Полученные клоны могут быть использованы в фармакологии для расширения сырьевой базы при производстве препарата аллапинин. Однако особенно важно то, что вносится вклад в сохранение природных популяций этого растения.

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PECULIARITIES OF SPHAEROBLAST FORMATION AND DEVELOPMENT IN *OLEA EUROPAEA* L.

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Introduction

Sphaeroblasts are adventitious buds consisting of a woody, more or less globular structure connected to the vascular system of the plant through a pointed end and capable of differentiating vegetative meristems giving growth to juvenile shoots (Fig. 1 C, D, E). Their life is between three to four years and if during this life period do not differentiate meristems and/or give new growth they loose their viability. The first to describe sphaeroblasts was Theophrastos (371 – 287 BC), in his surviving work «*Enquiry into Plants*». He named them γόγγρο (singular), γόγγροι (plural) after their shape, looking like the beet root (γογγύλη). The first and perhaps the only study on sphaeroblast formation in olive trees have been made by Baldini and Mosse [1].

Sphaeroblasts are formed on various tree species under the effect of various factors and

they constitute a mechanism of survival and/or regeneration in plants. Among the various species *Olea europaea* L. is showing a high frequency of sphaeroblast formation.

Semihardwood leafy cuttings of olives root at higher percentages if they are made from juvenile shoots [4]. Shoots arising from sphaeroblasts are having strong juvenile characters and constitute ideal propagating material. Therefore it is very important to know first the conditions under which sphaeroblasts are differentiated; second the conditions under which sphaeroblasts develop shoots because not all of them differentiate vegetative meristems.

The purpose of this study is to investigate sphaeroblast formation in three main olive varieties cultivated in two different areas of Greece.

Objects and methods of investigation

The experiment was carried out in two olive groves located in different areas during the years 2000-2006. The first is located in the district of New Moudania city, county of Chalkidiki in north Greece; the second is located on mount Pelion, county of Magnesia, central Greece. Both olive groves included the varieties Chondrolia Chalkidikis, Megaritiki and Koroneiki. These varieties are among the main varieties cultivated in Greece the first for processed edible olives, the third for olive oil production and the second for both i.e. edible olives and olive oil. For each variety there were 36 and 60 trees in the olive groves of New Moudania and mount Pelion respectively. All the trees were distributed randomly in space. The age of the olive trees in both groves is forty years old. In New Moudania the trees are irrigated and pruned annually, on mount Pelion the trees are non irrigated and pruned every second year.

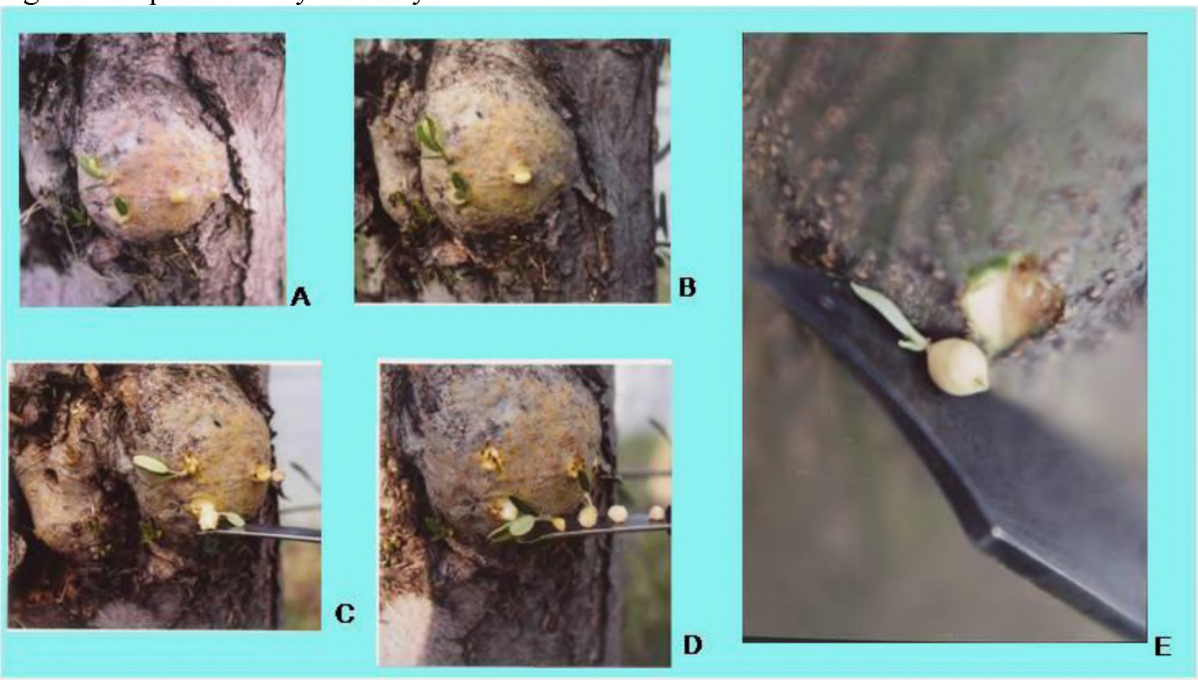

Fig. 1: Sphaeroblast formation in *Olea europaea* var. Chondrolia Chalkidikis: A – sphaeroblast formation is observed mainly on hyperplasias along and around the trunk; B,C,D – sphaeroblast excision in olive trees can be easily done with the use of a pointed tool during the vegetative period; C, D, E – sphaeroblasts in olive trees are woody structures having the form of a beet root, their size varies from 3-7 mm and they can differentiate shoot meristems on top of them

The experimental design was a factorial one 3 x 2 x 7 with the trees completely randomized in space. Seven of them were taken randomly to form the sample for recording data. Observations were made in the second half of May with the beginning of flowering. for the seven years and the number of sphaeroblasts formed in each tree was recorded.

A factorial analysis of variance (ANOVA) was performed, with variety and location as independent fixed factors and years as random factor. Homogeneity of variances was tested with Levene's test. Analysis of variance was conducted with SPSS 12 (SPSS Inc. 2003).

Results

Sphaeroblasts could be easily traced because the phloem at their position is raised to a hemispherical dome or knot. They could also be excised easily with the use of a sharp or pointed tool (Fig. 1). Usually the sphaeroblasts were observed at high numbers and formation of a single sphaeroblast in a certain place rarely occured (Fig. 2).Sphaeroblast formation was mainly observed on hyperplasias of the trunk (Fig. 1) and secondarily on shoots of all ages except the very young ones bearing leaves.

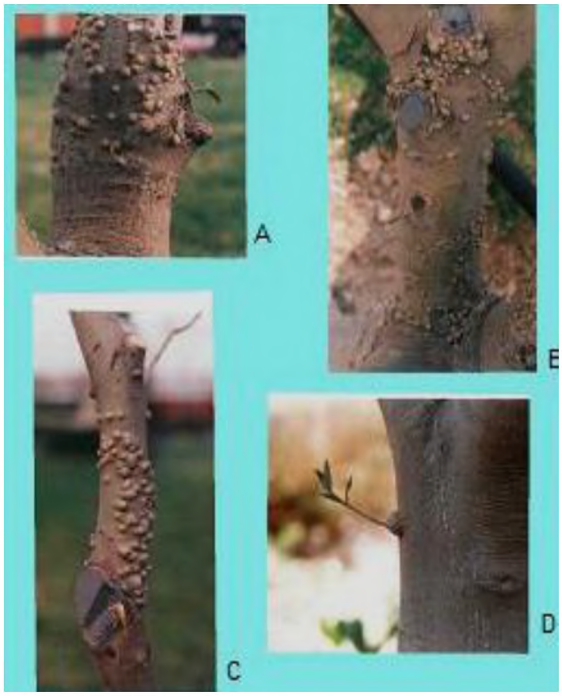

Fig. 2. Sphaeroblast formation on branches and young shoots of trees of *Olea europaea* var. Chondrolia Chalkidikis

Under certain conditions sphaeroblasts were contaminated by Pseudomonas savastanoi either before or while their shoot meristems were developing to shoots and were emerging through the phloem. Contaminated sphaeroblasts were converted to a callus mass. (Fig. 3 B, D).

Factorial analysis of variance indicated that varieties (Fig. 4) and location (Fig. 5) influenced significantly the sphaeroblast formation.

Analysis of variance determined that differences exist among varieties (MS = 810.167; df = 2,12; F = 27.034; $p < 0.0001$). According to S-N-K multiple comparison test, Megaritiki differs significantly from Koroneiki (p = 0.045). Variety Chondrolia Chalkidikis shows significantly higher sphaeroblast formation than the other varieties ($p < 0.0001$). Moreover, the average sphaeroblast formation differs significantly by location (MS = 240.667; df = 1, 6; F = 7.665; p = 0.032).

Furthermore, a significant interaction between years and locations on sphaeroblasts exist (MS = 31.397; df = 6, 264; F = 2.145; p = 0.049). It seems that on mount Pelion the sphaeroblast formation shows a significantly more fluctuation during the seven years than in New Moudania. This phenomenon could be due to the pruning practice in this location (Fig. 6).

There is a different response of the varieties to the locations (MS = 158.024; df = 2,264; F = 10.798; $p < 0.0001$). The varieties Megaritiki and Koroneiki had significantly more sphaeroblasts in New Moudania than on mount Pelion, while the variety Chondrolia Chalkidikis had more sphaeroblasts on mount Pelion (Fig.7).

Interactions between varieties and locations on sphaeroblast formation, are shown in Fig. 8. The stability of the variety Chondrolia Chalkidikis is characteristic, showing significantly more sphaeroblasts on both locations. The variety Koroneiki responds better in New Moudania, while on mount Pelion showed a considerably low sphaeroblast formation.

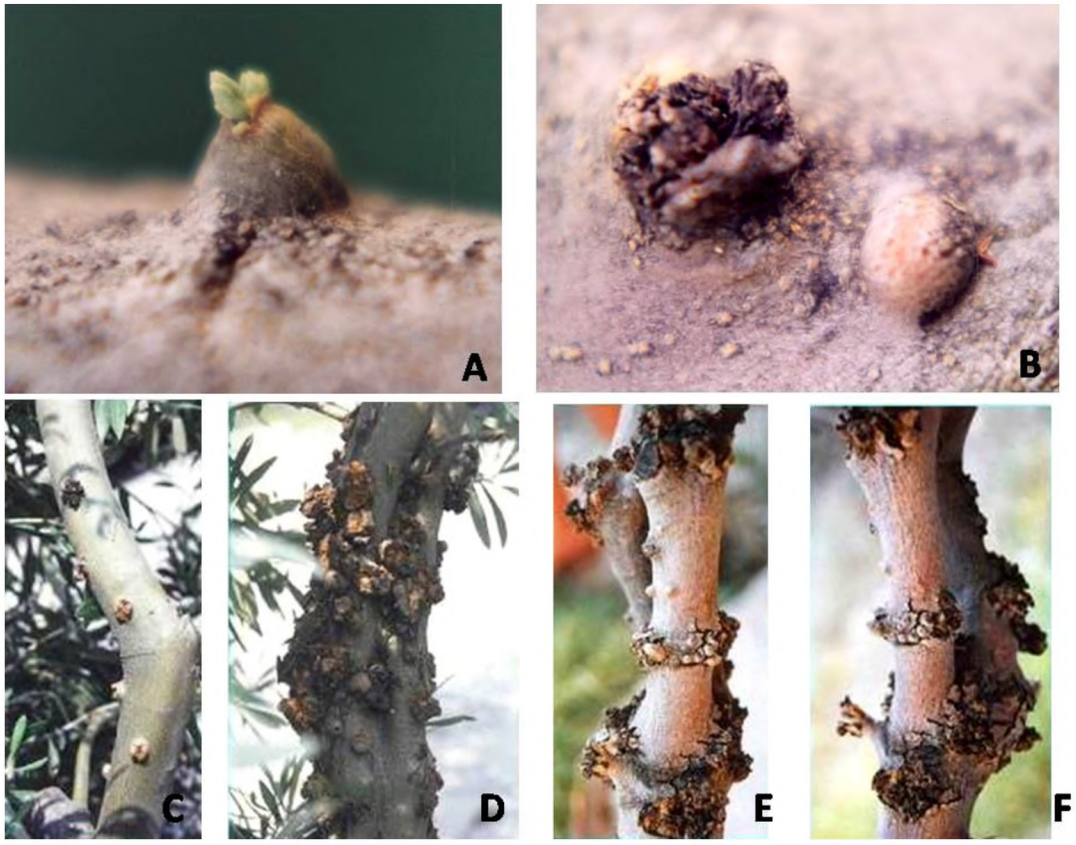

Fig. 3. Shoot formation from sphaeroblasts: A – sphaeroblasts and soot meristems arising from sphaeroblasts and emerging through the phloem in *O. europaea* var. Chonrdolia Chalkidikis; B – intact sphaeroblasts on the left and sphaeroblast infected by *Pseudomonas savastanoi* and converted to callus mass (canker) on the right; C, D – sphaeroblasts infected by *Pseudomonas savastanoi* in *O. europaea* var. Chondrolia Chalkidikis; E, F – intact sphaeroblasts and callus mass produced from infected by *Pseudomonas savastanoi* sphaeroblasts in *O. europaea* var. Chondrolia Chalkidikis

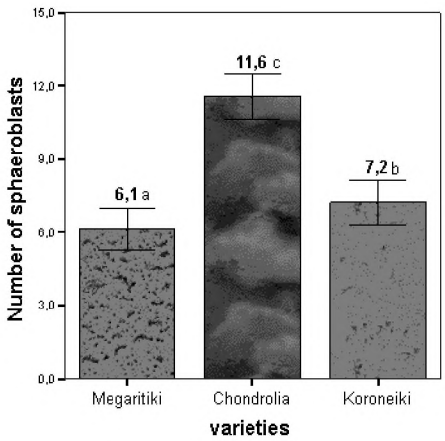

Fig. 4. Average number of sphaeroblasts formed in three olive varieties (Chondrolia Chalkidikis, Megaritiki and Koroneiki), cultivated in two different areas in Greece

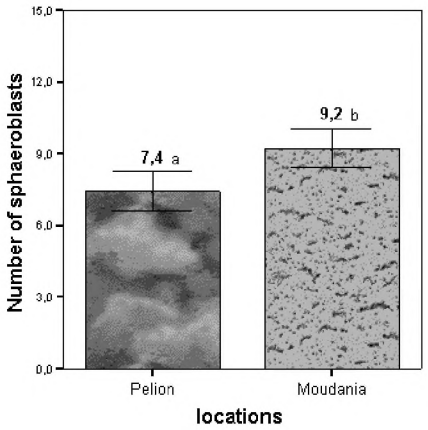

Fig. 5. The effect of the area of cultivation on the number of sphaeroblasts formed in three olive varieties (Chondrolia Chalkidikis, Megaritiki and Koroneiki)

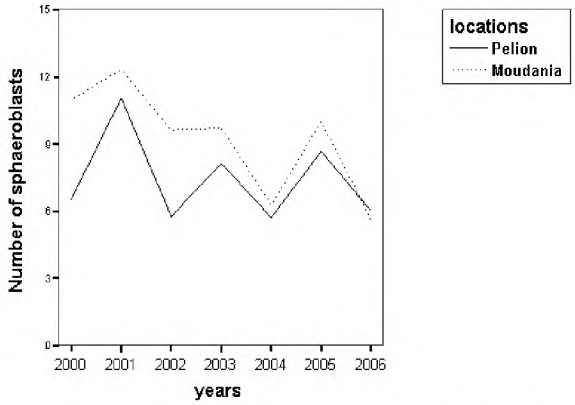

Fig. 6. Fluctuation of the number of sphaeroblasts formed in three olive varieties cultivated in the areas of New Moudania and mount Pelion in Greece

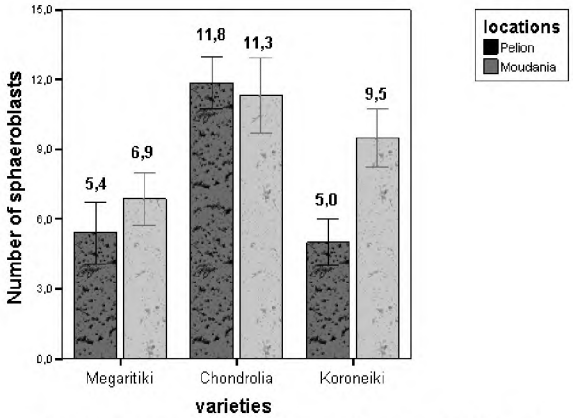

Fig. 7. Average number of sphaeroblasts formed in three olive varieties (Chondrolia Chalkidikis, Megaritiki and Koroneiki), cultivated in the areas of New Moudania and mount Pelion in Greece

The response of the varieties along the seven years was different (MS = 29.968; df = 12,264; F = 2.048; p = 0.021). The variety Chondrolia Chalkidikis showed the smallest fluctuation from year to year, being so more homeostatic and steadier in production. Variability in was biggest in the variety Megaritiki, having negative picks, making this variety very unsteady in sphaeroblast formation (Fig. 9).

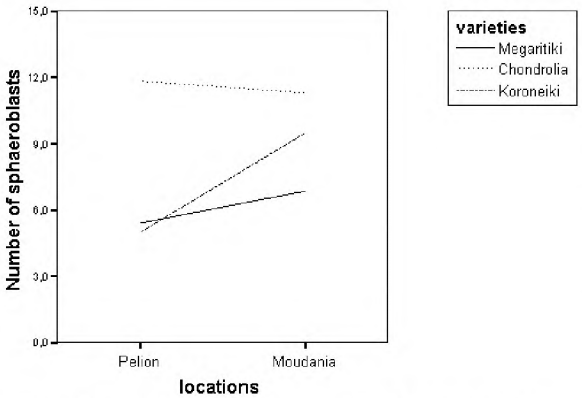

Fig. 8. Interaction between olive varieties and area of cultivation on sphaeroblast formation

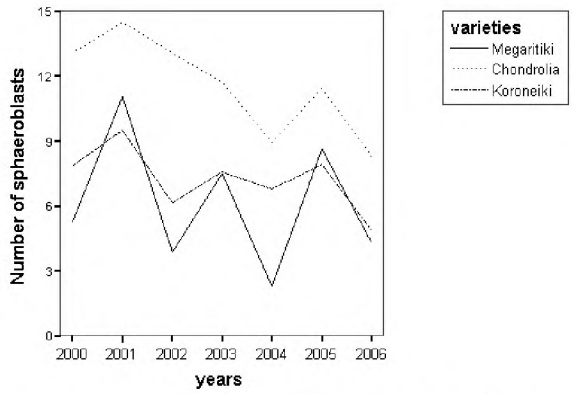

Fig. 9. The response of the olive varieties to the number of sphaeroblast formation by time

Discussions

The number of sphaeroblast formation is significantly affected by variety and area of cultivation. Among the three olive varieties used in this experiment the variety Chondrolia Chalkidikis is showing the highest and most steady number of sphaeroblast formation in the two areas of their cultivation. The response of the varieties to the same factors seems to be different. For example the varieties Megaritiki and Koroneiki had significantly more sphaeroblasts formed in New Moudania than on mount Pelion, while the variety Chondrolia Chalkidikis had more sphaeroblasts on mount Pelion. This is probably related to differences between the varieties. For example Chondrolia Chalkidikis is more vigorous variety in comparison to Megaririki and Koroneiki, while Koroneiki is better adapted to very dry, non fertile areas. Cultivation techniques have an effect on sphaeroblast formation. The higher fluctuation on mount Pelion seems to be related to the every second year pruning.

With apple species disbudding can cause sphaeroblast formation [2]. The olive tree is having the ability to form sphaeroblasts under conditions which are not known. It is probable that drought, low temperatures, temporary water logging, pruning and other factors may cause sphaeroblast formation and their shoot development but this needs to be investigated experimentally.

For a more detailed study olive trees of the variety Chondrolia Chalkidikis are cultivated in pots and subjected to various factors in order to study the effect of certain factors on sphaeroblast formation.

Sphaeroblasts or knots on shoots of olive trees of the varieties Chondrolia Chalkidikis and Megaritiki after excision in some of them the woody globular structure of a sphaeroblast is appearing [3], in some others a colored mass is revealed [6], containing obviously colonies of bacteria, which cause the following canker development. In other cases while the shoot of the sphaeroblast is coming through the phloem contamination occurs and canker development follows. Rojas with colleagues [5] reported that *Pseudomonas savastanoi* in olive trees induced knot formation from which a total of nine endophytic bacterial strains were isolated, each from inside a different tree knot. From the results of A.M. Rojas investigation [5] and the results of present work the question arising is whether the knots on shoots of olives are induced by the bacteria or sphaeroblasts at a certain stage of their development are contaminated and disorganized by the endophytic bacteria and together with the surrounding tissues develop to canker. The authors believe that the second view is the more probable.

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ЭКСПЕРИМЕНТАЛЬНЫЙ МУТАГЕНЕЗ В СЕЛЕКЦИИ ПЕРСИКА

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Введение

Экспериментальный мутагенез является важным направлением в выведении новых форм и сортов плодовых культур [2, 3, 5]. Он повышает частоту изменчивости признаков растений и расширяет возможности селекционера для отбора хозяйственно-ценных форм. Мутанты могут индуцироваться физическими и химическими мутагенами. Использование гамма-радиации на персике показало свою эффективность [6]. Перспективно также облучение пыльцы для включения в гибридизацию [4].

В последние годы начаты исследования по изучению химического мутагенеза в