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FIXED HETEROSIS: EFFECTIVE GENETIC BASIS IN BREEDING OF THE *SALVIA SCLAREA* L. SPECIES

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Abstract. It has been demonstrated that the use of fixed heterosis is an effective genetic basis in breeding of the *Salvia sclarea* species. The phenotype of inbred lines derived from the varieties by hybrid origin with fixed heterosis Ambra Plus and Nataly Clary was varied and genetic segregation was expressed in the diversity of quantitative traits such as plant height, inflorescence length and their structure, essential oil content. Along with the inbred lines S3 generations in which the content of essential oil is lower than that in the varieties they originate from, lines have been produced with high (1.001-1.350%) and very high (1.351-1.600%) content, the latter ones making 20% of the lines derived from the variety Ambra Plus and 11% of the total number of the lines derived from Nataly Clary.
Inbreeding results in phenologic changes: the S_2- S_3 inbred lines fall into three groups of maturation; early-middle- and late-ripening that constitute 24, 48, and 28% respectively, in the lines derived from Ambra Plus and 33% in each group of the lines derived from Nataly Clary. The inbred lines with high and very high content of essential oil are valuable genotypes in view of the improvement of raw material quality and the increase of the productivity of new varieties of *Salvia sclarea*.
Keywords: Salvia sclarea, genotype, variety, hybrid, fixed heterosis, inbred lines, essential oil

Introduction

Salvia sclarea L. (Clary Sage) is a medicinal and aromatic species in the family Lamiaceae of Mediterranean origin known and appreciated in Ancient Egypt and the Roman Empire where it had been cultivated. The species synthesizes and accumulates secondary metabolites (Legrand et al., 2010) essential oil in inflorescences, flowers. In folk medicine, sage flowers are used externally to cure wounds, to bathe, to make laundry, to take care of skin and ulceration and edema of hair (Kintzios, 2000). Inflorescences have anticatarrhal and antispasmodic action (Soldatcenco et al., 1999; Lattoo et al, 2006; Goncearіuc, 2008), in addition to carminative and oestrogenic one (Lattoo et al, 2006). The antioxidant, antimicrobial (Gülçin, 2004; Hyo Jung

Yang et al. 2014), antibacterial and cytotoxic activity (Hayet et al., 2007); sedative, emenagogous and anticonvulsive action (Păun, 1995; Gonceariuc, 2000, 2013) are also mentioned for this species. The pharmacological properties of the essential oil are well known. So, the essential oil of *S.sclarea* is used in osteoarthrosis and rheumatic arthritis treatment (Rusu & Calinina, 1999; Rusu & Caminschi, 2006). It is also employed in the treatment of arterial hypertension (Seol et al. 2010, 2016), catarrhal inflammation, tonsillitis, and inflammation of teguments (Păun, 1995; Gonceariuc, 2002), in aromatherapy (Setzer, 2009; Hyo Jung Yang et al. 2014). The utilization of this oil is beneficial due to the analgesics, anti-inflammatory (Moretti et al., 1997), antioxidant (Pitarokili, 2002; Gülçin, 2004; Lattoo et al, 2006; Setzer, 2009), antifungal (Pitarokili, 2002; Simic et al., 2004; Lattoo et al, 2006; Jirovetz et al, 2007; Dzamic et al.,2008) antimicrobiene actions (Peana et al., 1999; Pitarokili, 2002; Gonceariuc, 2002; Gülçin, 2004; Lattoo et al, 2006; Jirovetz et al, 2007). As a nice nervous tonic, the essential oil of Clary Sage is used to treat depression (Seol, 2010). Not only the essential oil but each of its components has a certain beneficial action in different treatments. For example, some researchers found antibacterial, antifungal and growth regulating activity of the essential oil of *S. sclarea* due to sclareol (Sybille Van Den Brûle et al., 2002; Caniard; et al., 2012), others attribute the antibacterial action to linalool (Simic et al.,2004) etc. As a nice nervous tonic, the essential oil of Clary Sage is used to treat depression (Stevič et al., 2004; Seol, 2010). The essential oil and inflorescences of *S.sclarea* appeared to have a beneficial action in the treatment of cancer (Simon et al., 1984) and extracts from inflorescences are beneficial in the treatment of Alzheimer's disease (Filipa et al., 2013).

The carbon bonds of terpenes in the essential oil of Clary Sage are known to constitute intermediate products in the biosynthesis of steroid hormones, enzymes, antioxidants, and vitamins etc., the terpenes demonstrating analgetic, anti-inflammatory, antimicrobial, antiviral, diuretic, hypotensive, sedative, spasmolytic, expectorant, antirheumatic actions listed above. The therapeutic properties depend on the combinations of natural compounds that occur in the form of monoterpenes to polyterpenes (Krylov et al., 1992).

The essential oil of *S. sclarea* is also used in food industry to produce beer, tonic beverages, liqueurs, as well as Muscat and Vermouth type wines. In addition, this essential oil is widely employed in perfumery (Voitkevici, 1999; Kintzios, 2000; Clebsch, 2013), where it is greatly appreciated for both odorous qualities and as an excellent fixer (Păun, 1995; Gonceariuc, 2002, 2008).

Processing of fresh material or wastes from the distillation of the essential oil through extraction with organic solvents affords a product named *concret* which, along with other components, contains sclareol, a labdanein type diterpene alcohol (Dimas, et al., 2007; Caniard et al., 2012). It is a minor component in the essential oil, while it is a major one in concret. Sclareol is considered a refined odorant in perfumery (Laville et al., 2013), and especially a fixer. In perfume industry, sclareol is a principal bioactive component that may be also used to produce Ambrox (Decorzant, 1987; Günnewich et al., 2013), a chemical compound in the class of tetralabdanoxides considered as one of the most valuable perfumes of animal origin Zibet and Moschus. Previously, the source of Ambrox was Ambra, a waxy substance from the digestive tract of the whale.

The importance of the species, its multiple utilizations and the fact that *S. sclarea* has been cultivated and processed for over 65 years in the Republic of Moldova, and essential oil and concret are designed for export impel a number of researchers to develop new genotypes, lines, hybrids, cultivars that would synthesize and accumulate essential oil in the contents as high as possible. A number of the cultivars of hybrid origin have been already developed, registered, patented and employed (Goncearuc, 2013, 2014), and the development of initial material for prospective improvement is in process. This work is dedicated to the findings of these studies.

Material and methods

The biological material was represented by 124 inbred lines S_2-S_3 generations not affected by inbreeding depression. They have been derived from two cultivars of hybrid origin: Ambra Plus, early-ripening which is a complex backcross hybrid and the cultivar Nataly Clary, a triple late-ripening hybrid. The both cultivars (hybrids) are characterized by the presence of fixed heterosis. The plants selected as genotypes, the genitors of future inbred lines, were subject to forced self-pollination at the beginning of the flowering stage after the inflorescences had a special toilet treatment. The phenotypic differences were studied in each line through evaluation of the phenological development stages and of the morphological characters (qualitative) that directly influence the productivity. The essential oil content was measured in the samples of fresh inflorescences (100 g) through hydro distillation for 60 min in the Ginsberg apparatus, the results recalculated for standard humidity (70%) and dry matter (d.m.). Varieties Ambra Plus and Nataly Clary from which the lines have been derived were used as witnesses for the inbred lines produced.

Results and discussion

In order to efficiently exploit the *S. sclarea* species, it is necessary to improve the initial breeding material necessary to create the hybrids, the varieties of performance.

It provides research aimed at obtaining new genotypes with a high content of essential oil, supported by valuable quantitative characters, all of which ensure excellent qualitative and quantitative composition of the essential oil (Gonceariuc, 2002). We have employed two methods: inbreeding and hybridizations of different types to diversify the genetic basis for *S. sclarea* quality through raising the capacity of essential oil accumulation.

These studies have been initiated because the hybrids we have developed earlier are not simple androsterile hybrids and may be used only in the F_1 generation when the heterotic effect is highest. In *S. sclarea*, utilization of F_1 hybrids is not feasible due to the complications at producing F_1 seeds caused by the morphological traits of the cloggy pollen of relatively large size and of the flowers adapted to entomophilous pollination (Goncearіuc, 2000, 2002, 2008, and 2013). Taking into consideration the importance of heterosis employment (Kirpichnikov, 1967; Goncearіuc, 2000, 2016; Fu et al. 2014), including in plant breeding (Gallais, 1988) and to take advantage of this phenomenon, we have developed a large number of hybrids of different types, including very complex and heterotic ones. Among them, we have selected hybrids in which the heterotic effect in a number of quantitative traits, especially in the essential oil content, is manifested not only in the F_1 but in the F_2-Fn generations (Goncearіuc, 2000, 2002, 2008, 2013, 2014). It is well known that unstable heterosis is manifested only in F_1 and loses its amplitude in further generations. It is also known that transmissible, fixed heterosis (Mac Key, 1976; Abel et al., 2005; Wespel & Becker, 2008; Wespel et al., 2009; Payal Bansal et al., 2012) consolidates in the genetic systems of the organism becoming a value of evolution (Mac Key, 1976).

Our varieties developed using the hybrids in which the fixed heterosis is attested in the important quantitative traits (Goncearіuc, 2000, 2002, 2008, 2013), including those of Ambra Plus and Nataly Cary, are distinct, uniform, stable and high-productive (Goncearіuc, 2014), while the production of seeds poses no difficulties. All these are explained by the fact that cross-fertilized heterozygous hybrid populations reduce the variability to a mean value necessary for adaptation of the population to the conditions of local cultivation (Lewis, 1953, 1954). By ensuring the lability retention, the heterozygous population (in our case, the varieties Ambra Plus and Nataly

Clary) is the cause of the fact that the phenotypic traits of a large number of genotypes are optimally adapted to a specific environment of the population existence (Mather, 1955, 1955a), which results in a balanced situation where natural selection occurs, that is genetic homeostasis takes place (Lerner, 1954; Turbin, 1967). It is also known that inbreeding allows identification of forms with recessive traits and selection of desired and promising ones (Goncearuc, 2002, 2008, 2013, with the reference to N.I. Vavilov). Thus, inbreeding is also well known as a method of development of the initial material for breeding of cross pollinated species, such as *S. sclarea*. Therefore, diversification of the genetic basis for the quality of *Salvia sclarea* was achieved using this method. Genitors, the donors of the quality – the varieties Ambra Plus (early-ripening) and Nataly Clary (late-ripening) are the most productive, the both of them representing complex hybrids with a fixed heterotic effect. The both varieties meet the requirements of the International Union for the Protection of New Varieties of Plants for the DUS factors: distinct, uniform, stable.

It is a knowledge that inbreeding leads to phenologic, phenotypic and genetic changes in selected and inbred genotypes.

Assessment of the inbred lines derived from both Ambra Plus and Nataly Clary shows that they are divided into three groups, early-, mid, and late-ripening (Goncearuc et al.,2016) (Fig. 1, 2).

The inbred lines derived from both varieties are divided into early-, middle-, and late ripening duration of the vegetation period. Our studies have demonstrated that the quantitative traits that determine the phenotype in inbred lines differ from those of the original variety. The Tables present only the inbred lines derived from the same variety, that exhibit pronounced phenotypic diversity. For example, in 2017, the height of the variety Ambra Plus is 116.3 cm. The values for plant height in the early-ripening lines derived from this variety range from 98.9 (AP114-11S_3) to 127.0 cm (AP37-11S_3). The inbred lines with the lowest values for height were recorded in the set of lines with a medium period of maturation (AP103-11S_2) – 90.6 cm and late-ripening ones (AP30-11S_2) – 88.8 cm (Tab.1).

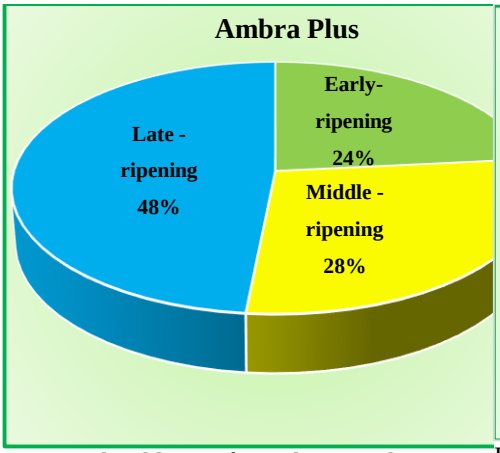

Fig. 1. Inbred lines of *S. sclarea* with a different period of vegetation derived from the early-ripening variety Ambra Plus.

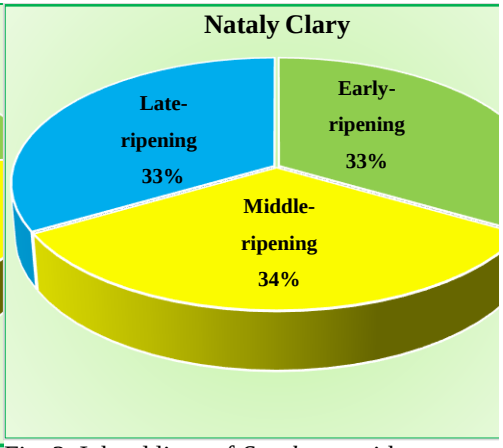

Fig. 2. Inbred lines of *S. sclarea* with a different period of vegetation derived from the early-ripening variety Nataly Clary.

Table 1.

Diversification of quantitative trait values in the inbred lines S_3 generation of *Salvia sclarea* derived from early-ripening Ambra Plus variety, the 2-nd year of vegetation, 2017

Inbred lines	Plant height, cm	Inflorescence length, cm	Ramifications of inflorescence		Essential oil content, %	
	X±sX	X±sX	primary	secondary	Humidity 70%	Dry matter
early-ripening						
AP 10-11 S_3	116.2±8.3	65.4±5.5	14.0±1.4	19.2±3.6	0.245	0.851
AP 37-11 S_3	**127.0±10.1**	**70.1±9.4**	13.8±2.2	19.7±6.3	0.245	0.908
AP 62-11 S_3	112.6±5.3	63.9±3.3	14.5±2.0	27.3±9.9	0.516	**1.507**
AP 77-11 S_3	119.7±11.2	64.0±8.8	15.6±1.7	27.5±9.3	0.180	0.579
AP 114-11 S_3	98.9±9.2	60.7±5.2	13.6±1.5	24.4±6.2	0.212	0.762
AP 115-11 S_3	103.4±8.8	68.0±5.7	13.2±1.7	20.6±7.1	0.337	**1.394**
middle-ripening						
AP 34-11 S_3	112.8±5.4	**68.2±4.6**	15.2±3.0	27.4±13.1	0.337	**1.271**
AP 49-11 S_3	**112.6±8.5**	**63.0±9.5**	**13.7±1.6**	**22.6±5.8**	0.374	**1.166**
AP 54-11 S_3	116.1±6.6	64.8±4.8	17.3±2.0	25.9±4.4	0.539	**1.237**
AP 66-11 S_3	109.5±11.7	69.5±9.4	16.2±1.7	32.7±10.0	0.135	0.522
AP 103-11 S_3	**90.6±5.3**	**57.2±9.1**	**13.6±1.5**	**24.1±8.7**	0.412	**1.349**
late-ripening						
AP 28-11 S_3	127.4.0±5.1	73.7±7.1	16.0±1.5	30.0±7.4	0.245	0.777
AP 30-11 S_3	**88.8±4.4**	**57.0±5.7**	**12.8±1.7**	**16.2±7.2**	0.381	**1.385**
AP 52-11 S_3	110.9±9.3	61.6±8.7	13.0±2.3	19.4±3.4	0.245	0.713
AP 60-11 S_3	**113.9±6.5**	**63.0±5.7**	**14.9±2.0**	**24.6±5.9**	0.337	**1.074**
Ambra Plus, st.	116.3±8.9	62.2±6.2	13.9±1.6	21.1±8.6	**0.337**	**1.078**

The other important quantitative trait is inflorescence length. In 2017 it makes 62.2 cm for Ambra Plus, while the lines derived from this variety have developed inflorescences with a length ranging from 57.0 cm to 73.7 cm. These lines also differ for the number of inflorescence ramifications, especially of the first and second degree. The number of glands oleiferous placed on their epidermis (Caissard et al., 2012) is known to be more significant and their density reaches that of glands oleiferous on the flower calyx.

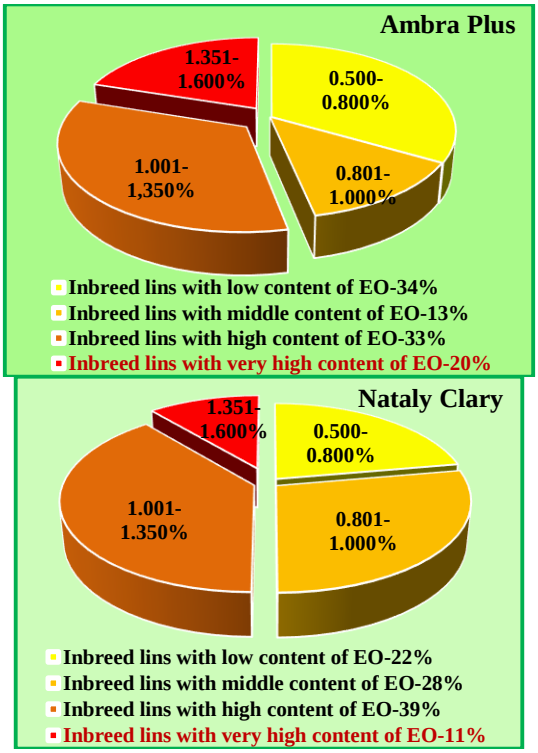

Fig.3. The content of essential oil (d.m.) in the inbred lines S_3 generations in 2017of *Salvia sclarea* derived from Ambra Plus and Nataly Clary.

The inbred lines derived from the late-ripening variety Nataly Clary, as well as those derived from early-ripening Ambra Plus differ phenological providing early- and mid-ripening genotypes, the other lines remain late-ripening as the initial variety (Tab.2, Fig.3). The segregation of the quantitative indices of the traits is evident also in the inbred lines derived from the Nataly Clary variety. As shown above, phenological and phenotypic traits, including quantitative traits that determine the line phenotype are subject to

diversification. Inbred lines with enhanced and much enhanced content (1.027-1.391%, dry matter) of essential oil in comparison with 1.020% of the initial variety Nataly Clary have been obtained and attested from the variety Nataly Clary as in the case of the lines derived from early-ripening Ambra Plus (Tab. 2).

All these traits influence the synthesis and accumulation of essential oil, the content of which in the inbred lines derived from Ambra Plus makes 0.135-0.539% at a standard humidity (70% humidity) and 0.522 in the middle-ripening line AP66-11S_3 and 1.507% (d.m.) in the line with an early period of vegetation AP62-11S_3. The oil content in Ambra Plus variety is 0.337% (70% humidity) and 1.078% (d.m.). Thus, inbreeding allowed us to also obtain variability for the content of essential oil, it being much higher in some lines, and much lower in others in comparison with the initial Ambra Plus variety (Fig.3). We conventionally divided the lines in four groups for the essential oil content: with a low (0.500-0.800% (d.m.), medium (0.801-1.000% (d.m.), high (1.001-1.350% (d.m.), and very high (1.351-1.600% (d.m.) content of oil (Fig.3). It should be mentioned that in *Salvia sclarea*, the content of essential oil of more than 1% (dry matter) is considered high. The lines with the content of essential oil of over 1% derived from the variety Ambra Plus constitute 33% (Fig. 3). Thus, by using inbreeding we have obtained lines with a very high content of essential oil, they making 20% of the total lines derived from this variety.

Differences were recorded in the trait of plant height, two inbred lines being higher (120. cm and 126.6 cm), the other lines developing lower (71.1-92.9cm) plants. The inbred lines derived from the late-ripening variety Nataly Clary, similarly with those derived from early-ripening Ambra Plus, are conventionally divided into four groups for the content of essential oil: 22% of lines with low content, 28% of medium content, 39% of high content, and 11% of very high content (Fig. 3).

The results regarding the diversification of the all quantitative characters, including the essential oil content and the duration of vegetation of inbreed lines S_2 generations, derived from varieties with constant heterosis Ambra Plus and Nataly Clary (Goncearіuc et al., 2016), were confirmed in the S_3 generation. The 2015 and 2017 pedoclimatic conditions were different: 2015 was a drought year, and 2017 was an ordinary year. In the dry years *Salvia sclarea*, but also other aromatic species such as *Lavandula angustifolia*, accumulates essential oil more than in ordinary or rainy years. However, the differences between the initial varieties and the inbred lines derived from them were confirmed in S_3 generation not only to

Table 2

Diversification of quantitative trait values in the inbred lines S_3 of *Salvia sclarea* L. derived from late-ripening Nataly Clary variety, 2017

Inbred lines	Plant height, cm	Inflorescence length, cm	Ramifications of inflorescence		Essential oil content, %	
	X±sX	X±sX	primary	secondary	Humidity 70%	Dry matter
early-ripening						
NC 6-11 S_3	105.0±10.0	61.2±10.3	13.6±2.2	23.4±4.2	0.314	**1.391**
NC 8 -11 S_3	**115.0±12.4**	**62.3±11.2**	**15.1±2.6**	**24.8±10.9**	**0.348**	**1.151**
NC 4 -11 S_3	119.7±9.1	**63.6±11.0**	**16.2±2.5**	**30.0±8.0**	0.247	0.741
NC 10 -11 S_3	**117.5±6.7**	**73.2±12.2**	**14.9±1.7**	**26.8±6.2**	**0.247**	**0.754**
NC 20 -11 S_3	**114.5±6.4**	**57.3±6.5**	**13.8±2.5**	**22.9±11.3**	**0.398**	**1.092**
NC 19 -11 S_3	**120.0±6.6**	**74.4±7.5**	**16.1±2.2**	**32.4±8.0**	**0.291**	**1.035**
NC 26 -11 S_3	**120.0±12.2**	**64.8±7.9**	**13.0±1.4**	**17.6±3.9**	**0.247**	**0.925**
NC 104-11S_3	112.2±16.1	**63.0±4.4**	**15.4±1.3**	**26.2±3.7**	0.269	0.865
middle-ripening						
NC 61-11 S_3	112.0±6.7	**61.0±7.9**	**13.8±1.8**	**25.4±9.3**	0.247	0.827
NC 34 -11 S_3	117.0±7.1	**57.5±7.1**	**14.0±1.8**	**27.3±3.4**	**0.292**	**0.943**
NC 96-11 S_3	**103.0±7.5**	**56.9±10.8**	**14.8±1.3**	**26.5±7.2**	**0.292**	**1.027**
late-ripening						
NC 13-11 S_3	**126.5±7.8**	**73.7±10.0**	**19.3±4.4**	**34.8±13.7**	**0.314**	**0.984**
NC 21 -11 S_3	121.5±8.5	69.5±6.0	**17.6±2.7**	**35.0±11.6**	**0.245**	**0.688**
NC 60-11 S_3	**92.9±8.6**	**64.6±8.8**	**16.5±2.5**	**24.6±10.0**	**0.292**	**1.039**
NC 77-11 S_3	71.1±6.9	38.7±7.3	**11.6±1.5**	**14.3±2.2**	**0.337**	**1.196**
NC 99-11 S_3	122.7±7.0	**46.4±6.5**	11.4±1.3	16.8±5.9	**0.381**	**1.365**
NC 100-11S_3	116.6±7.8	57.2±5.8	13.5±1.5	18.8±6.4	**0.359**	**1.292**
Nataly Clary, st.	119.6±4.4	64.9±4.1	16.8±4.3	19.5±3.4	0.314	**1.020**

the essential oil content but also to other quantitative characters on which the degree of oil accumulation depends.

All the inbred lines with increased and very high content of essential oil derived from the both varieties are promising and will be included in the programs and plans of hybridization in different hybrid combinations as donors of genes to produce hybrid genotypes with enhanced content of essential oil, which ensures a superior quality of raw material of *Salvia sclarea* and a higher producing capacity of future hybrids and varieties.

Conclusion

1. It has been demonstrated that the use of fixed heterosis is an effective method of obtaining variability, for creating new genotypes with valuable quantitative characters necessary for the development of performance varieties of *Salvia sclarea* species.
2. The phenotype of inbred lines derived from the varieties with fixed heterosis Ambra Plus and Nataly Clary was varied and genetic segregation expressed in the diversity of quantitative trait values such as plant height, inflorescence length and structure and in the essential oil content supported by these characters.
3. Along with the inbred lines S_3 generations in which the content of essential oil is lower than that in the lines they originate from, lines have been produced with high (1.001-1.350%) and very high (1.351-1.600%) content, the latter ones making 20% of the lines derived from the variety Ambra Plus and 11% of the total number of the lines derived from Nataly Clary.
4. Inbreeding results in phenologic changes: the S_3 inbred lines fall into three groups of maturation; early-, mid- and late-ripening that constitute 24, 48, and 28% respectively, in the lines derived from Ambra Plus and 33% in each group of the lines derived from Nataly Clary.
5. The inbred lines with high and very high content of essential oil are valuable genotypes in view of the improvement of raw material quality and the increase of the productivity of new varieties of *Salvia sclarea* L. varieties.

References

1. Abel, S., Möllers, C., Becker, H.C. (2005). Development of synthetic Brassica napus lines for the analysis of “fixed

heterosis" in allopolyploid plants. Euphytica - Springer, 146 (1): 157-163.

2. Caissard, J., Olivier, T., Delbecque, C., Palle, S., Garry, P-P., Audran, A., Valot, N., Moja, S., Nicole, F., Magnard, J. L., Legrand, S., Baudino, S., Jullien, F. (2012). Extracellular Localization of the Diterpene Sclareol in Clary Sage (Salvia sclarea L., Lamiaceae). PLOS One Journal. 7(10): e48253.
3. Caniard, A., Zerbe, P., Legrand, S. (2012). Discovery and functional characterization of two diterpene syntheses for sclareol biosynthesis in Salvia sclarea L. and their relevance for perfume manufacture. BMC Plant Biology, UK, 129(119): 1-13.
4. Clebsch, B. (2013). The New Book of Salvias. Timber Press. From Better World Books (Mishawaka, IN, U.S.A.), 344p.
5. Decorzant, R. et al. (1987). A short synthesis of Ambrox from sclareol. Tetrahedron. 43: 1871-1879.
6. Dimas, K., Hatziantoniou, S., Tseleni, S. (2007). Sclareol induces apoptosis in human HCT116 colon cancer cells in vitro and suppression of HCT116 tumor growth in immunodeficient mice. Apoptosis. Springer, 12(4): 685-694.
7. Dzamic, A, Sokovic, M., Ristic, M., Grujic-Jovanovich, S., Vukojevic, J., Marin, P.D. (2008). Chemical composition and antifungal activity of Salvia sclarea (Lamiaceae) essential oil. Arch. Biol. Sci. Belgrade Journal. 60 (2): 233-237.
8. Filipa, Marcelo, Dias, C., Martins, A., Madeira, P., J., Tiago Jorge, M., Helena Florêncio, F., Cañada,J., Cabrita, E., J., Jiménez-Barbero, J., Rauter, A., P. (2013). Molecular Recognition of Rosmarinic Acid from Salvia sclareoides Extracts by Acetyl cholinesterase: A New Binding Site Detected by NMR Spectroscopy. Chemistry - A European Journal. 19(21):6641–6649.
9. Fu, D., Xiao, M., Hayward, A., Fu, Y., Liu, G., Jiang, G. (2014). Utilization of crop heterosis: a review. Euphytica. Springer, 197: 161–173.
10. Gallais, A. (1988). Heterosis: its genetic basis and its utilization in plant breeding. Euphytica- Springer, Oct. Vol. 39, 2, pp.95-104.

11. Goncearіuc, M. (2000). Particularităţile expresiei heterozisului la hibrizii triliniari şi dubli de Salvia sclarea L. Cercetări de Genetică Vegetală şi Animală. România. 8:84-97.

12. Goncearіuc, M. 2002. Efectul heterozisului la hibrizi backcross de Salvia sclarea L. Simpozion Naţional de Genetică vegetală şi animală, Agris - Redacţia Rev. Agricole S.A., Bucureşti, 12:27,28.

13. Goncearіuc, M. (2008).Genetics and breeding of Salvia sclarea L. species. J. Hop and medicinal plants, XVI, 1-2 (31-32), Printing house Academic Pres, Cluj-Napoca, Romania: 132-139.

14. Goncearіuc, M. (2013). Cercetări de genetică şi ameliorare la Salvia sclarea L. Akademos, 3 (30):77-84. Editat la Tipografia ASM.

15. Goncearіuc, M. (2014). Moldavian medicinal and aromatic plants varieties. J. Hop and Medicinal Plants, XXII, 1-2: 51-62.

16. Goncearіuc, M., Balmuş, Z., Cotelea, L. (2016). Genetic diversification of Salvia sclarea L. quality by increasing the storage capacity of the essential oil. Oltenia Journal for Studies in Natural Sciences 2016, (Proceedings of the 23rd International Conference of the Oltenia Museum), XXXII, No. 1/2016, ISSN 1454-6914 B+, Thomson Reuters pp.29-36.

17. Gülçin, I. (2004). Evaluation of the antioxidant and antimicrobial activities of clary sage (Salvia sclarea L.). Turk. J. Agric. For. 28: 25-33.

18. Günnewich, N., Higashi, Y., Feng, X., Choi, KB., Schmidt, J., Kutchan, TM. (2013). A diterpene syntheses from the clary sage Salvia sclarea catalyzes the cyclization of geranyl diphosphate to (8R)-hydroxy-copalyl diphosphate. Phytochemistry. 91:93-9.

19. Hayet, E., Fatma, B., Souhir, I., Waheb, F., A., Abderaouf, K., Mahjoub, A., Maha, M. (2007). Antibacterial and cytotoxic activity of the acetone extract of the flowers of Salvia sclarea and some natural products. Pakistan Journal of Pharmaceutical Sciences. 20(2): 146-148.

20. Hyo, Jung Yang, Ka, Young Kim, Purum, Kang, Hui Su Lee, Seol G., H. (2014). Effects of Salvia sclarea on chronic immobilization

stress induced endothelial dysfunction in rats. BioMed Central Complementary Alternative Medicine. 14: 396.
21. Jirovetz, L.K., Wicek, G., Buchbauer, V., Gochev, T., Girova, A., Stoyanova, E., Schmidt, Geissler, M. (2007). Antifungal activities of essential oils of *S. lavandulifolia, S. officinalis* and *S. sclarea* against various pathogenic *Candida species*, J. Essential Oil-Bearing Plants. 10: 430-439.
22. Kintzios, S., E. (2000). Sage – The Genus *Salvia*. Harwood, Academic publishers: 20-21.
23. Kirpichnikov, V. S. (1967). Общая теория гетерозиса. Rus. The general theory of the heterosis. Генетика, т.3, № 10, pp.167-180
24. Krylov, A., Marcenko, V. (1992). Фитотерапия в комплексном лечение заболеваний
внутренних органов. (Rus.) Herbal medicine in the complex treatment of diseases of
internal organs. Киев, 198 p.
25. Lattoo, S. K., Dhar, R. S., Dhar, A. K., Sharma, P. R., Agarwal, S. G. (2006). Dynamics of essential oil biosynthesis in relation to inflorescence and glandular ontogeny in *Salvia sclarea.* Flavour and Fragrance Journal. 21 (5) : 817–821.
26. Laville, R., Castel, C., Fattarsi, K., Roy, C., Legendre, L., Delbecque, C., Garry, P., Ph., Audran, A., Fernandez, X. (2013). Low sclareol by-product of clary sage concrete: chemical analysis of a waste product of the perfume industry. Flavour and Fragrance J. 28 (2): 93–101.
27. Legrand, S., Valot, N., Nicolé, F., Moja, S., Baudino, S., Jullien, F., Magnard, J.L., Caissard, J. C., Legendre, L. (2010). One-step identification of conserved miRNAs, their targets, potential transcription factors and effectors genes of complete secondary metabolism pathways after 454 pyrosequencing of calyx cDNAs from the Labiatae S. sclarea gene. Jan 15; 450(1-2):55-62.
28. Lerner, I.M. (1954). Reprinted 1970. *Genetic Homeostasis*. Edinburgh: Oliver and Boyd. American edition, New York: John Wiley & Sons, New York: Dover Publications.
29. Lewis, D. (1953). A relationship between dominance, phenotypic stability and variability, and a theory of alternative genetic pathways. Nature, 172: 1136–1137
30. Lewis, D. (1954). Gene interaction environment and hybrid vigour. Proceeding of the Royal Society of London, B: 43-45.

31. Mac Key. (1976). Genetic and evolutionary principles of heterosis. Heterosis in plant breeding. Proceeding VII-th Congress Eucarpia, Budapest: 37-41.
32. Mather, K. (1955). Response to selection. Cold Spring Harb. Symp. Quant Biol. 20: 197-212.
33. Mather, K. (1955a). The Genetical Basis of Heterosis. Proceedings of the Royal Society of London. B, Biological Sciences, 144 (915): 143-150.
34. Moretti, M., Peana, A.T., Satta, M. (1997). A study on anti-inflammatory and peripheral analgesic action of Salvia sclarea oil and its main component. J. Essential Oil Res. 9: 199-204.
35. Payal, Bansal, Shashi, Banga, S. S. (2012). Heterosis as Investigated in Terms of Polyploidy and Genetic Diversity Using Designed *Brassica juncea* Amphiploid and Its Progenitor Diploid Species. PLoS One. 7(2): e29607.
36. Păun, E. (1995). Şerlaiul *(Salvia sclarea)*. Sănătatea Carpaţilor. Edit. Arta Grafică S.A.: 218-222.
37. Peana, A.T., Moretti, M.D.L., Juliano, C. (1999). Chemical composition and antimicrobial action of the essential oils of *Salvia desoleana* and *Salvia sclarea.* J. Medicinal Plant and Natural Product Research. 65: 752-754.
38. Pitarokili, D., Couladis, M., Petsikos-Panayotarou, N., Tzakou, O. (2002). Composition and antifungal activity on soil-borne pathogens of the essential oil of Salvia sclarea from Greece, J. Agric. Food Chem. 50: 6688-6691.
39. Rusu, M., Calinina, L. (1999). Reacţia indicilor imuni sub influenţa masaj-magnetoforeză cu ulei eteric de salvie în tratamentul complex al artritei reumatice. J. Curier Medical, Chisinau, 7-9:31-37.
40. Rusu, M., Caminschi, V. (2006). Electroforeza concretului de Salvia sclarea L. în tratamentul complex al ostioartrozei la etapa medicinei primare. Anale Ştiinţifice ale USMF, Chișinău. 3: 83-86.
41. Seol, G.H., Shim, H.S., Kim, P.J., Moon, H.K., Lee, K.H., Shim, I., Suh, S.H., Min, S.S. (2010). Antidepressant-like effect of Salvia sclarea is explained by modulation of dopamine activities in rats. J Ethnopharmacology. Jul 6;130 (1):187-90.
42. Seol, G.H., Purum Kang, Hui Su Lee. (2016). Antioxidant activity of linalool in patients with carpal tunnel syndrome. BMC Neurol. 16: 17.
43. Setzer, W.N. (2009). Essential oils and anxiolytic aromatherapy. Nat. Prod. Commun. 4: 1305-1316.

44. Simić, A., Soković, M., Ristić, M., Grujić-Jovanović, S., Vukojević, J., Marin, D.P. (2004). *Antifungal activity of essential oil of Salvia sclarea*, 11th OPTIMA Meeting, September 5-11, Belgrade (Serbia and Montenegro), P-61 (PS-2: 137).
45. Simon, J.E., Chadwick, A., Craker, L.E. (1984). The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Archon Books. 770 pp.
46. Soldatcenko, S. S., Kashcenko, G. F., Pidaev, A. B. (1999). Aromaterapia. Profilactica i lecenie zabolevanii ăfirnîmi maslami. Edit. Tavrida, Simferopol. 139p.
47. Stević, T., Tomaši, O., Kostić, M., Stanković, S., Soković, M., Nikčević, S., Ristić, M. (2004). Biological activity of linalool. Proceedings from the 3rd CMAPSEEC, Belgrade, Serbia: 102-106.
48. Sybille Van Den Brûle, Müller, A., Fleming, A.J., Cheryl, C., Smart. (2002). The ABC transporter SpTUR2 confers resistance to the antifungal diterpene sclareol. Article online: 13 Jun. DOI: 10.1046/j.1365-313X.2002.01321.x
49. Turbin, N.V. (1967). Genetics of heterosis. (rus. Genetika geterozisa). (1967). J. Vestnik selihoz nauki. Moskwa 3: 23-29.
50. Voitkevici, S.A., (1999). *Ăfirnîe Masla v Parfiumerii i Aromaterapii*. (Rus.) *Essential oils in perfumes and aromatherapy*. Edit. Pishchevaia prom., Moscow: 264-266.
51. Wespel, F., Becker, H. C. (2008). Raps als Modell zur Untersuchung der „fixierten Heterosis“ bei allopolyploiden. Pflanzen der Vereinigung Pflanzenzüchter und Saatgutkaufleute Österreichs: 111--- 114.
52. Wespel, F., Abel, S., Becker, H. C. (2009). Analyzing fixed heterosis by comparative mapping of QTL for early biomass in Brassica napus, *B. rapa* and *B. oleracea*. Proceeding International Conference on Heterosis in Plants University of Hohenheim, Stuttgart, Germany, Sep. 7 – 9:11.