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Article

Description of Two Promising Walnut (*Juglans regia* L.) Selections with Lateral Bud Fruitfulness and Large Nuts

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Abstract: Walnut (*Juglans regia*) populations are considered a valuable genetic resource for genetic variability conservation and walnut cultivar improvement. In the current study, two walnut selections from Central Greece, "FM3" and "FM6", were evaluated for their characteristics to determine their perspective as new cultivars. Their main phenological and pomological characteristics were assessed according to IPGR (1994) and UPOV-TG/125/6 (1999) criteria for 10 consecutive years and compared with "Chandler". Results revealed that "FM3" presents high lateral flowering (70–80%), whereas "FM6" is 30–35%. Although the date of bud break and male blooming of both selections did not differ from "Chandler" ($p > 0.05$), the female blooming phase of "FM6" occurred earlier ($p < 0.05$) than "Chandler" and nut harvest was earlier than both "Chandler" and "FM3" ($p < 0.05$). Nut dimensions of both selections were higher than "Chandler" ($p < 0.001$). The mean in-shell nut weight and kernel weight of "FM3" were higher than "FM6" and "Chandler", and those of "FM6" were higher than "Chandler" ($p < 0.001$). Other positive nut characteristics were their light kernel color, the well kernel fill, and the easy removal of the kernel halves. Thus, the current results indicate that "FM3" and "FM6" could be considered promising gene pools in crossbreeding activities.

Keywords: walnut selection; *Juglans regia*; native population; phenological description; pomological description

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1. Introduction

In Greece, walnut (*Juglans regia* L.) has been traditionally cultivated in landraces, where the population is well adapted to local climatic conditions, forming a very rich genetic material of local walnut populations [1]. The largest production of walnuts comes from seedling populations, 70% of which are found in mountainous regions of the country (altitude > 500 m). At the same time, in the last 30 years, significant areas have been planted with fine walnut cultivars of large commercial interest, such as Serr, Gustine, Amigo, Vina, Hartley, Chandler, Lara and Franquette in irrigated orchards, which are progressively changing the traditional Greek form of walnut cultivation to a more enterprising one [2,3]. The annual walnut production of the country for the year 2021 amounted to 36,400 metric tons [4] with wide distribution (Thessaly 26%, Peloponnese 16.46%, Central Greece 15.5%, Central Macedonia 11.60%, Thrace 10.00%, West Greece 9.91%, West Macedonia 9.63%, Epirus 4%; remaining areas 2.66% [5]). However, the intensification and modernization of walnut cultivation with commercial, highly productive cultivars pose the risk of genetic diversity loss.

Due to its breeding characteristics, the walnut has developed a large number of genetic diversities via a lengthy history in a challenging environment [6]. Whether applied in conventional or modern breeding, genetic engineering, or traditional farming systems, plant genetic resources, including walnut ones, constitute a worldwide resource of walnut diversity of incalculable importance [7]. Therefore, it is convenient to take advantage of the existing variability to generate appropriate selections considering traits such as climatic adaptations, precocity, high productivity, good quality of nuts and kernels, and resistance to major diseases, such as anthracnose, which severely cause damage to several walnut cultivars [8,9]. The study of the plural Greek walnut seedling population and the strict selection of individuals with interesting characteristics, their propagation, and their establishment in collections for further evaluation in comparison with known walnut cultivars is of great interest because new valuable cultivars could generate from selected seedlings with crossings [1]. Germain (1997) [10] asserts that some characteristics, such as bud breaking, flowering season, harvest date, average fruit and kernel weight, and a strong adherence of the two halves, are easily inherited, while others, such as lateral fruit bearing habit, kernel color, and productivity are difficult to do so. Furthermore, selected seedling populations could serve as pollinators for common commercially cultivated walnut cultivars. Moreover, in the cases where a selection exhibits superior tree and fruit characteristics it can be recommended as a newcultivar. Additionally, seedling populations can be studied and used as selections resistant to diseases, more importantly to walnut blight.

In the past, local walnut populations were detected, after systematic explorations into walnut cultivation regions of Greece, in the frame of nuts European projects [1,11,12]. Data from the research revealed that walnut trees were distributed throughout the country, most of which were found in Peloponnesus, Thessaly, Central Macedonia, and Crete. After in situ evaluation of local walnut populations given predetermined criteria (good fruit characteristics, good productivity, desirable lateral fruitfulness, resistance to pests and diseases, early harvest dates, etc.), twenty-seven and one hundred thirty-six [1,10] individuals were selected to create a germplasm bank, many of which presented lateral bud fruitfulness and often produced good quality nuts [1]. Subsequently, thirteen and seventy populations were finally chosen, respectively, and grafted and cultivated ex situ at the Department of Nut Trees of Hellenic Agricultural Organization (ELGO)–DIMITRA for further evaluation [1,2,11,12]. Some of these native populations from Peloponnesus, Macedonia, Crete, Central Greece, and Epirus, as well as internationally cultivated walnut genotypes, kept in selections, were further evaluated for their genetic diversity using inter-simple sequence repeat (ISSR) markers. Results revealed that the Greek native population genotypes presented higher genetic diversity than the international cultivars [13]. According to the authors, the considerable genetic diversity of native walnut populations across the country provides excellent potential for walnut breeding to directly obtain new cultivars or to exploit them in cross-breeding efforts. Thus, Greece could be considered a long-established region of walnut diversity with a vast gene pool resource.

A number of the native walnut selections that were cultivated in the frame of the evaluation of Greek seedling populations are still kept in collections at the Department of Nut Trees of ELGO–DIMITRA for systemic evaluation. After the first early preliminary evaluation of Rouskas et al. (1997) [11] and Rouskas and Zakynthinos (2001) [12], two selections coded "FM3" and "FM6" were noticed for their agronomic traits. Both selections presented mid-early bud break, large nuts, and good to very good kernel quality. Thus, the aim of the present study was the further systemic evaluation of these selections for their phenological and pomological characteristics. The agronomic value of these selections, such as lateral bud fruitfulness, large fruits, kernel quality, and other traits, might give promising results that are very important in walnut breeding activities.

2. Materials and Methods

2.1. General Information

This study was conducted at the Department of Nut Trees of the Institute of Plant Breeding and Genetic Resources of ELGO–DIMITRA. In an effort to find and select walnut individuals from the Greek local populations as genitors with high yield, high-quality nuts, and desirable phenological traits, past research programs were carried out in which walnut seedling genotypes were detected and studied in situ in the mountainous areas of Central Greece [1,12]. A number of genotypes were selected based on predetermined criteria (good fruit characteristics, good productivity, desirable lateral fruitfulness, pest and disease resistance, etc.) and checked for Cherry leaf roll virus (CLRV) by the Institut national de la recherche agronomique (INRA, Bordeaux, France). From these genotypes, thirteen selections were finally selected and were grafted onto seedling rootstocks of the Hartley cultivar because they combine robustness with uniform growth. Grafted plants were established in 1989 at a 10 × 10 m planting distance for systemic evaluation ex situ. Collections of the "Chandler" cultivar of similar age were also established in the same experimental orchard of the Department of Nut Trees and were included in the trial as a reference cultivar. After preliminary evaluation at early ages, among all selections, two coded "FM3" and "FM6" were noticed for their phenological and pomological characteristics, i.e., both selections presented mid-early bud break, large nuts, and good to very good kernel quality and high kernel efficiency [12]. For these reasons, they were further systematically evaluated from 2013 to 2022. Both selections are established only in the Department of Nut Trees.

All trees of walnut genotypes evaluated received the same cultivation care (such as fertilization, irrigation, pruning, and pest and disease control) and maintenance. Irrigation was conducted by pipes with micro drippers, and the applied pruning system was cup-shaped with a central axis.

Soil, after examination, was characterized as clayey (clay 52–56%, silt 32–36%, sand 8–14%) [14]. Moreover, soil provided water saturation of 65–70%, electrical conductivity of 0.55–0.59 mS/cm, total salts of 0.2%, and pH of 7.7 [15].

2.2. Meteorological Data

During the whole experimental period (2013–2022), meteorological data concerning average monthly temperature, minimum and maximum temperature, average monthly precipitation, and relative humidity were recorded by a meteorological station that is established in the institute, and mean values (±SD) were calculated, as shown in Figures 1 and 2.

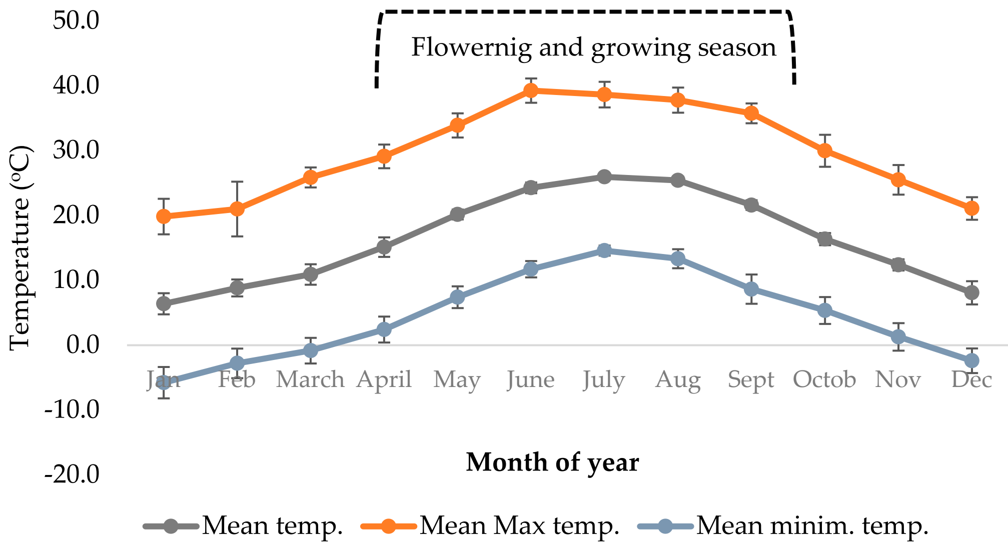

Figure 1. Mean temperature (°C), mean minimum and maximum temperature (°C), per month, for the years studied (2013–2022).

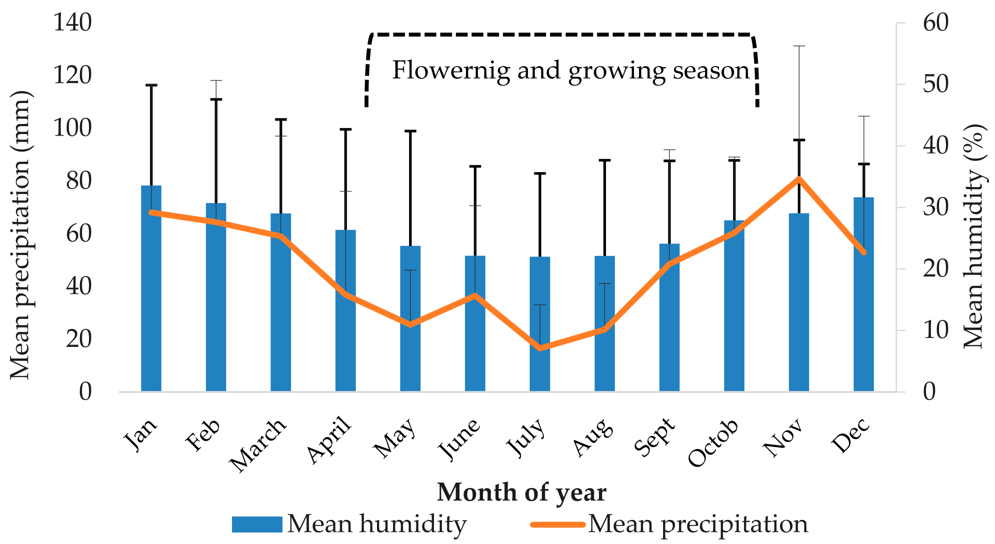

Figure 2. Mean precipitation (mm) and mean relative humidity (%), per month, for the years studied (2013–2022).

2.3. Phenological Characteristics Observation

Phenological characteristics of FM3", "FM6", and "Chandler" (six trees per genotype) were evaluated according to International Plant Genetic Resources Institute (IPGR, 1994) [16] criteria for ten consecutive years, from 2013 to 2022 (Tables 1 and 2). Lateral bud flowering is expressed as the percentage (%) of current season lateral shoots with female flowers in young trees according to IPGR (1994) [16] and characterized as terminal <30%, intermediate <60%, and lateral >60% [16]. For the characterization of the precocity of the leafing, according to the climatic conditions of the area, the following scale was followed: too early up to 21 of March, early from 22 of March to 27 of March, mid-early from 28 of March to 2 of April, mid-late from 3 of April to 10 of March, and late from 11 of April to 18 of April.

Table 1. Average phenological characteristics of "FM3" and "FM6" walnuts compared to the reference cultivar "Chandler".

Characteristics [1,2]	**"FM3"**	**"FM6"**	**"Chandler"**
Lateral bud flowering [3] (%)	70–80	30–35	90
Tree vigor	Intermediate (5)	Intermediate (5)	Intermediate (5)
Growth habit	Semi-erect (2)	Semi-erect (2)	Semi-erect (2)
Branching	Intermediate (5)	Intermediate (5)	Dense (7)
Dichogamy	Protandrous	Protogynous	Protandrous
Hull dehiscence	Dehiscent (3)	Dehiscent (3)	Dehiscent (3)
Sunburn susceptibility of hull [4]	Low (3)	Low (3)	Low (3)
Susceptibility to codling moth (*Cydia pomonella* L.) [5]	Intermediate (5)	Intermediate (5)	Intermediate (5)
Susceptibility to walnut blight (*Xanthomonas campestris*) [5]	Low (3)	Low (3)	Low (3)

[1] According to IPGR (1994) descriptors for walnut. [2] Numbers given in parentheses refer to the respective description according to IPGR (1994). Data are presented as means ± SD. [3] Lateral bud flowering percentage (%) of current season lateral shoots with female flowers in young trees, according to the IPGRI descriptor. [4] Sunburn susceptibility scale: 1, 0–20%; 3, 21–40%; 5, 41–60%; 7, 61–80%; and 9, 91–100% according to IPGR (1994) descriptor. [5] Susceptibility to codling moth and walnut blight scale: 0, no signs of susceptibility; 1, 0–20%; 3, 21–40%; 5, 41–60%; 7, 61–80%; and 9, 91–100% according to IPGR (1994) descriptor.

2.4. Nut Trait Measurements and Observations

Pomological characteristics of "FM3", "FM6", and the reference cultivars "Chandler" (Tables 3 and 4) evaluation was conducted on 20 nuts per genotype randomly collected at harvest time from 2013 to 2022. Nuts were evaluated in accordance with the criteria of IPGR (1994) [16] and UPOV-TG/125/6 (1999) [17]. Nut height, nut width, and nut thickness were measured using a digital caliper of 0.01 mm, and nut roundness index was calculated according to the equation; nut width + nut thickness)/2) × nut height (UPOV (1999) [17]). In-shell nut weight and kernel weight were determined with a digital scale (0.01 g). The kernel weight to in-shell nut weight ratio was calculated as kernel percentage (%).

Table 2. Average phenological characteristics concerning blooming and harvesting time of "FM3" and "FM6" walnuts compared to the reference cultivar "Chandler".

Characteristics [1]	"FM3"	"FM6"	"Chandler"	*p*-Value
Date of bud break	91.90 ± 10.09 days (2 April ± 10.09 days)	90.18 ± 10.23 days (31 March ± 10.23 days)	97.09 ± 9.25 days (7 April ± 9.25 days)	0.249
First female bloom date	109.80 ± 7.42 days [ab] (20 April ± 7.42 days [ab])	105.55 ± 8.82 days [a] (16 April ± 8.82 days [a])	115.18 ± 7.03 days [b] (25 April ± 7.03 days [b])	0.025
Last female bloom date	119.60 ± 7.24 days [a] (30 April ± 7.24 days [a])	116.27 ± 8.92 days [a] (26 April ± 8.92 days [a])	126.82 ± 6.55 days [b] (6 May ± 6.55 days [b])	0.010
First male bloom date	104.60 ± 7.60 days (15 April ± 7.60 days)	110.55 ± 8.58 days (20 April ± 8.58 days)	110.55 ± 7.84 days (20 April ± 7.84 days)	0.170
Last male bloom date	114.90 ± 7.09 days (25 April ± 7.09 days)	120.36 ± 8.88 days (30 April ± 8.88 days)	120.27 ± 7.90 days (30 April ± 7.90 days)	0.225
Harvest date	273.00 ± 2.36 days [b] (30 September ± 2.36 days [b])	268.36 ± 4.54 days [a] (25 September ± 4.54 days [a])	273.45 ± 5.41 days [b] (30 September ± 5.41 days [b])	0.018

[1] According to IPGR (1994) descriptors for walnut. Data are presented as means ± SD. Data are presented as day of the year and as calendar dates (day, month). Different superscripts denote significant differences.

Table 3. Nut and kernel traits of "FM3" and "FM6" walnuts compared to the reference cultivar "Chandler".

Characteristics	"FM3"	"FM6"	"Chandler"
Nut size [1]	Very large (9)	Very large (9)	Medium (5)
Nut shape [2]	Broad elliptic (7)	Broad elliptic (7)	Ovate (4)
Shell texture [2]	Rough (7)	Medium (5)	Smooth (3)
Shell color [2]	Medium (5)	Medium (5)	Medium (5)
Shell integrity [2]	Intermediate (2)	Intermediate (2)	Complete shell (3)
Nut: shape in longitudinal section through suture [1]	Broad elliptic (7)	Broad elliptic (7)	Ovate (4)
Nut: shape in longitudinal section perpendicular to suture [1]	Broad elliptic (7)	Broad elliptic (7)	Ovate (4)
Nut: shape in cross-section [1]	Oblate (1)	Oblate (1)	Circular (2)
Nut: shape of base perpendicular to suture [1]	Cuneate (1)	Cuneate (1)	Rounded (2)
Nut: shape of apex perpendicular to suture [1]	Truncate (3)	Truncate (3)	Rounded (2)
Nut: prominence of apical tip [1]	Weak (3)	Weak (3)	Weak (3)
Nut: position of pad on suture [1]	On upper 2/3 of nut (2)	On upper half of nut (1)	On upper half of nut (1)
Nut: prominence of pad on suture [1]	Medium (5)	Strong (7)	Medium (5)
Nut: width of pad on suture [1]	Broad (7)	Broad (7)	Narrow (3)
Nut: depth of groove along pad on suture [1]	Medium (5)	Deep (7)	Shallow (3)
Nut: structure of surface of shell [1]	Strongly grooved (3)	Moderate grooved (2)	Slightly grooved (1)
Nut: thickness of shell	Medium (5)	Medium (5)	Very thin (1)
Nut: adherence of two halves of shell [1]	Strong (7)	Strong (7)	Weak (3)
Nut: thickness of primary and secondary membranes [1]	Medium (5)	Medium (5)	Thin (3)
Kernel color [1]	Light (3)	Light (3)	Very light (1)
Kernel size [1]	Very large (9)	Very large (9)	Medium (5)
Kernel fill [2]	Well (7)	Well (7)	Well (7)
Kernel flavor [2]	Satisfactory (1)	Satisfactory (1)	Satisfactory (1)
Ease of removal of kernel halves [1]	Easy (3)	Easy (3)	Very Easy (1)

[1] According to UPOV (1999) descriptors for walnut. Numbers given in parentheses refer to the respective description. [2] According to IPGR (1994) descriptors for walnut. Numbers given in parentheses refer to the respective description.

Table 4. Average nut and kernel traits of "FM3" and "FM6" walnuts compared to the reference cultivar "Chandler".

Characteristics	"FM3"	"FM6"	"Chandler"	*p*-Value
Nut height (mm) [1]	45.44 ± 0.324 [b]	48.54 ± 0.424 [c]	41.81 ± 0.400 [a]	<0.001
Nut width (mm) [1]	37.34 ± 0.233 [b]	40.55 ± 0.192 [c]	32.99 ± 0.429 [a]	<0.002
Nut thickness (mm) [1]	43.36 ± 0.385 [b]	42.62 ± 0.284 [b]	35.02 ± 0.217 [a]	<0.001
Nut roundness [1]	1830.0 ± 24.06 [b]	2018.2 ± 18.73 [c]	1422.3 ± 19.06 [a]	<0.001
Shell thickness (mm) [2]	2.52 ± 0.058 [c]	2.10 ± 0.038 [b]	1.85 ± 0.042 [a]	<0.001
In-shell nut weight (g) [2]	23.70 ± 0.220 [c]	21.36 ± 0.199 [b]	13.41 ± 0.257 [a]	<0.001
Kernel weight (g) [2]	11.75 ± 0.200 [c]	10.16 ± 0.100 [b]	6.67 ± 0.171 [a]	<0.001
Kernel percentage (%) [2]	49.56 ± 0.663 [b]	47.61 ± 0.468 [a]	49.94 ± 0.348 [b]	0.023

[1] According to UPOV (1999) descriptors for walnut. [2] According to IPGR (1994) descriptors for walnut. Data are presented as means ± SE. Different superscripts denote significant differences.

2.5. Assessment of Susceptibility to Abiotic and Biotic Factors

For two years (2020–2021) susceptibility of hulls to sunburn, susceptibility of nuts to codling moth (*Cydia pomonella* L.), and walnut blight (*Xanthomonas arboricola* pv. *juglandis*) was studied (Table 1), according to IPGR (1994) [16]. Particularly, in order to evaluate the degree (%) of susceptibility to sunburn, nuts that were not protected by tree foliage and exposed to sunlight at temperatures above 42 °C were examined for sunburn symptoms under natural conditions. The susceptibility of nuts to both codling moth (*Cydia pomonella* L.) and walnut blight (*Xanthomonas arboricola* pv. *juglandis*) was assessed under the usual phytosanitary orchard control. At harvest, 100 nuts were randomly collected from each genotype, and the percentage (%) of infected nuts was determined.

2.6. Statistical Analysis

Bud break and flowering dates of the genotypes studied were determined as calendar days (from 1 January as day 1). The data are expressed as mean calendar day, as well as date ± standard deviation (SD). Nuts measurements are expressed as means ± error deviation (SE). The statistical analysis of the data was performed using the Statistical Package for the Social Sciences (2011) [18].

3. Results

3.1. Phenological Evaluation

According to phenological data collected, "FM3" walnut presented 70–80% lateral bearing habit, close to "Chandler", while that of "FM6" was found at 30–35%. Both "FM3" and "FM6" trees presented intermediate vigor and semi-erect habit, similar to "Chandler", and intermediate branching. The flowering of "FM3" is protandrous, whereas that of "FM6" is protogynous. (Table 1).

As shown in Table 2, phenological observations revealed that the mean date of bud break of both selections did not differ significantly from that of "Chandler" ($p > 0.05$). The first female blooming of "FM6" occurred significantly earlier (9.64 days earlier; $p < 0.05$) than the "Chandler" cultivar, while the last female blooming occurred significantly earlier ($p = 0.01$) in both "FM3" (7.22 days earlier) and "FM6" (10.55 days earlier) selections, compared to "Chandler". The mean date of the beginning and the end of the male blooming of "FM3" and "FM6" did not differ significantly ($p > 0.05$) either between them or with "Chandler" (Table 2; Figure 3). The mean date of harvest of nuts of "FM6" occurred significantly earlier than "FM3" (4.64 days earlier) and "Chandler" (5.09 days) ($p < 0.05$), while the mean date of harvest of nuts of "FM3" did not differ ($p > 0.05$) with that of "Chandler" (Table 2).

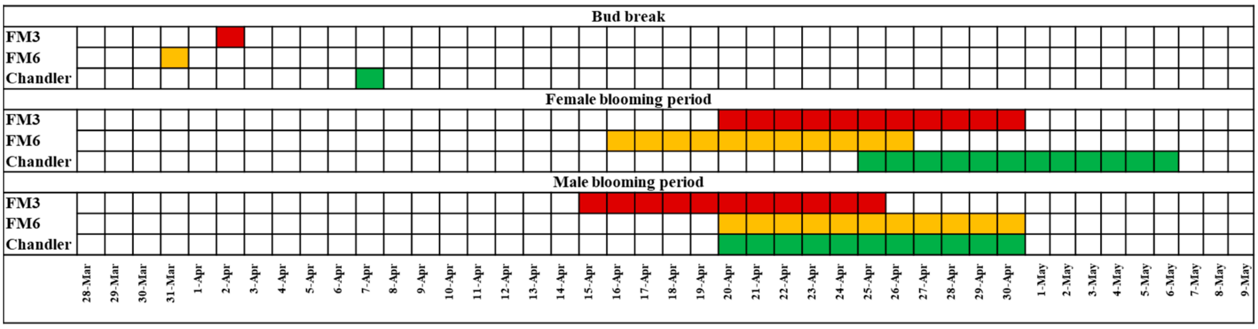

Figure 3. Phenological characteristics concerning the date of bud break, first and last female bloom dates, and first and last male bloom dates of "FM3" (red color) and "FM6" (orange color) walnuts, compared to the reference cultivar "Chandler" (green color).

Both "FM3" and "FM6" presented easy hull dehiscence, low sunburn susceptibility of the hull, intermediate susceptibility to codling moth (*Cydia pomonella* L.), and low susceptibility to walnut blight (*Xanthomonas campestris*), similar to "Chandler" (Table 1).

3.2. Nut Characteristics

Fruit characteristics are presented in Tables 3 and 4. Both "FM3" and "FM6" nuts were of very large size and of broad elliptic shape, whereas those of "Chandler" were of medium size and ovate shape (Table 3; Figure 4). Indeed, the nuts' heights, widths, and roundnesses were found to be higher in "FM6" relative to "FM3" and "Chandler", and those of "FM3" were higher than "Chandler" ($p < 0.001$). Nuts thicknesses of both "FM3" and "FM6" differed significantly ($p < 0.001$) from those of "Chandler" (Table 4). The shell texture of "FM3" was evaluated as rough, whereas that of "FM6" medium. The shell color of nuts of both studied walnuts was evaluated as medium with intermediate shell integrity (Table 3; Figure 2). The shell thickness of nuts of "FM3" was found to be higher than "FM6" and "Chandler" nuts, and that of "FM6" was higher than "Chandler" nuts ($p < 0.001$; Table 4).

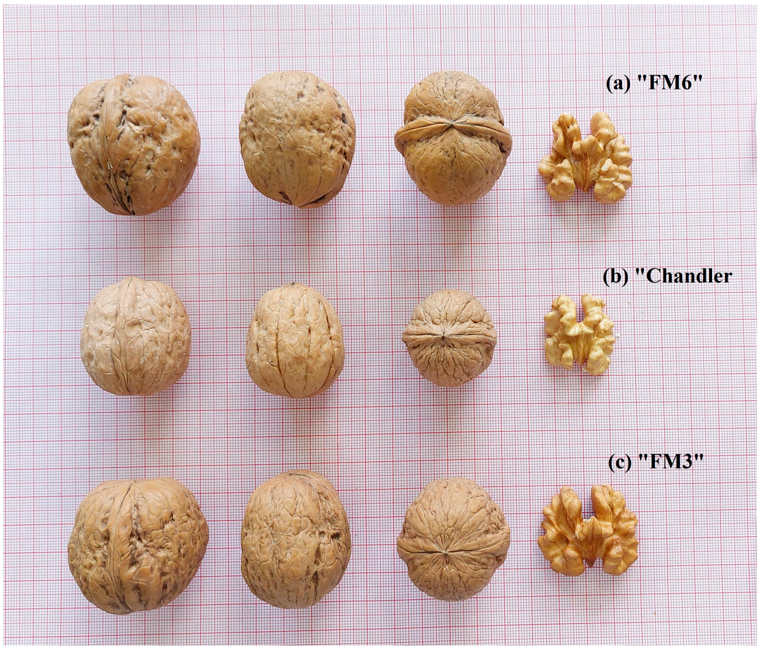

Figure 4. Nut and kernel size of (**a**) "FM6" selection (very large nut size; very large kernel size), (**b**) "Chandler" cultivar (medium nut size; medium kernel size), and (**c**) "FM3" selection (very large nut size; very large kernel size).

Both "FM3" and "FM6" nuts' shapes in the longitudinal section through the suture and in the longitudinal section perpendicular to the suture were evaluated as broad elliptic. The shape in the cross-section was evaluated as oblate, and the shape of the base perpendicular to the suture as cuneate. The shape of the apex perpendicular to the suture was truncated, and the prominence of the apical tip was found to be weak. The position of the pad on the suture was found on the upper 2/3 of the nut in "FM3" and on the upper half of the nut in the "FM6" selection. The prominence of the pad on the suture was medium in "FM3" and strong in "FM6"; the width of the pad was broad in both selections, whereas the depth of the groove along the pad on the suture was medium in "FM3" and deep in "FM6". The structure of the surface of the "FM3" nuts' shells was strongly grooved, and that of "FM6" was moderately grooved, and the nut shell thickness was medium in both of them. The nuts of both walnuts studied presented strong adherence of the two halves of the shell and medium thicknesses of the primary and secondary membranes (Table 3; Figure 2).

Interestingly, the mean dried in-shell nut weight and kernel weight of "FM3" (23.70 ± 0.220 g and 11.75 ± 0.200 g, respectively) was higher ($p < 0.001$) relative to "FM6" (21.36 ± 0.199 g and 10.16 ± 0.100 g, respectively) and "Chandler" (13.41 ± 0.257 g and 6.67 ± 0.171 g, respectively) and that of "FM6" higher ($p < 0.001$) than "Chandler". However, nut kernel efficiency was found higher in "FM3" (49.56 ± 0.663 g) and "Chandler"

(49.94 ± 0.348 g) relative to "FM6" (47.61 ± 0.468 g) ($p < 0.05$; Table 4). In both "FM3" and "FM6", nuts presented very large size, light kernel color, with well kernel fill, satisfactory flavor, and easy removal of the kernel halves (Table 3; Figure 4).

4. Discussion

When evaluating new walnut selections, phenotypic characteristics are crucial. During walnut breeding activities, traits like lateral fruiting, date of bud break, blooming and harvest, as well as fruit weight, kernel weight, efficiency, and quality, have been selection criteria. Phenological description of the studied selections coded "FM3" and "FM6" revealed that "FM3" presents a high percentage of fruitful lateral buds, similar to "Chandler", and it is characterized as lateral according to IPGR (1994) [15], whereas "FM3" presents a lower percentage and is characterized as intermediate. Lateral bud fruitfulness is a significant characteristic and criterion in walnut breeding programs [10,19] since cultivars or selections with high lateral fruitfulness are considered more productive than those with a low (0–10%) percentage [20] and even more, they bear at earlier ages [21–23]. Although according to Germain (1997) [10], lateral bud fruitfulness is a trait that is not easy to inherit, it could be manipulated through breeding to produce high-yield cultivars [24]. Relevant studies in other countries have found that of the 540 Persian walnut tree accessions selected and ex situ evaluated in the Neiriz region of Iran, 106 of all accessions were terminal fruitfulness, 167 were lateral fruitfulness, and 267 of all accessions were mixed and had terminal and lateral fruitfulness [24]. Moreover, from 232 walnut genotypes selected from the six different parts of the Fars province in Iran, the mean lateral bud fruitfulness was 74.16% [25]. Evaluation of 19 walnut types after selection from the middle Black Sea region of Turkey revealed a lateral fruitfulness ranging from 10 to 70%; four out of 19 walnut types presented lateral fruitfulness lower than 30%, fourteen up to 60%, and only one higher than 60% [26].

In our study, both "FM3" and "FM6" selections are classified as mid-early genotypes according to their bud break date, which occurs earlier than "Chandler". Hendricks et al. (1998) [21] assert that when selecting a cultivar, the period of leafing and the date of blooming should be considered. Early leafing cultivars are prone to late spring frosts that reduce tree fruiting and yield, or they are susceptible to diseases related to rainy conditions, such as walnut blight. Thus, as late leafing is a valuable characteristic, walnut genotypes that escape bacterial blight, insect pests, and early spring frosts should be developed by selection for late leafing. According to climatic diversity and late spring frosts, four regions for walnut production might be identified in Greece. The first includes the areas where the last spring frosts occur the first fifteen days of March. In the second one, the last spring frosts occur at the end of March. The third and fourth zones include areas where the last spring frosts occur in the first fifteen days of April and at the end of April, respectively [11]. Therefore, the studied selections, as mid-early genotypes, are acceptable for cultivation in areas where the last spring frosts occur in late March or the first few days of April.

"FM6" is a protogynous genotype, as its female flowers bloom 4 days earlier than male ones, and it presents intermediate tree vigor and intermediate branching. Although the male flowering period of "FM6" occurs close to "FM3" and "Chandler", as protogynous, female blooming occurs earlier than others. "FM3" presents intermediate tree vigor and intermediate branching, it is protandrous, and the blooming of female and male flowers occurs earlier than "Chandler" but not significantly.

Harvest of nuts of both selections and "Chandler" occurs the last ten days of September, earlier in the "FM6" selection. In other relevant studies, for most of the native superior selections that were studied in experimental plots in Romania and released as cultivars, harvest occurs the last half of September [27]. The harvest date from the selected trees of the study of Khadivi-Khub et al. (2015) [24] and Khadivi-Khub et al. (2015) [25] varied from early September to the beginning of October. The date of nut harvest is another important factor that impacts the economic potential of a walnut genotype and consequently affects the market value of the products. As it also applies to walnuts, an early harvest date must be regarded as a protective measure against early-autumn frost occurrences [28]. Furthermore, early harvest dates give the opportunity to walnut producers to get introduced earlier to the walnut market, thus gaining higher market prices. Another advantage of an early harvest date is the lower need for the process of walnut drying, thus gaining energy and reducing drying costs. According to McGranahan and Leslie (2009) [29], when choosing a walnut genotype, the timing of leafing and harvesting has always been a crucial factor. Although early-leafing genotypes are more vulnerable to frost, walnut blight, and pests, early-leafing and early blooming genotypes offer the advantage of early harvest (in September) and early entry into the holiday nut trade. On the other hand, late-harvesting genotypes (in the middle of October) are susceptible to challenges like rain, which can obstruct harvesting activities; moreover, vigorous late-harvesting genotypes may also be more vulnerable to damage from early frosts and winter injury. In this regard, the harvest season of an ideal walnut cultivar would end in early October [20], as in the case of "FM3" and "FM6" genotypes in the present study.

Both walnut selections present easy hull dehiscence, low sunburn susceptibility of the hull, intermediate susceptibility to codling moth, and low susceptibility to walnut blight like "Chandler". All these traits of these genotypes could be considered as important so as to be selected in breeding programs.

Pomological evaluation helps determine fruit quality, and thus, it has been widely used in many breeding studies to identify fruit genotypes with superior traits. According to the current results, the nuts of both "FM3" and "FM6" are of very large size and broad elliptic shape, with rough and medium shell texture, respectively, medium shell color, medium shell thickness, and a strong adherence of the two halves of the shell. According to McGranahan and Leslie (2012) [30], the ideal nut should have a clean, strong, thin shell and a tight seal, traits that both selections in the present study fulfill. All dimensions of nuts measured were higher in both "FM3" and "FM6" in relation to "Chandler".

The most important traits when considering a walnut genotype are nut weight, kernel weight, kernel percentage, kernel color, kernel fill, and ease of removal of kernel halves [20]. According to McGranahan and Leslie (2012) [30], nut and kernel quality is strongly affected by genotype, environment, and their interaction. The ideal nut should weigh between 12 and 18 g; the kernel should be easily removable from the shell, uniformly light in color, clean, and weigh 6–10 g or at least 50% of the entire nut weight. Indeed, in the present study, the nuts of both selections have light kernel color, well kernel fill, and easy removal of the kernel halves. More interestingly, the mean dried nut weight (in shell) was found to be higher than 21 g in both "FM3" and "FM6" selections, significantly higher than "Chandler". Kernel weight was also found to be higher than 10 g in both "FM3" and "FM6" (10.16 ± 0.100 g) genotypes, both being significantly higher than "Chandler". Moreover, the size of the kernel of both selections was very large; it had satisfactory flavor, it was easily removable from the shell, light in color, uniformly lighted, and the kernel efficiency was 50% for the "FM3" selection and close to 50% for the "FM6" selection. Other studies by Khadivi-Khub et al. (2015) [25] reported nut weights from 8.00 to 23.00 g (mean 14.40 g), kernel weights from 4.00 to 14.00 g (mean 7.26 g), and kernel percentages from 40.00 to 72.22% (mean 50.20%) from 232 walnut genotypes selected from the six different parts of Fars province in Iran. However, in another study of 540 Persian walnut tree accessions selected and evaluated ex situ in the Neiriz region of Iran [24], nut weights ranged from 3.60 to 20.28 g (mean 9.61 g), kernel weights from 1.32 to 10 g (mean 4.55 g), and kernel percentages from 17.44 to 83.88% (mean 47.14%). Vasilescu and Botu (1997) [27] reported kernel percentage from 44.00 to 53.00% of 16 native superior selections that were studied in experimental plots in Romania and released as cultivars. Thus, "FM3" and "FM6" could be regarded as superior to "Chandler" concerning their nut size and the nut and kernel weight. Moreover, all together, the above qualitative and quantitative pomological characteristics of these selections satisfy the specifications for walnut genotypes [30] and support the notion that "FM3" and "FM6" could be considered promising genotypes of great interest and high breeding value.

Taking into consideration all the data together, both selected genotypes, "FM3" and "FM6", present satisfactory characteristics in terms of tree vigor, hull dehiscence, susceptibility to sunburn and diseases, date of bud break, flowering, and harvest, as well as superior nut qualitative and quantitative characteristics. Among the two genotypes, "FM3" presents higher lateral fruitfulness and later leafing and blooming, thus could escape early spring frosts and bacterial blight, insects, and pest insults. Moreover, "FM3" has better pomological traits, as indicated by the higher in-shell nut and kernel weights and kernel efficiency. However, the high breeding value of "FM6" could not be ignored and diminished due to the early harvest date and the superior to "Chandler" quantitative traits, such as in-shell nut and kernel weights.

5. Conclusions

Evaluation of local walnut genotypes from various areas in Greece that presents high genetic diversity, collection of the superior of them, and incorporation in breeding programs could be a successful tool for the creation of new cultivars with desirable phenological and pomological characteristics. In this regard, the two walnut selections, "FM3" and "FM6", could be regarded as genetic materials of significant value due to their favorable agronomic traits like lateral fruitfulness, precocity, adaptability to given soil and climate conditions, tolerance/resistance to main diseases and pests, very big size and weight and very good quality of nuts and kernels, kernel efficiency, etc. They are unique, each one from the genetic point of view. They have been selected initially in the local walnut populations and are considered as high-value germplasm because it is adapted to the specific environmental conditions and can provide future cultivars to choose from, along with consecrated ones, for efficient use in commercial orchards in the context of actual climatic changes.

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