

PERFORMANT NEW VARIETIES OF *SALVIA SCLAREA* L. WITH DIFFERENT PERIOD OF VEGETATION CARRIED OUT IN THE REPUBLIC OF MOLDOVA

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Abstract. *Salvia sclarea* L. varieties of hybrid origin have been developed; they blossom abundantly in the first, second, and third year of vegetation. The varieties ensure inflorescence production up to 30 t/ha during the three years of the plantation exploitation. The production of essential oil during the three years of plantation exploitation ranges between 54.9 kg/ha in the late-ripening variety M-404 and 73.3 kg/ha in the early-ripening variety Ambra Plus. A simultaneous cultivation of the early-, mid-, and late-ripening varieties permits an extension of the harvesting period up to 25-27 days. The varieties form a conveyor during harvesting. The productivity of industrial plantations increases by 20% due to the fact that each variety is harvested on optimal dates when the inflorescence production and the content of essential oil are the highest. The essential oil quality is high. The content of linalyl acetate is 65%-70% and up to 75%-76% during the favourable years. All the varieties developed are applicable for two processing technologies – production of essential oil through involvement of vapours and that of concrete through organic solvent extraction.

Keywords: *Salvia sclarea* L., varieties, essential oil.

Rezumat. Noi soiuri performante de *Salvia sclarea* L., cu perioada diferită de vegetație, obținute în Republica Moldova. Au fost create soiuri de proveniență hibridă de *Salvia sclarea* L., care înfloresc abundent în anul I, al II-lea și al III-lea de vegetație. În trei ani de exploatare a plantației soiurile asigură obținerea producției de inflorescențe de până la 30 t/ha. Producția de ulei esențial în trei ani de exploatare a plantației este de la 54.9 kg/ha la soiul tardiv M-404 până la 73.3 la soiul timpuriu Ambra Plus. Cultivarea concomitentă a soiurilor timpurii, intermediare și tardive permite extinderea perioadei de recoltare până la 25-27 zile. Soiurile în timpul recoltării formează un conveier. Productivitatea plantațiilor industriale se majorează cu 20% datorită faptului că fiecare soi se recoltează în termeni optimi, atunci când producția de inflorescențe este cea mai înaltă și conținutul de ulei esențial este cel mai ridicat. Calitatea uleiului esențial este înaltă. Conținutul de acetat de linalilă este de 65-70 %, iar în anii favorabil până la 75-76 %. Toate soiurile create sunt pretabile pentru 2 tehnologii de procesare: obținerea uleiului esențial prin antrenare cu vapori de apă și fabricarea concretului prin extracție cu solvenți organici.

Cuvinte cheie: *Salvia sclarea* L., soiuri, ulei esențial.

INTRODUCTION

Salvia sclarea (LINNAEUS, 1758), Clary sage, has been known and used as a medicinal plant in phytotherapy since the antiquity. In folk medicine, sage flowers are used externally, for ulceration and edema. Inflorescences have an antispastic and anticataral action (GONCEARIUC, 2002, 2008). The sedative, emmenagogue and anticonvulsive action are also mentioned for this species. Sage inflorescences have been proved to have a beneficial action in the treatment of cancer (SIMON et al., 1984). The essential oil contained in inflorescences of the species accounts for all these qualities. The essential oil is used in osteoarthritis treatment and rheumatic arthritis (RUSU & KALININA, 1999). It is also utilized in wines industry for processing the Muscat type, aromatization of vermouth and especially in perfumery, where it is greatly appreciated for both its odorous qualities and as an excellent fixer (GONCEARIUC, 2002). For the Republic of Moldova, the species is important for the fact that its essential oil and concrete are meant for export. Processing of larger quantities of inflorescences without major industrial capacities of equipment requires varieties with different vegetation period: from early- to late-ripening, which would extend the harvest by 10-15 days. For these reasons and to increase profit from the cultivation and processing of species, a research has been carried out to develop new varieties with increased productivity, adapted to the cultivation conditions of Moldova.

MATERIALS AND METHODS

Salvia sclarea L. varieties and cultivars of hybrid origin were used as biological material. The experiments of testing varieties and hybrids were carried out on the experimental field of the Institute of Genetics and Plant Physiology on common chernozem with a humus content of 2.8%. Wheat harvested for grain was a precursor of the sage. The sowing took place in autumn, in the second half of October. Phenological evaluations, examination of quantitative characters, determination of the resistance to wintering, drought, producing capacity studies, statistical analysis were performed according to the variety testing methods used by the State Commission for Plant Variety Testing of the Republic of Moldova. The content of essential oil was determined three times per season through hydro distillation in Ginsberg apparatus and recalculated for dry matter. The concrete was produced through extraction using 'Soxhlet' type apparatus. Petrol ether was used as a solvent. The concentration of the major components in essential oil and concrete was evaluated using GC-MS and HPLC.

RESULTS AND DISCUSSIONS

The investigation was carried out to develop new hybrids and varieties with an increased inflorescence and essential oil productivity (raw material), differing in industrial ripening dates, adapted to pedoclimatic conditions of the Republic of Moldova. In order to fulfill the aim, vast initial breeding material has been developed, characterized by valuable qualities that includes inbred and male sterility lines, the fertile lines that consolidate male sterility, hybrids of different types. Keeping this in mind, it was also necessary to study the consequences of inbreeding in *Salvia sclarea* L.; male sterility, its types and the frequency of male sterility form appearance; general and specific combining capacity of the parental forms selected for hybridization (1994); manifestation of heterosis in different hybrid types (GONCEARIUC & BALMUŞ, 2006; GONCEARIUC et al., 2007), as well as effective methods of seed production (GONCEARIUC, 1994).

The lines possessing increased general combining capacity were selected as parental forms. The highest heterosis effect was registered for the ramification number (59-106%) and essential oil content (up to 123.6%). The majority of the lines studied are also characterized by high specific combining capacity, the heterosis for some indices being 96-132%.

Salvia sclarea L. hybrids have been developed using a method of hybridization between male sterile and inbred lines, which manifest heterosis in F_1 to row of important character's for productivity, but the setting of F_1 hybrid seeds is very low on spatially isolated sectors. The production of F_1 hybrid seeds does not exceed 50 kg/ha, which makes the utilization of these hybrids not profitable. Double hybrids have been developed to increase seed production, but these hybrids are not effective either for the same reason. The investigation carried out on a large number of hybrids demonstrated that along with the hybrids in which essential oil content dropped and heterosis effect decreased in the F_2 - F_n generations, there were hybrids in which the essential oil content increased considerably, while the heterosis manifestation was corroborated by the high indices of heterosis effect (GONCEARIUC, 2002a).

Taking this into account, hybrid populations have been developed, in which seed production is similar to that of common cultivars, while their producing capacity is higher. Therefore, forms with remarkable quantitative characters, and, in the first place, with an increased content of essential oil have been selected in the F_2 generation of the heterosis manifesting hybrids. Negative selections were conducted in all the following generations on spatially isolated sectors to eliminate the plants differing in the genotype selected in F_2 . Some hybrid populations demonstrated heterosis effect in all the subsequent generations. Thus, the heterosis has become transmissible, fixed and constant from an instable form. The manifestation of the heterosis in F_2 - F_n presents a great interest. Unlike the unstable heterosis, which is expressed only in the F_1 generation and loses its scope in the subsequent generations, the fixed, transmissible heterosis is consolidated in the genetic systems of the organism and becomes an evolution value (GONCEARIUC, 1994). Hybrid populations used as a basis to develop the varieties of hybrid origin, registered up to the present in this country have been developed using some simple heterotic hybrids. Among them, early ripening Dacia-50 and middle ripening Dacia-43 are the most remarkable.

All the investigations have been carried out to develop productive hybrids and cultivars. The employment of *Salvia sclarea* L. F_1 hybrids is not effective; thus, the development of the different hybrid types, some of them very complex, has allowed us to obtain varieties of hybrid origin with a high producing capacity, superior quality of essential oil, with different ripening dates, resistant to wintering, drought, diseases. Some of them have been registered; others are being tested to be registered. The concentration of the principal compounds in essential oil is high. To your attention seven varieties of hybrid origin of *Salvia sclarea* L. were created in the Institute of Genetics and Plant Physiology and included in the State Register of Moldova: Early-ripening varieties – Dacia – 50, Ambra Plus (Figs. 1, 2); Medium ripening varieties – Dacia – 99, V-junior; Late-ripening varieties – Victor, Natali-Clary, M-404.

The biodiversity of the developed *Salvia sclarea* L. varieties of hybrid origin is evident (Fig. 3). First of all, the varieties are distinguished by the length of the vegetative period – early-ripening, medium-ripening and late-ripening (Fig. 4). When the early-ripening varieties, hybrids, lines are at the stage of flowering, the medium-ripening ones are at the stage of flower bud opening, while the late-ripening ones have a lance-shaped floral bud.

The determination of the quantitative character indices that have a direct influence on the of inflorescences and essential oil productivity of the *Salvia sclarea* L. varieties (hybrids) has demonstrated that the values of those indices are significantly increased. The plant length is above 120 cm. No significant difference has been attested between the varieties and hybrids tested, with the exception of the late-ripening variety Natali-Clary in which this index is the highest reaching 129.2 cm (Table 1). The inflorescences are more compact with a larger number of primary and secondary ramifications (35.5; 35.7) in the varieties Victor and Natali-Clary. The inflorescences of the Ambra Plus variety are less ramificated. The number of verticiles on the central spike of the panicle, as well as the inflorescence length do not differ significantly in different varieties.

All the tested varieties bear blossom in the first, second and third year of vegetation. The Ambra Plus variety forms the highest inflorescence production in the first year of vegetation (9.4 t/ha), while the Victor and Natali-Clary varieties in the second year (14.3-14.5 t/ha). The average inflorescence production during the three years of plantation exploitation of the developed varieties ranges from 23.6 t/ha in the late-ripening variety to 30.0 t/ha in the early-ripening variety Ambra Plus (Fig. 1).



Figure 1. Early variety Ambra Plus, first year of vegetation, isolate lot (original).

Figura 1. Soiul timpuriu Ambra Plus, primul an de vegetație, lot izolat (original).



Figure 2. Variety Ambra Plus secondary year of vegetation (original).

Figura 2. Soiul Ambra Plus, anul al doilea de vegetație (original).



Figure 3. Biodiversity of the *Salvia sclarea* varieties (original).
Figura 3. Biodiversitatea varietăților de *Salvia sclarea* (original).



Figure 4. *Salvia sclarea* varieties with different period of vegetation (original).

Figura 4. Varietățile de *Salvia sclarea* cu diferite perioada de vegetație (original).

Table 1. Values of some productivity indices of the *Salvia sclarea* L. varieties in comparative competitive varieties (TCCV), the year of vegetation 2009.

Tabel 1. Valorile unor indici de productivitate la soiurile de *Salvia sclarea* L. în testarea în culturi competitive de concurs (TCCC), anul de vegetație 2009.

Varieties	Plant height -cm-	Inflorescence length -cm-	Number of ramifications		No. verticils on main axis
	X ± Sx	X ± Sx	primary X ± Sx	secondary X ± Sx	
Early-ripening varieties					
Dacia-50, standard	123.7 ± 5.4	53.4 ± 5.2	13.9 ± 2.8	19.1 ± 5.6	8.0 ± 0.8
Ambra Plus	123.5 ± 6.1	53.9 ± 2.0	12.9 ± 3.5	16.0 ± 6.0	7.9 ± 0.7
Medium ripening varieties					
Dacia-99, standard	125.3 ± 6.0	54.8 ± 5.8	14.4 ± 1.9	20.1 ± 8.2	8.0 ± 0.6
V-Junior	125.7 ± 6.8	54.5 ± 5.1	14.5 ± 1.7	17.9 ± 5.8	8.1 ± 0.8
Late-ripening varieties					
Victor	125.6 ± 6.2	54.0 ± 4.2	14.3 ± 2.6	21.2 ± 4.2	7.8 ± 1.0
Natali-Clary	129.2 ± 6.2	55.0 ± 3.2	14.3 ± 1.8	20.3 ± 5.4	8.4 ± 0.9
M-404, standard	123.6 ± 6.7	54.5 ± 7.7	13.6 ± 1.7	18.4 ± 5.4	8.4 ± 1.1

The content of essential oil varies depending on the pedoclimatic conditions. For example, it was relatively lower in 2009 than in the preceding years. The higher concentration of essential oil were accumulated by the varieties Dacia-50 and V-Junior making 1.106% and 1.021% (dry materials), respectively. The highest production of essential oil in the first year of vegetation was registered in the variety Ambra Plus in 2005 constituting 26.8 kg/ha and in 2008 - 21.4 kg/ha, while the average essential oil production calculated for three years (2005-2009) was very high – from 54.9 kg/ha in the late-ripening variety M-404 to 73.3 kg/ha in the early-ripening variety Ambra Plus (Fig. 1). The late-ripening varieties Victor and Natali-Clary exceed the witness variety by 8.9% and 20.6 %, ensuring the production of 59.8 kg/ha (Victor variety) - 66.2 kg/ha (Natali-Clary variety) of essential oil per hectare. Both Dacia-99 and V-Junior varieties with an intermediary vegetation period that blossom and achieve the stage of industrial maturation (harvesting) 5-6 days later than the early-ripening varieties and 6-7 days earlier than the late-ripening varieties are characterized by a very high productivity of 62.0 kg/ha and 61.7 kg/ha of essential oil, respectively.

A simultaneous cultivation of the early-, intermediary-, and late-ripening varieties that form a conveyor during the harvesting period permits an increase of the productivity of industrial plantations by 20% due only to the fact that each variety is harvested on optimal dates, while the inflorescence production and the content of essential oil are the highest. The developed varieties of hybrid origin, including those in State Registered, are perfect for processing through organic solvent extraction. One ton of inflorescences or wastes of essential oil manufacturing can provide for 7-8 to 8-10 kg of concrete in relation to the performance of extraction installation and in the case the processing technology is respected.

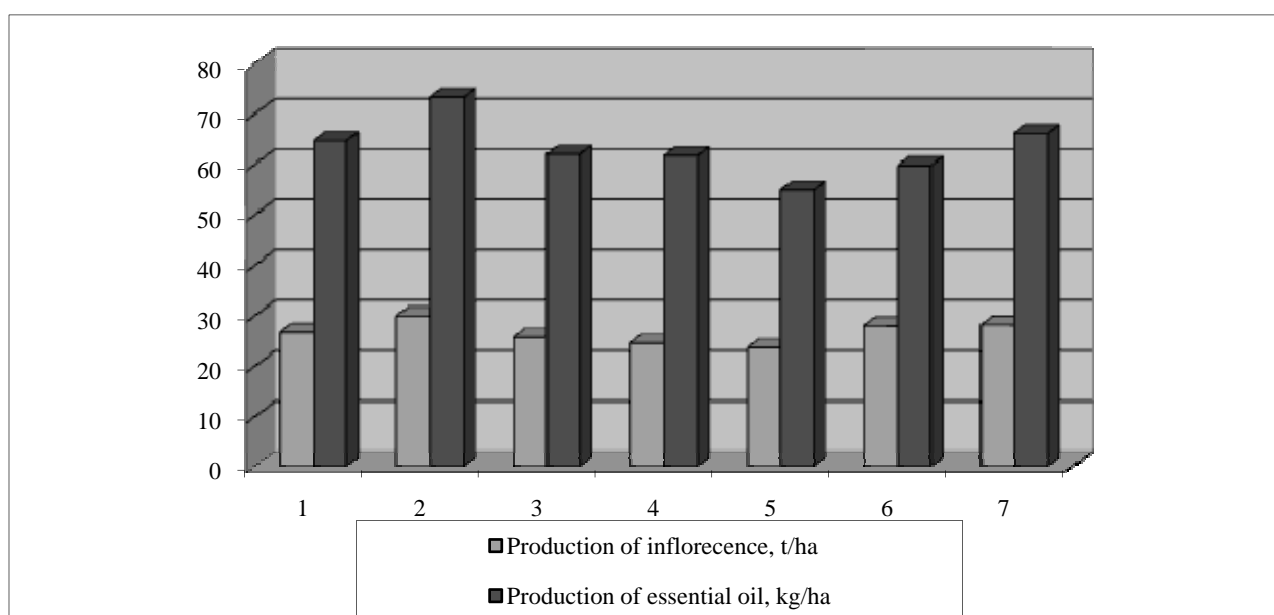


Figure 5. Inflorescences and essential oil productivity of *Salvia sclarea* L. varieties (3 years exploitation of plantation).
Figura 5. Producția de inflorescențele și de ulei esențial la soiurile de *Salvia sclarea* L. (exploatarea plantației trei ani).

Legend: 1- Dacia-50; 2-Ambra Plus; #-Dacia-99; 4- V-Junior; 5- M-404; 6- Victor; 7- Natali-Clary.

The essential oil quality is excellent: the concentration of linalyl acetate is 65-70 %; in some years it equals 75-76 % and sclareol, the compound on which perfumery value and property to fix and freshen other aromatic substance depends on makes 6.0%-12.0 %. The potential sclareol concentration in the concrete is 68-70%.

CONCLUSIONS

1. *Salvia sclarea* L. varieties of hybrid origin have been developed, which blossom abundantly in the first, second, and third year of vegetation.
2. The varieties ensure inflorescence production up to 30 t/ha during three years of plantation exploitation.
3. The production of essential oil during the three years of plantation exploitation ranges between 54.9 kg/ha in the late-ripening variety M-404 and 73.3 kg/ha in the early-ripening variety Ambra Plus.
4. A simultaneous cultivation of the early-, mid-, and late-ripening varieties permits an extension of the harvesting period up to 25-27 days. The varieties form a conveyor during harvesting.
5. The productivity of industrial plantations increases by 20% due to the fact that each variety is harvested on optimal dates, while the inflorescence production and the content of essential oil are the highest.
6. The essential oil quality is high: the content of linalyl acetate is 65%-70% and up to 75%-76% during the favourable years.

7. All the varieties developed are applicable for two processing technologies – production of essential oil through involvement of vapours and that of concrete through organic solvent extraction.

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Received: April 30, 2010

Accepted: June 2, 2010