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MOLDAVIAN MEDICINAL AND AROMATIC PLANTS VARIETIES

GONCEARIUC Maria
Laboratory of Medicinal and Aromatic Plants Institute of Genetics, Physiology and Plant Protection Academy of Sciences of Moldova
mgonceariuc@yahoo.com

Abstract: The studies carried out in the area of genetics and breeding of aromatic and medicinal plants in the Republic of Moldova have resulted in the development of new high-efficient cultivars. The new cultivars of *Salvia sclarea* that are early-, medium- and late-ripening allow the production of 18-24.8 t/ha of inflorescences and 67-79 kg/ha of essential oil depending on the variety. New *Lavandula angustifolia* clone cultivars, named Moldoveanca-4, Alba-7, and Vis Magic-10 with a different harvesting time have been developed. Their producing capacity is 125-245 kg/ha of essential oil and varies with the variety. The works carried out on the breeding of *Anethum graveolens* have afforded a cultivar named Ambassador with a producing capacity of 10.5 t/ha of raw material and 88.9 kg/ha of essential oil with a carvone content of 39.8%. A new cultivar named Miracol has been developed in *Salvia officinalis*, which contributes to a producing capacity of 900 kg/ha of dry leaves or 18 kg/ha of essential oil for a single harvesting. The research conducted on *Silybum marianum* has provided a new cultivar named Argintiu, which is early-ripening with a fruit producing capacity of approximately 890-1,000 kg/ha. Hybridizations and individual selections have produced two new cultivars of *Calendula officinalis* – Nataly and Diana with a production making more than 1,000 kg/ha of dry inflorescences and a content of flavones and polyphenols of 0.624-0.873% and 0.988-1.038% respectively, depending on the variety.
Keywords: medicinal and aromatic plants, variety, essential oil, yield, polyphenols, flavones

Introduction

The importance of medicinal and aromatic plants is indisputable in view of the revival of phytopharmacy and herbal treatments. Thus, the share of drug products from medicinal and aromatic plants and their derivatives has been constantly growing. More than 50% of the prescribed drugs are chemical derivatives identified for the first time in medicinal plants. Estimated 50 -70 thousand of plant species are used in medicine throughout

the world (Rose,1981). On the other hand, further harvesting of these plant species from wild flora is currently uncontrolled in many countries, thus damaging irreparably wild flora. Many of the medicinal plant species from the flora of the Republic of Moldova (Negru, 2007), as well as of other countries (Murphy, 2008; Biodiversity, EC., 20013) have become rare endangered species. Preservation of vegetal biodiversity, including the biodiversity of medicinal plants may be accomplished only through cultivation of these species. Taking this into consideration and because the quality of raw vegetal material does not meet the standards of the European Pharmacopoeia in many cases, it is imperative to cultivate these species, while the cultivation requires development of varieties with specific characters and properties and a certain content of chemical components in a particular ratio for the specific pedoclimatic conditions of cultivation.

Given the fact that about 3,500 tons of essential oil, 10,000 tons of food additives, 13,000 tons of perfumery additives are produced in the world, development and cultivation of medicinal and aromatic plant varieties are strictly necessary, thus ensuring steadfast production of high quality pharmaceutical, perfumery, and food raw material with a much more enhanced content of active principles in comparison with the types collected in wild flora. Moreover, they provide an important source of income for population in rural areas.

The producing potential of the Republic of Moldova in the matter of pharmaceutical raw material, essential oil and concrete (200-250 tons), food, cosmetic, perfumery additives is relatively modest. However, the quality of the products derived from the medicinal and aromatic plants cultivated in the Republic of Moldova is very high due to the elevated concentration of active principles supported by the varieties developed here, that are distinguished by a unique ratio of the major components, as well as the pedoclimatic conditions specific to this area – abundant insolation, rich soils, reduced atmospheric depositions. For example, the potential of our *Salvia sclarea* L. varieties for the concentration of sclareol in the concrete is 65-70% or 10-15% higher than the requirements of the European standard in this sphere. All these make this branch very attractive.

Material and methods

The biological material is represented by varieties, hybrid genotypes of medicinal and aromatic plants belonging to the species *Salvia sclarea* L. (Clary Sage), *Lavandula angustifolia* Mill. (Lavender); *Salvia officinalis* L., (Common Sage); *Anethum graveolens* L. (Dill); *Glaucium flavum* Cr.

(Yellow poppy); *Silybum marianum* Gaerth. (Milk thistle) and *Calendula officinalis* L. (Common marigold).

S. sclarea varieties have been created through hybridization between depression non-affected inbred and male sterility lines (type 1). Simple, double, triple, backcross, and stepwise hybrids produced in the previous study were also used as parental forms. The general and specific combining capacity of the parental forms was assessed in top cross using two testers. The developed cultivars represent hybrids of different complexity. So, the varieties Dacia-50, Dacia-99, and Victor are simple hybrids; Nataly-Clay is a triple hybrid; Ambra Plus is a backcross hybrid; Balsam is a stepwise hybrid.

The initial *Lavandula angustifolia* materials were produced through different polycross hybridization methods to develop clonal varieties Moldoveanca 4, Vis magic 10, and Alba 7. Promising hybrid forms were used to produce clonal varieties by vegetative multiplication. The *Salvia officinalis* variety Miracol represents a simple hybrid between two genotypes originating from Moldova and Crimea. The *Calendula officinalis* varieties Nataly and Diana were developed by selection in the F_2 hybrid population. The population was obtained through hybridization between inbreeding lines $S_{2\text{-}12}$ of different provenience. Selections were carried out for quantitative traits. The varieties of the *Anethum graveolens, Glaucium flavum,* and *Silybum marianum* species were developed using individual selection methods.

The essential oil content was assessed by hydrodistillation and recalculated for dry matter. The qualitative and quantitative analyses were performed using Gas Chromatography (GC) coupled with Mass Spectrometry (GC-MS): gas chromatograph - Agilent Technologies 7890; mass selective detector 5975C Agilent Technologies with quadruple, capillary column (30m x 0.25mm i.d., film thickness 0.25 μм) with the HP-5ms non-polar stationary phase. The injector and detector temperatures were 250°C and 280°C, respectively, with a temperature gradient from T_1 = 70°C (2 min), T_2 = 200°C (5°C/min) to T_3 = 300°C (20°C/min, 5 min). Mobile phase: helium 1ml/min, the injected volume of essential oil - 0.03 μl, split rate - 1:100. The identification of the chromatographic peaks was performed using the software package AMDIS ™, coupled with NIST database. Flavones and polyphenols concentrations in *Calendula officinalis* inflorescences were determined by the methods described in the European Pharmacopeia.

The validation of the agronomic attributes, quantitative characters of the new varieties was done by testing in the comparative competitive crops by State Commission for Plant Cultivars Testing methods.

Results and discussions

The studies carried out in the area of genetics and breeding of medicinal and aromatic plants in the Republic of Moldova have resulted in the development, registration and patenting of the new varieties. The researches have been more profound and efficient in Sage Clary (*Salvia sclarea*) (Goncearіuc, 2002, 2005, 2005a, 2009, 2010).

The development, evaluation and use of the initial valuable material that includes inbreeding and male sterile lines, the lines that consolidate male sterility, simple hybrids, three line hybrids, double hybrids, backcrosses and stepwise hybrids have resulted in some high-efficient hybrids with an enhanced producing capacity. Among them, there are Dacia-50, Ambra Plus, Balsam, early- and late-ripening varieties named Victor and Nataly-Clary (Fig.1.), and a medium-ripening variety named Dacia-99. These varieties reach the ripening state gradually and together with the early-ripening varieties Dacia-50, Ambra Plus (Fig.2.), and Balsam, form a conveyer during harvesting, which allows a gradual harvesting of each variety and ensures a substantial reduction in raw material and essential oil losses. The varieties Victor (late), Dacia-50 (early) and Dacia-99 (medium) are simple hybrids, while Nataly-Clay (late) is a triple hybrid; Ambra Plus (early) and Balsam (early) are very complex hybrids, developed through stepwise and backcross hybridization (Goncearіuc, 2009, 2013; Goncearіuc, Balmuş, 2006). All the varieties are suitable for both processing of raw material (inflorescences) and production of essential oil through distillation, as well as of concrete through organic solvent extraction. The different vegetative period and gradual ripening allow the expansion of the harvesting period up to 25-27 days. In its turn, this contributes to the increase of the areas occupied with sage and processing of a higher quantity of raw material while expanding industrial processing capacities (Goncearіuc, 2010; Goncearіuc, Balmuş, 2006). When the plantations are sown with new sage cultivars, the seed rate is 4-5 kg/ha of the first class as opposed to other varieties, which need a sowing rate of 10-12 kg/ha of seeds.

The plantations of these varieties can be exploited three years. The yield of inflorescences of *S. sclarea* varieties in three years (2011-2013) of cultivation made 18.1-24.8 t/ha (Table 1). It should be mentioned that the

varieties listed have a producing capacity of up to 14 -15 t / ha of inflorescences in the second year of vegetation. The results obtained in 2012 were more modest because that year was very dry. Throughout the testing period, all the varieties accumulated high contents of essential oil (0.825-1.494%), especially in the second year of vegetation (1.253-1.494%), but the early-ripening variety Balsam was the best for that character.

The producing capacity of essential oil in the *S. sclarea* varieties developed is very high ranging between 63.8 and 79.5 kg/ha in three years of plantation exploitation. The early-ripening varieties Ambra Plus and Balsam are the most productive.

Figure 1. *Salvia sclarea,* variety Nataly Clary

Figure 2. *Salvia sclarea,* variety Ambra Plus

It is very important that the new varieties bloom in the first year of vegetation, while high resistance to wintering allows exploitation of plantation in the third year of vegetation. By the third year, their yielding capacity makes 14.1-16.2 kg/ha of essential oil depending on the variety. The quality of essential oil is high because the concentration of linalyl acetate in essential oil is 63-70% and sclareol – 6-12% (Goncearuc, 2010). All the *Salvia sclarea* varieties are registered in the Republic of Moldova. The varieties Dacia 99, Victor, and Nataly-Clary were patented in 2011. The variety Ambra Plus was patented in 2013.

The studies carried out in the area of genetics and breeding of lavender (*Lavandula angustifolia* Mill.) have started with the development of new genotypes through polycross hybridization using germ plasma resources from France, Crimea, and Moldova. Three new clone varieties of *L. angustifolia* named Moldoveanca-4, Alba-7, and Vis magic-10 (Fig.3) have been developed up to now. They are resistant to frost, wintering and drought (Goncearuc, 2005, 2005a; Goncearuc, Balmush, Kulcitki et al., 2012). All the lavender clone varieties were patented in 2010. Moldoveanca

4 and Vis magic 10 do not actually exceed the clone variety witness for the production of inflorescences and both have an increased content of essential oil (4.491, 4.224%), which provides yields of 178.7 and 125.3 kg/ha of essential oil respectively (Table 2).

Table 1

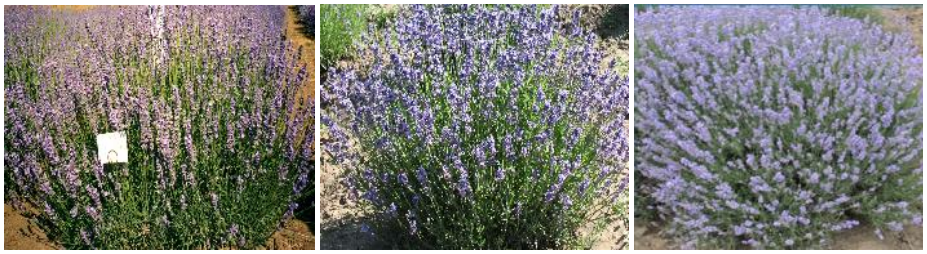
Inflorescence yield, essential oil content, and productivity of *Salvia sclarea* varieties in three years of vegetation (2011-2013)

Varieties	Yield of inflorescences, t/ha				Content of essential oil, % (dry matter)			Production of essential oil, kg/ha			
	I year 2011	II year 2012	III year 2013	Σ	I year 2011	II year 2012	III year 2013	I year 2011	II year 2012	III year 2013	Σ
Early-ripening varieties											
Dacia-50, st.	3.3	11.2	5.5	20	0.926	1.143	0.988	9	38.6	16.2	63.8
Ambra Plus	9	11	4.8	24.8	0.825	1.179	1.029	22.3	39.1	14.7	76.1
Balsam	6.3	10.4	4.4	21.2	1.009	1.494	1.078	18.9	46.5	14.1	79.5
Medium-ripening variety											
Dacia-99	3.9	10.4	3.8	18.1	1.003	1.411	1.023	11.7	44.1	11.3	67.1
Late-ripening varieties											
Victor	4.8	11.7	3.3	19.8	0.833	1.253	1.187	12.1	43.9	11.8	67.7
Nataly Clary	4.5	10.6	4.1	19.2	0.88	1.291	1.043	13.5	41	13.8	68.3

Table 2

The average productivity of *Lavandula angustifolia* Mill. clone-varieties

Varieties	Yield of inflorescences, t/ha	Essential oil content, % (dry matter)	Essential oil production, kg/ha	Variety efficiency, essential oil, kg/tonne
C-90, standard., late	8.9	2.629	93.5	10.5
Moldoveanca-4,early	8.7	4.491	178.7	14
Vis magic-10, medium	9	4.224	125.3	16.9
Alba-7, late	12.8	5.376	245.0	21.5



a b c

Figure 3. *Lavandula angustifolia* Mill. clone-varieties:
a- Moldoveanca- 4, b- Vis magic-10, c- Alba-10.

Alba 7 is the most efficient clone variety ensuring an inflorescence production of 12.8 t/ha with an essential oil content of 5.376%, which guarantees a very high production of essential oil of 245 kg/ha. One ton of inflorescences provides between 14 kg and 21.5 kg of essential oil depending on the variety through steam distillation. Thus, the efficiency of the new clone varieties is much higher than that of the witness (Goncearuc, 2005, 2005a).

Dill (*Anethum graveolens* L.) is another essential oil containing and medicinal species cultivated in Moldova. During 1996-2000, when the markets of essential oils had been lost by this country and the essential oil crop plantation was annihilated, dill oil was exported every year. An early-ripening dill variety, named Ambassador (Fig. 4), has been developed to increase the efficiency of cultivation and processing of this species. It is characterised by average yields of raw material making 10.5 t/ha, those of essential oil of 88.9 kg/ha with a carvone concentration of 39.8%. The variety was registered in 2004. The dill varieties that have been cultivated up to the present have a producing capacity of only 50-60 kg/ha of essential oil with a concentration of the principal component carvone making only 29.5% (Goncearuc, 2009; Balmush, 2006).

The works performed on *Salvia officinalis* L. have resulted in the development of an early ripening variety, named Miracol that is resistant to drought, frost and wintering (Fig. 5). It was registered in 2005. The variety can be used to produce pharmaceutical raw material of *Folium Salviae* and *Herba Salviae* and essential oil – *Oleum Salviae*. The producing capacity of the variety Miracol is 900 kg/ha of dry leaves (13% of humidity) or 18 kg/ha of essential oil (Table 3). In the case of two yields, the producing capacity of the variety is even higher (Goncearuc, 2008; Goncearuc, Balmush, Kulcitki, 2012, 2012a). Quantitative and qualitative analysis performed by GC GC-MS showed that the variety Miracol in the essential

oil contain 24 components. Major components in essential oils separated from variety Miracol are thujone (α-, β-), camphor, eucalyptol (Tab.4).

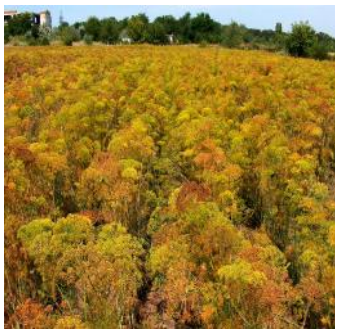


Figure 4. *Anethum graveolens*, variety Ambasador

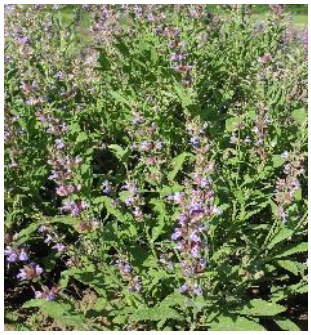


Figure 5. *Salvia officinalis*, variety Miracol

Table 3

Yield of *Salvia officinalis* L. variety Miracol.

Variety	Years	Yield of raw material, kg/ha		Essential oil content, % (dry matter)	Production of essential oil	
		Humidity 60%	Dry matter		kg/ha	%
Miracol	2012	2990	850	2.500	18.7	183.3
	2013	2930	960	2.240	17.4	153.9
	X	2960	905	2.362	18.0	168.2
Ghineţei, Witness	2012	2090	600	1.280	10.2	100.0
	2013	2010	570	1.691	11.3	100.0
	X	2050	590	1.485	10.7	100.0

Germ plasma resources of different genetic and geographic origin have been used to develop genotypes with new characteristics and properties in *Calendula officinalis* L. (Common marigold). A complex hybridization has produced many varieties, two of them – Nataly and Diana with large inflorescences, 7.0 cm in diameter and a high number of ligulate (Fig. 6) flowers, with a producing capacity of more than one ton per hectare. The varieties are distinguished by the tubular flower colour. Thus, the tubular as well as ligulate flowers are orange in Nataly variety, while the tubular flowers are brown and the ligulate flowers are orange in Diana variety. The concentration of the active matter in the new marigold variety is much higher than that in the local population cultivated in Moldova, which served as control. The distinction between them, as for this index, is as follows: the

flavones concentration in Nataly cultivar is 0.873%, being higher in Diana cultivar, which contains 0.624%, while the content of polyphenols is relatively lower (0.988%) than in Diana cultivar in which the polyphenols concentration is 1.038% (Gonceariuc, 2008, 2009).

Table 4

Qualitative and quantitative composition of the essential oil of the variety Miracol of *S.fficinalis*

Components		Variety Miracol		Components		Variety Miracol	
		shoots/ leafs	leafs			shoots/ leafs	leafs
1	α-Pinene	2.591	2.565	14	Camphor	24.59	19.144
2	Camphene	2.418	2.613	15	Borneol	3.302	3.472
3	Sabinene	0.253		16	4-Terpineol	0.538	0.554
4	β-pinene	1.365	1.68	17	α-Terpineol	0.285	-
5	β-Mircene	0.778	0.669	18	α-terpinylacetat	2.324	3.856
6	Limonene	1.505	1.302	19	β-caryophilene		0.27
7	Eucalyptol	8.416	10.372	20	α-caryophilene	3.929	5.44
8	γ-terpinene	0.311		21	Caryophilene oxide	2.617	3.367
9	α-terpinene	0.489	0.317	22	Viridiflorol		0.42
10	Linalool	0.497	0.483	23	Aroma dendrenoxid	3.063	3.653
11	α-thujone	33.791	21.239	24	Labdatriene	0.716	0.968
12	β-thujone	5.877	16.201	Components identifiend		22	20
13	*cis*-Sabinol	0.345		Total, %		100.0	98.585

a b c

Figure 6. *Calendula officinalis* inflorescences:
a- Diana variety, b- local population, c- Nataly variety

Genetic and breeding studies have also been conducted on *Glaucium flavum* Cr. (Yellow poppy), a very important species due to the fact that its *herba* contains 15 alkaloids, glaucine being the most important with an action similar to that of codeine but it is cheaper and has no depressing

action on respiration, no negative impact on the digestive system and, in contrast to codeine, produces no addiction. The studies have resulted in a new variety of *G. flavum*, named Agat (Fig. 7) with a vegetation period of three years. The average production of Agat fresh *herba* is 3.2 t/ha in the first year and 10.8 t/ha in the second year, while it makes 10.2 t/ha in the third year. Totally, the variety provides an average production of 24.0 t/ha in three years of exploitation.

Figure 7. *Glaucium flavum*, variety Agat

a

b

Figure 8. *Silybum marianum* variety Argintiu: a-plantation, b-seeds

Among the medicinal plant species under study, there is milk thistle (*Silybum marianum* (L) Gaertn). Individual selections and subsequent hybridization have afforded an early-ripening cultivar named Argintiu homologated in Moldova in 2004 and patented in 2013. The cultivar is resistant to drought, while simultaneous maturation of fruits, in the majority of inflorescences, contributes to mechanical harvesting. The average fruit producing capacity (*Fructus Cardui Marianus*) of Argintiu variety (Fig. 8) is 890-1,000 kg/ha under unfertilized conditions of cultivation (Goncearuc, 2009).

Conclusion

1. The studies carried out in the area of genetics and breeding of aromatic and medicinal plants in the Republic of Moldova have resulted in the development of new high-efficient cultivars.
2. The new cultivars of *Salvia sclarea* that are early-, medium- and late-ripening allow the production of 18-24.8 t/ha of inflorescences and 67-79 kg/ha of essential oil depending on the variety.
3. New cultivars of *Lavandula angustifolia* named Moldoveanca-4, Alba-7, and Vis Magic-10 have been developed. Their producing capacity is 125-245 kg/ha of essential oil and depends on the variety.
4. The works carried out on breeding of *Anethum graveolens* have resulted in the development of a cultivar named Ambassador with a producing

capacity of 10.5 t/ha of raw material and 88.9 kg/ha of essential oil with a carvone content of 39.8%.

5. A new cultivar named Miracol has been developed in *Salvia officinalis*, which contributes to a producing capacity of 900 kg/ha of dry leaves and 18 kg/ha of essential oil for a single harvesting.
6. The breeding works performed on *Glaucium flavum* have provided a variety named Agat with a yield of 24.0 t / ha of fresh *herba* in three years of plantation exploitation.
7. In *Silybum marianum*, the studies have afforded a new cultivar named Argintiu, which is early ripening with a producing capacity of approximately 890-1,000 kg/ha of fruits.
8. Hybridizations and individual selections have resulted in the development of two new cultivars of *Calendula officinalis* – Nataly and Diana with a production of more than 1,000 kg/ha of dry inflorescences, a content of flavones and polyphenols making 0.624-0.873% and 0.988-1.038% respectively.

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