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NEW SPICE FORMS OF *Thymus vulgaris* L. ssp. PROMOTED FOR IMPLEMENTATION IN THE REPUBLIC OF MOLDOVA

Lilia CHISNICEAN, Zina VORNICU

Institute of Genetics, Physiology and Plant Protection, MD 2002, 20 Padurii Street, Chisinau, Republic of Moldova

Corresponding author email: tatarlipa@gmail.com

Abstract

Thyme (Thymus vulgaris L.) is known and used in various directions since ancient times. Some time it was marginalized due to the impossibility of being cultivated industrially and mechanically harvested (small waist of the plant). Currently, the species returns both in terms of pharmaceutical remedy and good honey-bearing, as well a delicate spice, being absent from the landscape arrangements of parks, gardens and groves. Our research focused on the selection of two forms of Thymus vulgaris (CCT) and Thymus x citriodora (CFL) obtained by clonal selection. Which differ from the initial forms, by the increased productivity of the raw material, the waist of the plant, and production of essential oil. Have been identified the efficient methods of multiplying the seeding material, for various tests to promote and implement new forms.

Key words*: Thymus vulgaris, pharmaceutical remedy, productivity, multiplying, essential oil.*

INTRODUCTION

In the traditional and folk medicine in the countries of the EU and the Middle East, the cultivated thyme has been widely used due to its numerous health benefits, possessing antimicrobial, soothing, antitussive, antiseptic and anthelmintic properties (Chevallier, 1996; Menković et al., 2011; Benítez et al., 2010; Mati, de Boer, 2011; Kavita, 2011; Pieroni et al., 2013; Redžić, 2007; Rexhepi et al., 2013; Kayani et al., 2014; Mustafa et al., 2012).

The spasmolytic effect of flavonoids has been confirmed (Van Den Broucke & Lemli, 1983). Thyme has antimicrobial properties due to some compounds such as terpenoids, essential oil and flavonoids (Behidj-Benyounes et al., 2014; Askun et al., 2009; Horikrrisha et al., 2004). The cultivated species of thyme are used as natural food preservatives, inhibiting microbial growth and extending the shelf-life of food products, and thus they are used to prepare marinades, various canned foods and desserts.

Thymus x *citriodorus* (Pers.) Schreb. is currently used as an ornamental species in landscape architecture.

Some time ago, this plant was introduced as an aromatic species in Turkey (Bagdat et al., 2011) and Iran (Omidbaigi and Rezaei, 2000).

The strong antioxidant activity of thyme is due to phenols - flavonoids (Kulišić et al., 2005; Mihailovic-Stanojevic et al., 2013; Hossain et al., 2013). Hydro-extracts are very powerful antioxidants due to the rosmarinic acid content (Bauermann & Thomann, 2011).

Many varieties have been created, to which different flavours and morphological characters are specific: 'Silver-Edged' - with silver leaf margin, 'Aureus', 'Doone Valley', 'Golden Dwarf', 'Variegatus' - leaves with yellow and white spots.

Since it is a species with numerous medicinal, aromatic and ornamental qualities, it has been used as research object to identify, among various sources, one cultivar with a narrower direction of use (spice - aromatic) and higher resistance to the climatic conditions, which are changing from year to year.

MATERIALS AND METHODS

Three cultivars of *Thymus vulgaris* var. *citriodora* (Pers.) Schreb., which had been purchased at an exhibition with sale of propagating material, and a spontaneous hybrid between *Thymus pulegioides* L. *x T. vulgaris* L. were used as initial material for research.

As a result of the clonal selection, several forms were obtained, and two of them were

chosen because they had higher productivity of raw material and essential oil and the plants were 4-6 cm taller than the original forms.
The new forms are much more cold hardy and thus it is not necessary to protect (cover) them in winter. The two forms are also less vulnerable to the extended periods without rainfall, which have become more frequent in the recent years. The seeds of cultivated thyme are small and they maintain a rather high germination capacity of 65% for three years, under storage conditions without temperature and humidity control.
However, the growth energy, after two years of storage, decreases drastically with each year. The optimization of seed storage conditions makes it possible to extend this term.
Many researchers and growers of the given species propose to sow thyme seeds in early spring (Malankina, 2015), but there are also recommendations to sow them in late autumn or early winter (Rey, 1993).
Because the cultivated thyme is a very polymorphous species, in our opinion, it requires vegetative propagation in order to maintain the obtained characters.
Thus, it was necessary to initiate the vegetative propagation of the new forms with the application of several factors - root stimulators.
For the multiplication of the initial population, which was studied as control, as well as the new lemon-flavoured form, propagation works were initiated.
To identify the most efficient vegetative methods of rooting the forms of *Thymus vulgaris* L. var. *citriodora*, two variants were tested: rooting in filtered water at room temperature; processing of cuttings with Reglal G natural root stimulator solution (1:400 un. and 1:500 un. dilution) - a product obtained at the IGPPP and officially registered as a plant root stimulator.
The mother plants for collecting plant material were prepared (transplanted in pots stored indoors) in February.
The cuttings were taken at the beginning of March and were placed in glass tubes for rooting, filtered water and a natural root stimulator solution at a dilution of 1:400 and 1:500 units served as media.
For rooting, the plant material was freshly collected (cut) with sharp garden shears, the blade of which was treated with ethyl alcohol for purification.
The cuttings were collected in a container with 1% $KMnO_4$ solution. One hundred thirty cuttings of both forms of *Thymus vulgaris* ssp. were subjected to rooting.
Seventy cuttings were soaked in the solution with root stimulator at a dilution of 1:400 and 90 cuttings - at a dilution of 1:500 units, for 24 hours.
The plant material stored for rooting under laboratory conditions, at room temperature of 22-25°C, the humidity of even better results - the rooting rate was by the air being maintained by frequent spraying with filtered hot water (the filter of the "Nobil" brand) with a temperature of 35°C.
The cuttings subjected to the rooting process were kept in tubes placed on a support, being maintained the constant level of the rooting solution for 9 days, after which they were transplanted in a solarium under controlled conditions of humidity and temperature (under agril foil).
At the time of transplantation, at the base of the seedling, several outgrowths appeared - bulbous nodules, of which several actual roots developed in the soil.
The soil in which the cuttings were transplanted for rooting was prepared by adding a specially prepared industrial substrate for plants, peat with neutral acidity, crushed gravel and river sand in the ratio of 3: 3: 3: 1. Further, 20 days until the extraction from soil, the cuttings had been acclimated to the environmental conditions (temperature) and the agril foil was removed. The necessary humidity was provided by spraying the foliage, in the evening or morning, with a minimum amount of water.

RESULTS AND DISCUSSIONS

The cuttings were extracted from soil at the beginning of October, to be tested in the CCC. The obtained planting material was studied to determine the rooting rate and other important characteristics.
Thus, for the form *T. vulgaris* var. *citriodora* - CCT - the control consisted of 370 cuttings placed in filtered water to take roots and then transplanted into a solarium, so, 294 of them took roots and the rooting rate was 79.4%.

The cuttings treated with the natural root stimulator (Reglal G) at the dilution of 1:400 as well as 1:500 had practically the same rooting rate of 48.6 % and 46.7%, respectively (Table 1). As for the form selected of *T. vulgaris* var. *citriodora* - CFL - of 130 cuttings placed for rooting in filtered water, 76.9% took roots, and the rooting rate of those treated with the Reglal G root stimulator at a dilution of 1:400 and 1:500 was 55.7% and 52.2%, respectively. The treatment of cuttings of *Thymus vulgaris* ssp. with the Reglal G root stimulator had no advantages over the rooting in filtered water, which had even better results - the rooting rate was by 25% higher.

Table 1. The rooting rate of cuttings of *Thymus vulgaris* ssp. in various media

Name of the form	Filtered water			Natural root stimulator Reglal G, units					
				1:400 dilution			1:500 dilution		
	Number of cuttings		Rooting rate %	Number of cuttings		Rooting rate %	Number of cuttings		Rooting rate %
	Subjected to rooting	Rooted		Subjected to rooting	Rooted		Subjected to rooting	Rooted	
T. vulgaris var. *citriodora* - CCT - control	370	294	79.4	105	44	48.6	180	42	46.7
T. vulgaris var. *citriodora* - CFL selected form	130	100	76.9	105	39	55.7	180	47	52.2

At harvest, the cuttings were analysed morphologically according to the biometric characteristics - the size of the plants, the length of the roots, the number of the ramifications of the stem, the number of shoots formed on a plant (Table 2). Because the difference between the external morphological characteristics of the treated and untreated cuttings was not essential, the presented data constitute the averages (30 plants) from the measurements of three components in each sample (untreated, treated with Reglal G natural root stimulator solution with a dilution of 1:400 and 1:500).

Table 2. Biomorphological features of rooted cuttings of *Thymus vulgaris* var. *citriodora* ssp.

Name	Values	Height of the cutting, cm	Length of the root, cm	Number of stem ramifications	Number of shoot per cutting
T. vulgaris var. *citriodora* - CCT - control	min	24.0	13.0	20.0	2.1
	max	26.0	17.0	23.0	2.5
	X	24.4	14.0	21.0	2.4
	V%	21.4	28.1	23.0	17.9
	P%	3.8	3.9	3.2	2.3
	DL_{05}	2.3	1.7	2.1	1.0
T. vulgaris var. *citriodora* - CFL - selected form	min	25.0	13.0	19.0	2.0
	max	27.0	16.0	22.0	2.2
	X	25.7	14.7	21.7	2.6
	V	20.4	27.2	22.7	15.6
	P%	3.45	1.3	3.2	2.06
	DL_{05}	2.3	3.8	2.2	1.1

The height of the rooted cuttings was 24.4 cm in the control and 25.7 cm in the selected form. Both the length of the roots and the number of ramifications of the stem had slightly higher values in the selected form than in the control. Each of the cuttings subjected to rooting formed on average 2.4 shoots per plant in the control and up to 2.6 the selected form.

The size of the basal ramification (2-3 per plant) was practically equal to that of the mature plants, starting from growing from the base of the cuttings, the smaller apical ones (50% of the length of the basal ones).

Thus, the plant material of *Thymus vulgaris* ssp., necessary to start the testing experience in CCC, was multiplied during a growing season.

The treatment of the cuttings with the Reglal G natural root stimulator did not give any advantages as compared with the untreated cuttings, since the results obtained in all variants were almost identical.
The soil preparation works for planting cuttings consisted of fertilization with organic fertilizers as 60-80 kg/ha, applied under the plow, after planting and after the first harvest.
Plow at 28-30 cm, leveling, shredding and superficial loosening of the soil.
The planting norm is 160,000-180,000 threads per/ha, and the distance between nests is 20 cm.
After planting, a mandatory irrigation was applied, which needs to be repeated during the vegetation period.
The care work consists of soil remediation, weed destruction.
Harvesting was done only once because the plantation is young, by cutting the unaligned aerial part, before complete flowering.
During 2018, the planted cuttings developed into mature plants. In 2019, the test plantation was already created, and phenological observations and biometric measurements of the habitus of the plants were carried out.
The year 2019 was very difficult for the initiation of plant growth and development, and adverse weather conditions persisted throughout the growing season.
The lack of rainfall at key moments in plant development and the high temperatures that caused drought did not allow plants to develop normally, and thus productivity indices were very low.
The small stem of the plant, is one of the impediments, making mechanized harvesting impossible on industrial surfaces which prevents the introduction into this culture of this precious species.
During the researches, during several years, the selection on the character of the "plant size" was identified as a priority. The larger the size of the plant in the thyme species, the easier the harvesting, both the manual and the mechanized ones.
The lemon thyme (*Thymus vulgaris* var. *citriodora*) is a species of small plants, but this year, the plants were even smaller than usual - 20-23 cm in the control and 21-23 cm in the new cultivar. The diameter of the bush was equal to average values 19-22 cm from the control and 26-29 cm the new cultivar.
The other morphological indices also did not reach high values.
The number of productive stems at one plant was only 19-22 in the control and 26 in the new cultivar.
The length of the growing season until harvest was 85 days in both forms (Table 3).
The average fresh raw material production was slightly higher in the new cultivar, by 0.99 kg, and it was statistically confirmed.
At the full flowering stage (Figure 1) of plants, the content of essential oil was appreciated and it constituted 0.285%, in the control and 0.32% in the new cultivar, calculated per hectare, it would be equal to 7.24 kg and 10.3 kg/ha, respectively.

Figure 1. Testing CCT and CFL forms

Plants of both forms tested had abundant flowering, each shoot having flowers all its length. The plants in the plots predestined for seed collection were left until the disappearance of the floral components that constituted 35 days. The harvest for obtaining the seeds, however, was unsuccessful, with no seeds.
The binding of seeds may have been caused by the high temperatures (burning in air) of that period and the long-term lack of precipitation. Testing for seed production will be continued, to elucidate this case.

Table 3. Biomorphological and productivity indices of *Thymus vulgaris* var. *citriodora*, 2019

Name		Height of plants, cm	Diameter of a bush, cm	Number of stems per plant	Length of the growing season, days	Productivity of 10 m^2 of raw material		
						fresh, kg	dry, kg	essential oil, kg
CCT - control	min	20	19	148	85	2.04	1.06	
	max	23	22	156	-	2.73	1.61	
	x	21	21	153	85	2.54	1.46	0.724
CFL - new form	min	21	23	159	85	3.29	1.62	
	max	23	29	166	-	4.20	1.82	
	x	22	26	161	85	3.72	1.68	1.03

BD_{05} - 0.22 kg - for the production of dry raw material.

Lemon thyme is used as a spice in the well-known mixture of dried herbs "Herbs de Provence". It is also used as a medicinal plant, to produce effective medicinal preparations such as cough suppressants, bronchiolitis - syrups, drops, teas etc. Lemon thyme has high potential as honey plant, making it possible to obtain 160-180 kg/ha of honey. It is also commonly used as an ornamental plant to decorate alpine gardens.

CONCLUSIONS

The "premature" method (February - March) of vegetative propagation by cuttings of *Thymus vulgaris* var. *citriodor*a, transplanted later in soil, is quite efficient, obtaining a rooting rate equal to 77-79% and creating the possibility to reduce the loss of planting material by 25-30%, as compared with other methods of vegetative propagation (division of the mature plant, layering etc.)

The obtained cuttings are vigorous and are able to bloom even in the first year of vegetation.

The new cultivar of lemon thyme *Thymus vulgaris* var. *citriodora* (CFL) has higher productivity indices than the control cultivar (CCT), but it still needs testing to confirm these advantages, especially under adverse conditions of insufficient humidity, and to promote it as an aromatic plant.

The obtained cuttings are vigorous and are able to bloom even in the first year of vegetation.

The new cultivar of lemon thyme *Thymus vulgaris* var. *citriodora* (CLF) has higher productivity indices than the control cultivar (CCT), but it still needs testing to confirm these advantages, especially under adverse conditions of insufficient humidity, and to promote it as an aromatic plant, with unique medicinal properties.

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