DOI: 10.22620/agrisci.2017.21.004

CONNECTION BETWEEN THE LAUREL ESSENTIAL OIL CONTENT AND THE PLANT GENERATIVE REPRODUCTION FEATURES

Shorena Kapanadze*, Roland Kopaliani, Giorgi Getsadze

Akaki Tsereteli State University, Kutaisi, Georgia

***E-mail: shor-ka@mail.ru**

Abstract

The only species of the world wide industrially spread Laurel – Noble Laurel represents the difficult population, especially by the content of the essential oil. Because today the world market demand for Laurel products is high, the goal of the researches was to determine the connection between the previously selected high essential oil content forms of Laurel and the plant generative reproduction features.

The object of the research was the previously selected highly productive forms, and their vegetative and generative generation of commercial variations of Laurel spread in Western Georgia. Ginsberg's micro method and microscopic analysis method were used in the research. Based on the aforementioned, it was determined that only in the highly oil productive forms the oil content indicator was high in all cases of the same external morphological seedlings (according to the leaf morphology), and the difference between the features was in significant, while in other forms of the mother plant and their seedlings the oil content was different. It was also determined that the reexisted some correlation between the leaf thickness and oil content.

Keywords: generative generation, oil content, constant generation, seed plant, morphology.

INTRODUCTION

The national economic significance of the technical subtropical cultures production is big and diverse in our country, which gives different types of valuable products necessary for feeding, and raw materials for different fields of industry. Technical subtropical crops are widespread in the world and on an industrial scale, they are grown mainly in sub-tropical and tropical zones. The most important part of Subtropical technical cultures is the oil-bearing crops, from which the precious oils are produced in France, Italy, Bulgaria, Spain, Australia, the US, South America, India, China, Japan, Georgia and some other countries.

The noble Laurel belongs to the mentioned group of sub-tropical ether oil-bearing cultures. It is an evergreen, long lifetime plant and it has been included in the culture since ancient times. The Mediterranean coast and Asian countries are considered as the homeland of the Laurel. Laurel is not only included in the culture of Georgia, but it is also wildly widespread, so Georgia is considered as its homeland as well. At present, it grows in all regions of Georgia in the form of cultural plantations. (Kapanadze, 2014; Kintsurashvili and Kapanadze, 2014)

Sangun et al. (2007) mentioned earlier that, dried leaves of *Laurus nobilis* L. are used extensively in cooking, and the essential oil is used in a different area - flavorings industry, for a preparation of hair lotion due to its antidandruff activity and for the external treatment of psoriasis. Fruits are utilized for the production of perfumed soaps and candle manufacture because of their fatty acid content. The essential oil of leaves has antibacterial and antimicrobial properties.

A novel microwave method has been applied to the hydrothermal extraction of essential oil from *Laurus nobilis* L. leaves (Flamini et al., 2007).

The essential oils of the leaves and fruits from the bay (*Laurus nobilis* L.) grown in Antakya, Yayladagi and Samandagi were isolated from Sangun et al. (2007) by solvent extraction and analyzed by capillary gas chromatography (GC), gas chromatography and mass spectrometry (GC/MS). Although in both fruits and leaves the major component was found to be 1.8-Cineole in a concentration about 50%, compared with essential oils.

It should be noted that, at present, Laurel selective species exist neither in Georgia nor other parts of the world, and therefore on an industrial scale and in the form of units plantations (on homestead plots) significantly different forms of its species-populations are grown.

Georgian scientific research organizations conducted very large and important research work on subtropical crops, including the development of progressive technologies in Laurel cultivation, increasing of production level and reduction the

cost of product and improvement of the other economic indicators. As a result, today in Georgia Laurel cultivation is less laborious, gives a great deal of income and in subtropical agriculture, it contributes to a rational usage of natural and labor resources. At present, in the world market requirement for Laurel products is big and besides, it's increasing every year.

The worldwide industrially spread the only species of the Laurel - Laurel represents a difficult population. It consists of significantly different forms of morpho-biological characteristics, in particular by the content of essential oil. By these indicators, the taxons of Laurel population, widely-spread in Georgia, differ significantly from each other (Komakhidze, 1969).

It is known that the Laurel can be propagated by the generative and vegetative mod. Positive sides of seed propagation are a high rate of reproduction, simplicity, better ability to adapt to the changed environmental conditions, longer lifetime duration and less danger of disease and pests spreading, while the negative side is - that plants vary. On the other hand, the positive side of vegetative propagation is product equalling, negative – the complexity of the work fulfillment and expensiveness, shorter lifetime duration, more danger of spreading of diseases and pests (Vorontsov, 1979). Therefore, the main goal of that study was to determine the connection of oil content in previously selected high oil content Laurel forms with the plant generative reproduction features.

MATERIALS AND METHODS

The object of the research was taken from selected Laurel plants in different regions of the industrial plantations in West Georgia - commercial variations, as well as in homestead plantations and individual plantations. To some extent for the objects of experiments were taken the plants selected from natural Laurel plantations (Kheta, mountains of Urta and etc.).

Main studies have been conducted in Nosiri research farm of Akaki Tsereteli State University, where the soil is light clay to a depth of 20-40 cm, poorly expressed with good structure. The soil is humid, so on the farming plot drainage systems are arranged. It is with neutral reaction and averagely ensured to nutrient elements – nitrogen, phosphorus, and potassium. The soil is low productive concerning the humus amount (Getsadze, 2011; Getsadze and Kapanadze, 2013). Experimental trials of the same department in Meskhetian Agriculture, the soil is with light brown color, carbonaceous light loamy with good structure. The soil reaction is weak alkaline, alluvial, carbonaceous, low productive (because of low humus content), average ensured with phosphorus and potassium (Getsadze, 2011; Getsadze and Kapanadze, 2013).

For the determination of the plant essential oil crude and dried Laurel leaves were used. The experiment was conducted in 4 replicates, with 100 leaves in each. As a control was used a state standard green mass. Studies for determination of essential oils were conducted at the Essential oil technology laboratory in Akaki Tsereteli State University. The laboratory is equipped with Ginsberg machinery, trinocular light microscope, thermostats, refractometers, micrometers (for determining the leaf thickness) and other laboratory equipment.

To fulfill the planned tasks, different research methods were used. In particular, for determination of essential oil content in raw materials micro method (Ginzberg, 1932; Khlipenko et al., 2015) was used. In parallel with Ginzberg method accelerated essential oil determination method was used and microscopic analysis. There is no need for pre-processing of the leaves. They can be tested raw as well as dried, but essential oil storage is better seen in dried leaves. Microscopic analysis is rather accurate and coincides with the test results obtained by the method of hydrodistillation.

For determination, the leave's thickness micrometers were used.

Seed's and young seedlings growing and development were observed by generally accepted methods.

RESULTS AND DISCUSSION

Phenological observations and biometric measurements were carried out during the vegetation period, to determine the quality of homogeneity of the selected generative forms of Laurel.

Besides, to determine their quality of homogeneity of selected generative forms of Laurel, their seeds were sown in cold greenhouses. The study was conducted on seed germination.

Phenological observations and biometric measurements were also conducted on the seedlings. It has been determined that the percent of germination is lower and its amount is - 46.6 %. Seedlings height, after a month growing period, amounted 4,1 cm and by the end of August – 8,5 cm. The observations showed that among the leaves forms, seedlings differ according to the leaves and morphology of the vegetative organs. There is also a lot of diversity among seedlings according to their oil content (0,9 - 2,1%).

For determining the homogeneous quality of previously selected generative forms of Laurel, relatively more perspective forms were cultivated at Akaki Tsereteli State University research laboratory greenhouse and in specially designed boxes as well. The 130 pieces of seedlings of Laurel form №1 have been planted, 100 pieces of №3 and 120 pieces of H-4. There were optimal conditions for seedlings rooting.

Also, for the same purpose the most perspective form - №18, of the previously selected forms, was cultivated by the vegetative way, through the branches bending. After mentioned arrangements, the vegetative generations were accepted, according to the separately selected forms. The forms received after vegetative propagation were used as control variant to determine the homogeneity of vegetative generation.

From the previously selected Laurel forms (in December 2014), as the most productive were determined № 1, 2, 4, 18 and HH-4 with fully ripened seeds (with dark blue, blackish color). Pre-cultivation of the seeds was done by soaking in warm water, then seeds were peeled of and dried. Stratification of seeds was performed by keeping them in wet sand before sowing (Fig. 1).

Kept-stratified seeds, from the forms № 1, 2, 3 and 4, were sowed in Laurel experimental collective plot of Educational Research Center, in the middle of February 2015.

Fig. 1. *Peeled seeds of form № 18*

Fig. 2. *The seedlings of form № 18 sown in plastic cups*

Table 1. Content of essential oil in identical seedlings of form № 18 (September 2015)

Seedling's №	Quantity of essential oil storage	Oil content, in %
1	1025	2,05
2	986	1,97
3	1014	2,03
4	966	1,93
5	899	1,80
6	1000	2,00
7	1012	2,02
8	917	1,83
9	890	1,78
10	995	1,97
11	997	1,99
12	1005	2,01
13	888	1,78
14	916	1,83
15	971	1,82
16	968	1,91
17	870	1,74
18	990	1,98
Average:		1,93

The chemical variations of the essential oil from the aerial parts of *Laurus nobilis* L. (*Lauraceae*) have also been studied by Verdian-rizi (2009). Plant material has been harvested at each phenological status (vegetative, before anthesis, full flowering and seed-bearing) and identified 39 compounds in the essential oils.

Table 2. Content of essential oil in the mother plants with a slightly different leaves, according to their groups (September 2015)

Group №	Plant №	Quantity of essential oil storage, per piece	Essential oil content, in %	Group №	Plant №	Quantity of essential oil storage, per piece	Essential oil content, in %
1 Seedlings with slightly more rounded leaf plate	1	897	1,79	6 Seedlings with broader base of leaf plate	1	946	1,89
	2	920	1,84		2	997	1,99
	3	867	1,73		3	791	1,58
	4	1006	2,01		4	939	1,88
	5	965	1,93		5	840	1,68
		Average:	1,86			Average:	1,79
2 Seedlings with relatively broadleaf plate	1	965	1,93	7 Seedlings with relatively wide base of leaf plate and the sword	1	986	1,97
	2	1006	2,01		2	924	1,85
	3	1003	2,01		3	941	1,88
	4	865	1,73		4	880	1,76
	5	850	1,70		5	750	1,50
		Average:	1,88			Average:	1,82
3 Seedlings with slightly bent leaf plate	1	910	1,82	8 Seedlings with leaves with relatively broad tip of the plate	1	877	1,75
	2	944	1,89		2	1011	2,01
	3	857	1,72		3	856	1,71
	4	990	1,98		4	1111	2,22
	5	905	1,81		5	905	1,81
		Average:	1,83			Average:	2,03
4 Seedlings with slightly wavy edge leaf plate	1	920	1,84	9 Seedlings with slightly wavy visible signs of leaf plate	1	1003	2,01
	2	1000	2,00		2	900	1,80
	3	816	1,63		3	897	1,79
	4	947	1,89		4	802	1,60
	5	963	1,93		5	893	1,79
		Average:	1,8			Average:	1,81
5 Seedlings with more visibly distinct leaf plate veins	1	964	1,93	10 Seedlings with leaf plate of slightly uneven edges	1	980	1,96
	2	999	2,00		2	975	1,96
	3	891	1,78		3	925	1,85
	4	997	2,00		4	878	1,76
	5	1015	2,03		5	973	1,94
		Average:	1,87			Average:	1,90

Seeds of the form H-4 were sown at University Research Laboratory greenhouse, while seeds of form №18 were sown in plastic glasses (Fig. 2).

As mentioned, Laurel is the heterozygous plant, so seed reproduction, even if these seeds are received through self-pollination, they disintegrated and produced divers forms. We obtained diversity according to the oil content in individual Laurel seedlings and we compared if this indicator is correlated to the leaf plate morphology. The conformity has been established according to the example of generative generation of highly oil productive form № 18. Oil content determination was conducted in September 2015.

In the conducted investigations, Marzouki et al. (2009 a) revealed a seasonal variation in the yield and composition of essential oil, while geographical variability was not pronounced and no chemotypes were observed among the samples of Laurel leaf oil examined.

Due to a few leaves of selected seedlings, instead of Ginsberg method, we used microscopic analysis using the trinocular microscope. In this method, essential oil storage of every 50 pieces of leaves corresponds to 0,1% content of essential oil.

Kovacevic et al. (2007) reported in their publication, that steam distilled oil from the shoots, separated leaves, and stem, as well as from the flower of Laurel (*Laurus nobilis* L.), grown in Montenegro were analyzed by GC and GC/MS. The yield of essential oil was as follow 1.4% in young shoots, 1.5% in the separated leaves, and 0.7% in separated stems.

The research shows, that besides the form №18, the other forms of generative high oil content generations showed different leaf plate morphology from mother plants and therefore it showed different oil content, as well. The seedlings of form №18 were identical to the mother plant, according to the morphology, but the smaller number of seedlings differ slightly from the mother plant. Therefore form №18 is considered as perspective. To determine oil content, an analysis of different groups of seedlings was conducted. The results are shown in Table 1.

Shapes of the seedling' leaves from different groups from Table 2 are shown in Figures 3 and 4.

Fig. 3. *Shape of the seedling' leaves from groups № 1, 2, 3, 4 and 5*

Fig. 4. *Shape of the seedling' leaves from groups № 6, 7, 8, 9 and 10*

As it is shown in Table 2 and based on the oil content data we can conclude that generative generation of a distinctive highly oil productive Laurel form №18 is constant and almost repeats the mother plant's (Form № 18) indicators. The average oil content of ten group, of above-discussed seedlings, is 1.86%. Therefore, almost it is the same as the mother plant's average oil content of identical seedlings of highly oil content form №18 is 1,9 %.

Marzouki et al. (2009 b) analyzed stems, leaves, buds and flowers of *Laurus nobilis* L. for their essential oil composition. They found that the yields of essential oil of *Laurus nobilis* L. gathered from different stations in Tunisia, calculated by dry weight ranged between 0.4% and 1.1%.

135 seedlings of homogeneous plants were selected on the base of leaf external morphology (mainly mother plant type), and at the same time, there were selected plants which differ by oil content. Results are shown in Table 3.

Table 3. Essential oil content determination in leaves with light green color, collected from plants of № 18 generative generation

Plant №	Average thickness of leaves	Essential oil determined by the microscope	Essential oil content, in %
1	0,185 mcm	930	1,86
2	0,200 mcm	978	1,96
3	0,188 mcm	893	1,79
4	0,190 mcm	965	1,93
5	0,200 mcm	982	1,95
6	0,200 mcm	997	1,99
7	0,200 mcm	978	1,93
8	0,190 mcm	958	1,89
9	0,188 mcm	893	1,79
10	0,185 mcm	925	1,85
		Average:	1,90

The table shows that the oil content indicators of the same morphological seedlings (according to leaf morphology) in all cases is higher and differences between the rates are not significant.

CONCLUSIONS

After biometrical measurements of vegetative organs of the selected Laurel generative generation was established that according to these measurements of morphological traits there is a big diversity, indicating a high degree of initial heterozygosity. There is also diversity, in selected generative plants, according to the content of essential oils.

During the time of conducted investigations, it was revealed that in the various studied Laurel leaves from the evaluated plants, the storage of essential oils and the shape of the leaves are different. The content of the storage oils, for each Laurel plant, is individual and unchangeable. Therefore, perhaps it is genetically pre-determined.

The essential oil storage of Laurel leaves is with lysogenic origin.

It is found that, if leaf essential oil is a little in the time of conducted analysis, the storage amount of oil remains the same. That means essential oil storages of Laurel will be filled again in the time of good ecological conditions. If leaf veins created the wide mesh and there are only a few essential oil storages, no matter how favorable conditions will have for this plant, it will never be highly oil-bearing.

Concerning obtained results, in our study, we can conclude that it is possible to select plants with high oil content applying microscopic analysis.

Our investigations showed that the seedlings, from selected Laurel forms, differ according to the morphology of the seeds and vegetative organs. It was established a big diversity among seedlings, according to oil content (0,9-2,1%), found by microscopic analysis.

Results of the conducted study showed the correlation between the thickness of the leaves and oil content. On that base, selection should be carried out only on plants with thin leaves. Almost all of the thick-leaf forms don't have any high oil content, and that's why they have to be withdrawn from the selection process without any analysis.

REFERENCES

Flamini, G., Tebano M., Cioni P.L., Ceccarini L., Ricci A.S. and Longo I., 2007 - Comparison between the conventional method of extraction of essential oil of *Laurus nobilis* L. and a novel method which uses microwaves applied *in situ*, without resorting to an oven. J. Chromatogr. A., 1143, 36-40.

Getsadze, G., 2011 - Highly productive varieties of the sweet bay for receiving the results of selection - scientific conference on Grant № 511. Collected Works. Kutaisi, p. 13-25.

Getsadze, C., Kapanadze Sh. 2013 - From Laurel's a population of at high oil forms distinguishing results. Botanical Garden celebrates its 100th anniversary of the International Scientific Conference. Works. Batumi.Georgia.

Ginzberg, A. S. 1932 - A simplified method for determining the amount of essential oil in etherons. Jour. Him-farm-prom., No. 8-9, p. 326-329.

Kapanadze, Sh., 2014 – Laurel (*Laurus nobilis* L.) agrotechnology and genetically determined characteristics correlated with productivity. Monograph. Akaki Tsereteli State University Press. Kutaisi. ISBN 978-9941-453-13-7.

Khlypenko, L. A., Logvinenko L. A., Shevchuk O. M., Feskov S. A., Marko N.V., 2015 - Little-spread aromatic plants as a source of essential oils of a wide spectrum of action. C.57-59. In the collection. "Aroma correction of a person's psychophysical state". Materials V International Scientific and Practical Conference (Yalta, October 27-29, 2015). Yalta: 113 p.

Kintsurashvili, K. Kapanadze Sh. 2014 - the Ecological and economic importance of the introduction of new a highly productive varieties of bay Laurel in some regions of Turkmenistan. Works of the International scientific conference "Science, technology, and innovation in the era of power and happiness", the Academy of Sciences of Turkmenistan. Ashgabat.

Komakhidze, V., 1969 - Cultivation of Laurel. Publishing house "Ganatleba", Tbilisi, st. Kamo, №18.

Kovacevic, N. N., Simic, M. D., Risticb M. S. 2007 - Essential oil of Laurus nobilis from Montenegro. Chemistry of Natural Compounds, vol. 43, (4): 408-411.

Marzouki, H., Elaissi A., Khaldi A., Bouzid S., Falconieri D., Marongiu B., Piras A. and Porcedda S. 2009 a - Seasonal and Geographical Variation of *Laurus nobilis* L. Essential Oil from Tunisia - The Open Natural Products Journal, 2, 86-91.

Marzouki, H., Piras A., Bel Haj Salah K., Medini H., Pivetta T., Bouzid S., Marongiu B., Falconieri D. 2009 b - Essential oil composition and variability of *Laurus nobilis* L. growing in Tunisia, comparison and chemometric investigation of different plant organs. Natural Product Res23, 343-354.

Verdian-rizi, M., 2009 - Variation in the essential oil composition of *Laurus nobilis* L. of different growth stages cultivated in Ira - Journal of Basic and Applied Sciences, vol. 5, (1): 33-36, ISSN: 1814-8085.

Sangun, M. K., E. Aydin, M. Timur, H. Karadeniz, M. Caliskan and A. Ozkan. 2007 - Comparison of chemical composition of the essential oil of *Laurus nobilis* L. leaves and fruits from different regions of Hatay, Turkey - Journal of Environmental Biology October, vol. 28, (4): 731-733.

Vorontsov, V., 1979 - Biological bases of cultivation of Laurel. Monograph. Publishing house "Kaliningradskaya Pravda.