.*, 2017). The more intense green colour was often accompanied by a higher chlorophyll content in the leaves of tetraploid plants (Li *et al.*, 2021), suggesting that there is a close relationship between these two characteristics, as found in Chinese jujube (Wang *et al.*, 2019) and in apple (Hias *et al.*, 2017).

There is little information in the literature regarding SLA in olive cultivars and no data on Leccino. The value here found in L (50.5 cm^2 g^{-1}, Table 1) was slightly lower than previously observed in some Portuguese olive cultivars (ranging from 51.7 to 58.2 cm^2 g^{-1}) (Bacelar *et al.*, 2006). LC had significantly lower SLA than L (Table 1) due to the greater leaf thickness (Witkowski and Lamont, 1991). In the tetraploid leaves of *Eucalyptus polybractea* the specific leaf area (SLA) was also lower than in the diploid (Fernando *et al.*, 2019), while in poplar clones Alía *et al.* (2015) found no significant differences. Consistent with our result, Sun *et al.* (2009) found that the leaves of tetraploid pear trees had a higher specific leaf mass (SLM, which is the inverse of SLA) than in diploid plants.

LC is considered a slow growing genotype with reduced tree size, and therefore it is hypothesized that it could be used as a rootstock to reduce vigour in grafted cultivars (Pannelli *et al.*, 1992; Rugini *et al.*, 2020). However, there is no information why it grows more slowly. We hypothesized that tetraploidy might induce differences in partitioning between woody parts and leaves, reducing leaf area per unit of total biomass. This reduces light interception, canopy photosynthesis and thus resources for growth and/or fruiting (Rosati *et al.*, 2018c). The present results support this hypothesis, at least at the shoot level. The leaf mass ratio was lower for LC (Fig. 6b). Combining this with thicker leaves and lower SLA (Table 1), the leaf area ratio was even more reduced (about 18%) in LC, compared to L (Fig. 6a and Table 1). This means that for a total amount of biomass invested in shoots, LC has 18% less leaf area. In a small tree with little self-shading, this can significantly reduce tree carbon assimilation and thus tree growth. This effect adds exponentially, year after year, leading to increasingly larger differences in tree size year after year (Norby *et al.*, 2021). Thus, the observed shoot morphological differences here reported are sufficient alone to explain differences in tree growth rate.

However, the differences in partitioning here observed in the shoots are likely to extend to the rest of the woody structures of the tree. In fact, Umeda-Hara *et al.* (2022) studying poplar, observed that tetraploidy promotes basal growth of the stem predominantly in the radial direction, while growth is retarded in the longitudinal direction. In olive, Pannelli *et al.* (1992) found that the ratio of whole plant dry matter to leaf area ('Plant dw/Leaf area') was significantly higher in LC than in L, implying a lower leaf area ratio at the whole-tree level. Additionally, graphically re-elaborating data from Rugini *et al.* (2016b), we found larger TCSA (trunk cross-sectional area) in tetraploid olive trees than in diploid ones for the same plant height (Fig. 7), implying greater biomass partitioning into woody structures compared to the diploid plant.

This would add to the same mechanism described at the shoot level, further slowing down tree growth. It appears, therefore, that the slower growth of tetraploids might be simply explained with increased partitioning into woody structures and thus lower leaf

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area ratios, without resorting to the alternative hypothesis of a water stress mechanism (Fernando *et al.*, 2019).

Although a low vigour is desirable for high and very high-density olive groves, when growth is slowed down by greater proportional investments in woody structure and lower investments in leaf area, this is not advantageous, because slower growth due to lower resource availability also reduces and retards fruit production. The ideal tree grows quickly until reaching the desired canopy size, then slows down as a consequence of fruiting, i.e. partitioning more biomass into fruit, rather than partitioning more into wood (Famiani *et al.*, 2022; Paoletti *et al.*, 2023). Greater partitioning into wood slows down growth from the beginning, and then continues to reduce resource availability also later on, reducing yield. Therefore, tetraploid genotypes that invest proportionally more into woody structures might not be desirable genotypes. Nor the ability of slowing down growth would necessarily be transferred to the scion when using tetraploids as rootstock: the scion is likely to maintain its own partitioning habitus and grow normally.

While it is likely that tetraploidy will induce similar effects on other olive genotypes and/or in other environments, possible differences in the genotypic response to tetraploidy cannot be ruled out, nor possible genotype–environment interactions. Further experiments across different locations and genotypes are needed to investigate whether the present results can be generalized in olive.

Conclusions

This study allowed us to thoroughly characterize the effects of tetraploidy on the morphology of 1-year-old shoots. Tetraploidy induced significant changes in most of the observed parameters. As has been the case in other plants, all the changes found in this research due to tetraploidy level may be exploited to induce desirable agronomic characteristics. Generally, in addition to the morphological changes, polyploidy involves anatomical and physiological changes, and alterations of resistance to abiotic and biotic stresses, which in the case of LC, have yet to be thoroughly studied.

The results also suggest that LC (and tetraploid trees in general) grows slower than L because tetraploidy increases biomass partitioning into woody structures and reduces it in leaf mass and thus leaf area. For a total amount of biomass invested in shoots, LC has 18% less leaf area. At the whole tree level, the lower leaf area per unit of biomass is likely to be much further decreased by tetraploidy, given that not only the shoot stems are thicker, but also the branches and trunk. However, no data are available for the whole tree LAR. Future researches should explore this different partitioning of the biomass in woody structures at the whole tree level.

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