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MORPHOLOGICAL AND ANATOMICAL MARKERS OF PHASE CHANGE TO ADULT STAGE IN OLIVE SEEDLINGS

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By
E. S. Hegazi , A.A. Hegazi and A. M. Abd allatif

Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt

ABSTRACT

The current study was conducted during seasons 2009 to 2010 to investigate markers of phase change in olive seedlings. Three year old seedlings from open pollinated Koroneiki cv. seeds were used. Morphological and anatomical markers of phase change were measured in each seedling. Differences between the studied parameters were detected. The obtained results showed that the seedlings were divided into three groups (Juvenile, Intermediate-juvenile, Mature). In general, seedlings of the mature group recorded higher values of plant height, stem diameter, number of lateral shoots and internode length. Also while leaf length, width, area and L/D ratio were higher in the mature leaves. Leaf anatomy revealed that leaves of the adult seedlings had higher percentage of upper mesophyll, and additional layer of palisade mesophyll beside the lower epidermis. The studied characteristics could be used as preliminary markers to select seedlings of short juvenility in olive breeding programs.

Key words: *juvenility, leaf anatomy, olive, phase change.*

1. INTRODUCTION

Plants raised from seed germination enter a state of juvenility; during this stage the plant can not enter the flowering induction (Leopold and Kriedemann, 1975). The seedlings remained in juvenile stage for long period. In olive, the length of the juvenility period under natural conditions may last up to 15 years before transmission to the adult stage (Santos-Antunes *et al.*, 2005).

One of the main objectives in olive breeding programs is the selection of new lines which begin fruiting in short period (Leon *et al.*, 2007). Selection for the desired traits very early confers certain advantages for plant breeders because the sooner the screening of seedlings the more efficient the breeding programs (Pritsa *et al.*, 2003). Also, the earliness of flowering is correlated with precocity and high productivity of the new line (Zimmerman, 1972).

There are progressive physiological, morphological, and anatomical changes associated with the passing from juvenility to maturity (Hackett and Murray, 1993; and Murray *et al.*, 1994) which could be used as important markers of phase change (Basheer – Salimia *et al.*, 2004).

The transmission from juvenility to adult stage in fruit tree is usually associated with attainment of certain plant size measured as shoot height ackett, 1985; and Poethig, 1990) and stem diameter (Santos-Antunes *et al*. 2005).

The differences between leaves in adult and juvenile seedlings of many species were documented, and were included in phenomenon called “Heteroblasty” (Allsopp, 1967). The heteroblasty consists of genetically programmed structural changes that occurs during the whole plant ontogeny and are linked to changes from juvenile to adult phase (Jones, 1999).

Moreno-Alias *et al.,* (2009) reported that there was a significant difference between adult and juvenile leaves, leaf length, width, area and leaf length to width ratio consistently greater in the adult leaves.

Pritsa *et al.*, (2003) reported that 74% of early flowering olive seedlings derived from the single branched seedling and only 26% were from seedlings with lateral shoots. According to Lavee *et al.* (1996) the phase change from juvenile to adult stage in olive seedlings is associated with an increase in internode length. Moreno-Alias *et al.* (2009) reported that, in the transverse sections, total leaf thickness and thickness of both lower and upper mesophyll were higher in the adult than the juvenile leaves. As, the juvenile plant grows up structural differences along its stem may reflect the gradual change from juvenile to mature types of growth (Leopold and Kriedemann 1975). According to Hackett (1985) there is an over all spatial differences within the canopy in which the upper and peripheral parts of the plant mature while, the basal and inner parts remained juvenile.

The present study was designed to identify morphological and anatomical markers of phase change in olive seedling to assist selection of seedlings with short juvenility as early as possible.

2. MATERIALS AND METHODS

The present study was carried out during 2009 and 2010 seasons in a trial to investigate morphological and anatomical markers of phase change in olive seedlings. This study was conducted at the Nursery and Laboratory of Pomology Department, Faculty of Agriculture, Cairo University.

2.1. Plant materials:

The plant materials are consisted of 40 olive seedlings aged three years resulted from open pollination of cv. "Koroneiki" The different morphological parameters *i.e*. plant height, stem diameter at 10 cm from soil surface, number of lateral shoots, internode length, leaf length and width were estimated, and leaf area (cm²) was calculated using the following formula. Leaf Area = 0.53 (L×W) + 1.66 (Ahmed and Morsy, 1999), all the parameters were recorded during October of the two successive seasons (2009 and 2010).

To identify sites of phase change in the seedlings, each seedling was divided into 3 parts (basal , middle and terminal portion), internode length (cm) and leaf area (cm²) were recorded in each portion.

2.2. Anatomy of olive leaves

The anatomical studies were conducted on fully developed leaves from three sources:

Leaves of 8 month old olive plants cv. Koroneiki (derived from cuttings).

Leaves from olive seedlings that showed juvenile status.

Leaves from olive seedlings showed signs of transimition to adult stage.

The middle portion of leaf samples were fixed in mixture of F.A.A for 48 h. The leaf samples were dehydrated through a series of ethyl and butyl alcohol and embedded in paraffin wax. The tissues were sectioned at 20 µm with rotary microtome, mounted and stained with saffranin and light green. (Moreno-Alias *et al.*, 2009). The leaf sections were examined under light microscope. Images were captured by Olympous BX.40 microscope supplement with video camera (Panasonic WV-CP 220, Japan).

The leaf mesophyll was divided into adaxial mesophyll (composed of the major palisade parenchyma I) and abaxial mesophyll, (composed of the spongy parenchyma) and palisade parenchyma II. (Moreno-Alias *et al.*, 2009). Thickness of the different mesophyll types was measured using image analysis software and the percentage of adaxial to abaxial mesophyll was calculated.

2.3.Experimental design and data analysis

This study followed the complete randomized block design and data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980) using MSTAT C statistical package (Freed *et al*., 1990) software, and means of the treatments were compared by Least Significant Difference (L.S.D) according to Duncan (1955) at significance level of 0.05.

3. RESULTS AND DISCUSSION

3.1. Vegetative growth

The obtained results indicated that there were obvious variations in all the morphological parameters, and the studied seedlings fill into 3 categories (Juvenile, Intermediate-juvenile, Mature), as described by (Pritsa *et al*., 2003 and Santos-Antunes *et al*., 2005).

3.1.1. Seedling height

It is clear from the data presented in Table (1) that there was an obvious difference in seedlings height during the two studied seasons. The studied seedlings fill in three groups according to seedling height. Seedling in the adult category including (Line 6, 7, 9, 11, 12, 13, 17, 19, 21, 22, 23, 25, 27, 29, 33, 38) recorded the highest values in both season (130.3 and 152.3 cm, respectively), while seedlings in the 3rd group recorded the lowest values (63.64 and 91.4 cm, respectively), which including (Line 2, 3, 4, 5, 14, 18, 26, 36, 37, 39, 40).The Intermediate-juvenile seedlings including (Line1, 8,10,15,16,20,24,28,30,31,32,35).

According to Hackett (1985) the transmission from the juvenility to adult productive stage required the attainment of certain plant size measured as plant height. Kiran *et al.* (2008) used seedling height as a pre-selection criterion for the short juvenile stage in olive.

3.1.2. Stem diameter

The data in Table (1) show that seedlings of the mature group recorded the highest stem diameter category including (Line 6, 7, 9, 11, 12, 13, 17, 19, 21, 22, 23, 25, 27, 29, 33, 38), on the contrary, seedlings in the juvenility group recorded the lowest values (Line 2, 3, 4, 5, 14, 18, 26, 36, 37, 39, 40). The increase in stem diameter

Table (1). Vegetative characteristics of seedlings with different developmental stages.

Seedling	Plant height (cm)	Stem diameter (cm)	No. lateral shoots
Season 2009			
Juvenile	63.64 b	0.53 c	8.05 b
Intermediate-juvenile	68.58 b	0.61 b	18 ab
Adult	130.3 a	0.79 a	31.17 a
Season 2010			
Juvenile	91.4 c	1.63b	27.31
Intermediate-juvenile	120.5 b	1.86 a	38.25
Adult	152.3 a	1.92 a	45.82

was correlated with the increase of seedling percentage in the adult stage (Santos-Antunes *et al.,* 2005). According to Way (1971) apple with larger stem diameter come into flowering earlier than those with small diameter.

3.1.3 Number of lateral shoot/seedling

The data presented in Table (1) show a high difference between the seedlings in their ability to form lateral shoots.

Seedlings in the juvenility group (Line 2, 3, 4, 5, 14, 18, 26, 36, 37, 39, and 40) produced the least number of lateral shoots in both studied seasons (8.05 and 27.3, respectively). While seedlings of the mature stage (Line 6, 7, 9, 11, 12, 13, 17, 19, 21, 22, 23, 25, 27, 29, 33, 38) produced the highest number (31.17 and 45.82). Number of lateral shoots and frequency forming shoots had an effect of the seedling ability to flower and can be used as a morphological trait for transmission to adult stage (Ligeng *et al.,* 1995 and Pritsa *et al.,* 2003).

3.1.4. Leaf morphology

As shown in Tables (2 and 3) a significant difference between leaves belonging to the different categories were found for the size parameter, leaf length width and area were greater for the adult seedlings compared with the juvenile seedlings while leaves from the 2^{nd} group had an intermediate values.

The differences in leaf measurements were more obvious in the second season compared with the first one.

Concerning the effect of site on leaf characteristics, it was obvious that the markers of phase change appeared on the terminal portion of the seedlings and it was more noticeable on the seedlings in the adult stage.

According to Zimmerman (1972) the leaf form change when it reached the maturity stage and this change occurs during the whole plant ontogeny and is linked to change from the juvenile stage to adult phase (Jones, 1999). Moreno-Alias *et al.* (2009) reported that there was a significant difference between adult and juvenile leaves, leaf length, width and area were higher in the adult leaves.

3.1.5. Leaf shape

Data in Table (3) indicate that leaves from the different categories were included in the elliptical shape (L/W <4) and there were no significant differences between the leaves from the different categories during the 1^{st} season, while in the second season, leaves of the adult seedlings recorded the higher values compared with the seedling from the juvenile seedlings.

Also, the difference in shape index along the stem appeared only in the second season, and the L/W ratio was higher in the upper portion of the seedlings. Lavee *et al.* (1996) noted an increase in L/W ratio in the adult seedlings compared with juvenile seedlings. Also, Basheer-Salimia *et al.* (2004) reported that juvenile olive leaves were shorter and more rounded compared to the mature leaves.

3.1.6. Internode length

Internode length was significantly affected by seedling development stage and the site of measurement (Table, 4). Seedlings of the adult group recorded the highest internode length in both seasons compared with the other seedlings.

Also, the internode length was longer in the upper portion of the seedlings compared with the seedlings base . Lavee *et al.* (1996) reported that phase change from juvenility to adult stage in

Table (2) : Leaf length and width of seedlings with different developmental stage.

Season 2009						
Seedling	**leaf length (cm)**			**leaf width (cm)**		
	Terminal	**Mid.**	**Basal**	**Terminal**	**Mid.**	**basal**
Juvenile	**3.01 a-c**	**2.42 d**	**2.42d**	**1.04a**	**-**	**0.78a**
Transmission	**3.18 ab**	**2.7 b-d**	**2.56cd**	**1.04a**	**0.88a**	**0.79a**
Adult	**3.41 a**	**3.15 ab**	**2.63cd**	**1.03a**	**0.82a**	**0.87a**
Season 2010						
	Terminal	**Mid.**	**Basal**	**Terminal**	**Mid.**	**Basal**
Juvenility	**2.86cd**	**2.73d**	**2.73d**	**1.05b**	**-**	**0.94c-e**
Transmission	**3.18b**	**2.68de**	**2.68de**	**1 b-d**	**-**	**0.87e**
Adult	**3.68a**	**3.06bc**	**2.46e**	**1.20 a**	**1.03bc**	**0.91de**

Table (3) : Leaf area and shape of seedling with different developmental stage.

Season 2009						
Seedling	**leaf Area (cm)**			**leaf shape index**		
	Terminal	**Mid.**	**Basal**	**Terminal**	**Mid.**	**Basal**
Juvenile	**3.18a-c**	**-**	**2.67c**	**3.9b**	**-**	**3.13b**
Transmission	**3.16a-c**	**3.12a-c**	**2.75bc**	**3.07b**	**3.08b**	**3.24b**
Adult	**3.54a**	**3.35ab**	**2.86bc**	**3.29b**	**3.91a**	**3.09b**
Season 2010						
	Terminal	**Mid.**	**Basal**	**Terminal**	**Mid.**	**Basal**
Juvenile	**3.29bc**	**-**	**3.01cd**	**2.07bc**	**-**	**2.95ab**
transmission	**3.39b**	**-**	**2.9d**	**3.19a**	**-**	**3.07a**
Adult	**4.03c**	**3.34b**	**2.86d**	**3.05a**	**2.95ab**	**2.68c**

Table (4) : Internode length (cm) of seedlings with different development stage.

Season 2009				**Season 2010**			
	Terminal	**Mid.**	**Basal**		**Terminal**	**Mid.**	**Basal**
Juvenile	**1.77b**	**-**	**1.52b**	**Juvenile**	**2.18 a-c**	**2.02 bc**	**1.63 d**
Transmission	**2.32a**	**-**	**1.46b**	**Transmission**	**2.34 ab**	**2.35 ab**	**1.86 cd**
Adult	**2.37a**	**2.19a**	**1.49b**	**Adult**	**2.40 a**	**2.47 a**	**1.92 cd**

olive seedlings was associated with an increase in the internode length.

3.2. Leaf anatomy

In the transverse section, thickness of the upper mesophyll was higher for the adult leaves than the juvenile leaves as shown in Figs. (1 and 2) the percentage of leaf thickness occupied by the two mesophyll tissues were quite different between the two leaf types.

In the juvenile leaves the adaxial mesopyll occupied (36.38 % of the seeding tissue and 28.26 % of Koroneiki tissue) while, the upper

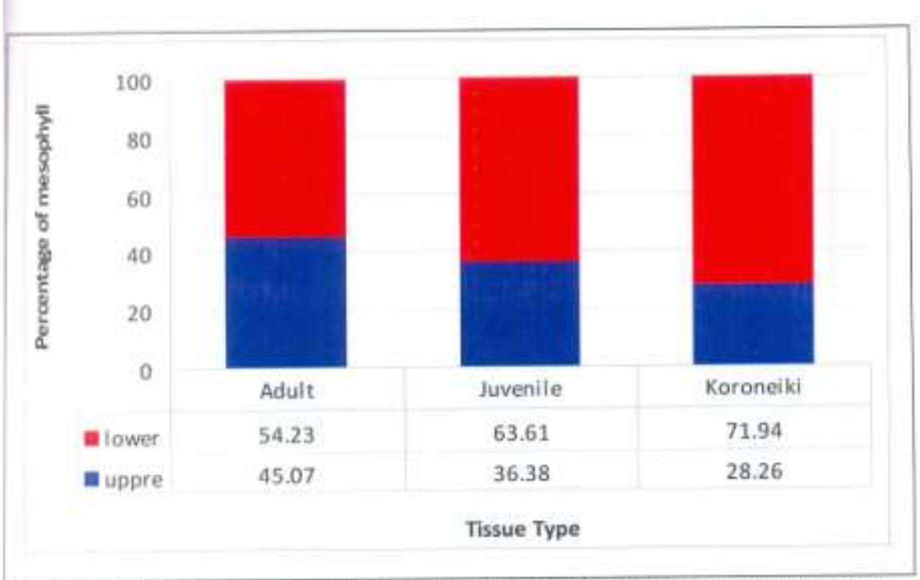

Fig. (1): Thickness of upper and lower mesophyll component in juvenile and adult seedlings compared to Koroneiki cutting.

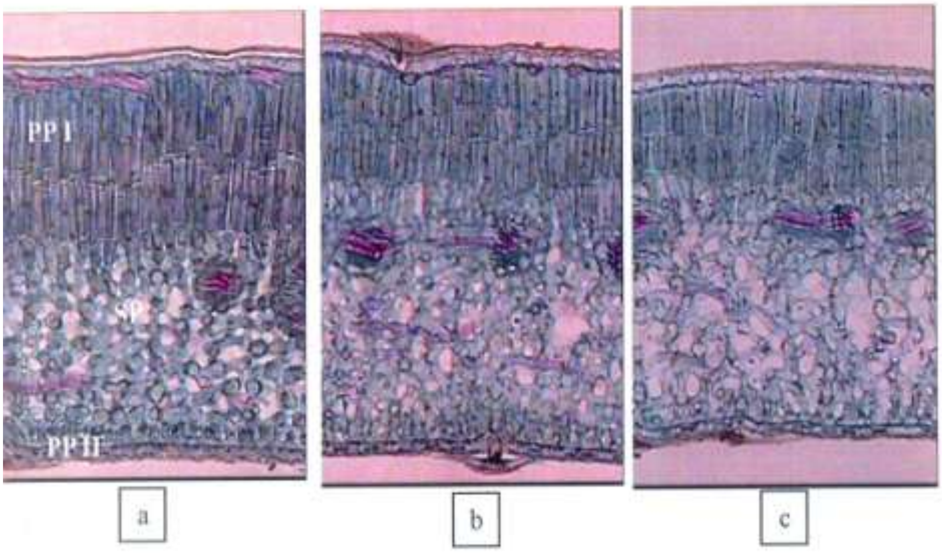

Fig. (2): Sections of olive leaves with different development status. a (Adult seedlings) b (Juven seedlings) c (Juvenile Koroneiki). Palisade parenchyma (PPI) palisade parenchyı (PPII), spongy parenchyma (SP).

mesophyll occupied 45.07% in the adult leaves. One of the major anatomical differences between adult and juvenile leaves was the layer of organized cells adjusted to the adaxial epidermis (palisade parenchyma PPII) which was present only in the adult leaves. This layer was reported in pervious studies on adult leaves (Chartzoulakis *et al.,* 1999 and Bosabalidis and Kofidis 2002).

The spongy parenchyma was different in both types of leaves, in all cases it showed large intercelluer spaces as well as vascular bundles, the degree of compactness of the spongy mesophyll varied greatly among both type of leaves, but in adult leaves it tended to be less compact than in juvenile leaves.

From the obtained results it can be concluded that, the morphological and anatomical parameters may be used as markers of phase change in olive seedling, which will help in the selection of new lines which begin fruiting in short period.

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الدلائل المورفولوجية و التشريحية للتحول لمرحلة البلوغ
في شتلات الزيتون البذرية

السعيد صادق حجازي - أيمن عبد المؤمن حجازي - عبده محمد عبد اللطيف

قسم الفاكهة - كلية الزراعة - جامعة القاهرة - مصر

ملخص

أجريت هذه الدراسة خلال موسمي 2009 و 2010 بهدف تحديد دلائل التحول لمرحلة البلوغ في شتلات الزيتون البذرية. أجريت الدراسة علي مجموعة من شتلات الزيتون البذرية الناتجة من التلقيح المفتوح للصنف كروناكي و قد تم تقدير الصفات المورفولوجية و التشريحية في كل شتلة علي حده. أظهرت الدراسة وجود اختلافات واضحة في الصفات المدروسة كما أمكن تقسيم الشتلات إلي ثلاث مجموعات في مراحل مختلفة من تطورها (البلوغ ، التحول والطفولة).

وبصفة عامة فقد سجلت الشتلات التي أظهرت علامات البلوغ قيم أعلي لكلا من طول النبات و قطر الساق و عدد الأفرع الجانبية و طول السلامية كما سجلت قيم أعلي لكل من طول، عرض و مساحة الورقة. أظهر تشريح الأوراق أن أوراق الشتلات التي أظهرت علامات البلوغ تميزت بإحتوائها علي نسبة أعلي من النسيج العمادي ووجود طبقة إضافية من النسيج العمادي ملاصقة للبشرة السفلية . و هذه الخصائص يمكن أن تستعمل كدلائل بسيطة لانتخاب الشتلات التي تتميز بقصر فترة الطفولة في برامج تربية الزيتون.

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