

GENETIC DIVERSIFICATION OF *SALVIA SCLAREA* L. QUALITY BY INCREASING THE STORAGE CAPACITY OF THE ESSENTIAL OIL

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Abstract. Genetic diversity of the quality in *S. sclarea* and development of genotypes with enhanced essential oil (EO) content have been conducted using the varieties of hybrid origin Ambra Plus (AP) and Nataly Clary (NC), both characterised by manifestation of fixed heterosis in a number of quantitative traits, including the content of EO. One hundred and twenty four inbred S_2 lines in the second year of vegetation not affected by degeneration through inbreeding have been developed and assessed through forced pollination. The findings show that the improvement of quality and the development of new genotypes with extremely high content of EO supported by the valuable quantitative traits through inbreeding in *S. sclarea* are efficient, the inbreeding provoking phenotypic, genotypic segregation of complex hybrid populations into a wide range of genotypes, some of them being promising. The phenotype of the inbred lines derived from the studied varieties is diversified, attesting genetic segregations expressed in the diversity of the values of quantitative trait indices such as plant height, inflorescence length and structure and in the content of EO supported by these traits. Along with the inbred lines in which the content of essential oil is lower than that in the lines they originate from, lines have been produced with enhanced (1.001-1.350%) and very enhanced (1.351-1.958%) content, the latter ones making 11% of the lines derived from AP and 7% of the total number of the lines derived from NC. Inbreeding causes phenological changes: the S_2 inbred lines fall into early-, mid- and late-ripening that constitutes 24, 48, 28%, respectively, in the lines derived from AP and 33% in each group of the lines derived from NC. The inbred lines with enhanced, very enhanced content of EO are valuable genotypes in view of the improvement of raw material quality and the increase of the productivity of *S. sclarea* cultivars.

Keywords: *Salvia sclarea*, genotype, inbred lines, essential oil.

Rezumat. Diversificarea bazei genetice a calității la *Salvia sclarea* L. prin creșterea capacității de acumulare a uleiului esențial. Diversificarea genetică a calității la *S. sclarea* și crearea genotipurilor cu conținut ridicat de ulei esențial s-a efectuat cu concursul soiurilor de proveniență hibridă Ambra Plus (AP) și Nataly Clary (NC), ambele caracterizându-se prin manifestarea heterozisului fixat la un șir de caractere cantitative, inclusiv, la conținutul de ulei esențial (UE). Prin polenizări forțate au fost create, evaluate 124 linii consangvinizate S_2 în anul al doilea de vegetație neafectate de degenerarea prin consangvinizare. Rezultatele obținute demonstrează că îmbunătățirea calității, crearea genotipurilor cu conținut foarte ridicat de UE susținut de caractere cantitative valoroase, prin metoda consangvinizării la *S. sclarea* este efectivă, consangvinizarea provocând segregarea fenotipică, genotipică a populațiilor hibride complexe într-o gamă largă de genotipuri, unele din acestea fiind foarte perspective. Fenotipul liniilor consangvinizate derivate de la soiurile AP și NC, este diversificat, atestându-se și segregări genetice exprimate în diversificarea valorilor indicilor caracterelor cantitative, cum ar fi talia plantei, lungimea, structura inflorescenței cât și a conținutului de UE, susținut de aceste caractere. De rând cu linii consangvinizate la care conținutul de UE este mai jos de cât la soiurile de la care provin, s-au obținut linii cu conținut ridicat (1.001-1.350 % (s.u.) și foarte ridicat (1.351-1.958% (s.u.), ultimele constituind 11% linii derivate de la soiul AP și 7% din numărul total de linii obținute de la soiul NC. În rezultatul consangvinizărilor au loc și schimbări fenologice: liniile consangvinizate S_2 , obținute se împart în 3 grupe de maturizare: timpurii, medii și tardive, care constituie 24, 48 și 28%, respectiv, la liniile derivate de la soiul AP și câte 33% în fiecare grup la liniile derivate de la soiul NC. Liniile consangvinizate cu conținut ridicat și foarte ridicat de UE sunt genotipuri valoroase în vederea îmbunătățirii calității materiei prime, ridicării randamentului soiurilor de *S. sclarea*.

Cuvinte cheie: *Salvia sclarea*, genotip, linii consangvinizate, ulei esențial.

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 was cultivated. The species synthesizes and accumulates secondary metabolites (LEGRAND et al., 2010), including 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 antiscorbutic and antispasmodic action (SOLDATCENCO et al., 1999; LATTOO et al., 2006; GONCEARIUC, 2008), in addition to carminative and oestrogenic one (LATTOO et al., 2006). The antioxidant, antimicrobial (GÜLÇİN, 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 essential oil of *S. sclarea* is used in osteoarthritis and rheumatic arthritis treatment (RUSU & CALININA, 1999; RUSU & CAMINSCHI, 2006). It is also employed in the treatment of arterial hypertension (SEOL et al., 2013), 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 analgesic, anti-inflammatory (MORETTI et al., 1997), antioxidant (PITAROKILI, 2002; GÜLÇİN, 2004; LATTOO et al., 2006; SETZER, 2009), antifungal (PITAROKILI, 2002; SIMIĆ et al., 2004; LATTOO et al., 2006; JIROVETZ et al., 2007; DZAMIC et al., 2008) antimicrobial actions (PEANA et al., 1999; PITAROKILI et al., 2002; GONCEARIUC, 2002; GÜLÇİN, 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). The essential oil and inflorescences of *S. sclarea* appeared to have a beneficial action in the treatment of cancer (SIMON et al., 1984). 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 (SIMIĆ et al., 2004), etc. As a nice nervous tonic, the essential oil of Clary Sage is used to treat depression (SEOL et al., 2010). The essential oil and inflorescences of *S. sclarea* appeared to have a beneficial action in the treatment of cancer 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 analgesic, 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; CAISSARD 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 et al., 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 (GONCEARIUC, 2013; 2014), and the development of the 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 S2 lines in the second year of vegetation not affected by inbreeding degeneration. 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. 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 hydrodistillation for 60 min in the Ginsberg apparatus, the results recalculated for standard humidity (70%) and dry matter (d.m.). Ambra Plus and Nataly Clary from which the lines were derived were used as witnesses for the inbred lines produced.

RESULTS AND DISCUSSION

The revaluation of aromatic and medicinal plants including the species *S. sclarea* through improvement of the genetic pool of the initial breeding material necessary for the development of new productive hybrids and varieties envisages research aimed at the production of new genotypes with an enhanced content of essential oil supported by the valuable quantitative traits, all these ensuring 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 rising 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 F₁ generation when the heterotic effect is the highest. In *S. sclarea*, the utilization of F₁ hybrids is not feasible due to the complications at producing F₁ seeds caused by the morphological traits of the cloggy pollen of relatively large size and of the flowers adapted to entomophilic pollination (GONCEARIUC, 2000; 2002; 2008; 2013). Taking into consideration the importance of heterosis employment (KIRPICHNIKOV, 1967; FU et al., 2014) 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 F₁ but in the F₂-F_n

generations (GONCEARIUC, 2000; 2002; 2002a; 2008; 2013). 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 (GONCEARIUC, 2000, 2002, 2002a, 2008, 2013), including those of Ambra Plus and Nataly Cary, are distinct, uniform, stable and highly-productive (GONCEARIUC, 2014; 2014a), 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; LEWIS, 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; MATHER, 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 (GONCEARIUC, 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 allogamic 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, both of them representing complex hybrids with a fixed heterotic effect. 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 known that inbreeding leads to phenological, phenotypic and genetic changes in the selected and inbred genotypes.

The 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 (Figs. 1 and 2; Tables 1, 2).

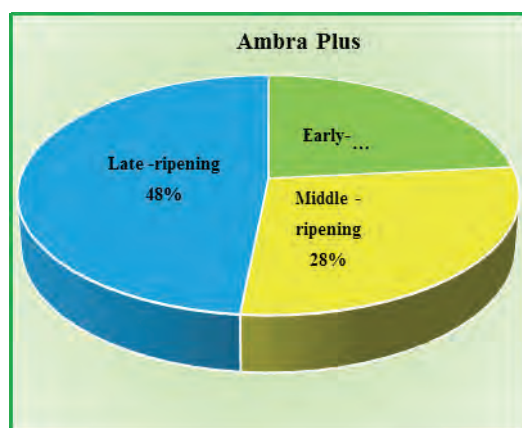


Figure 1. Inbred lines of *S. sclarea* derived from the early-ripening variety Ambra Plus with a different period of vegetation.

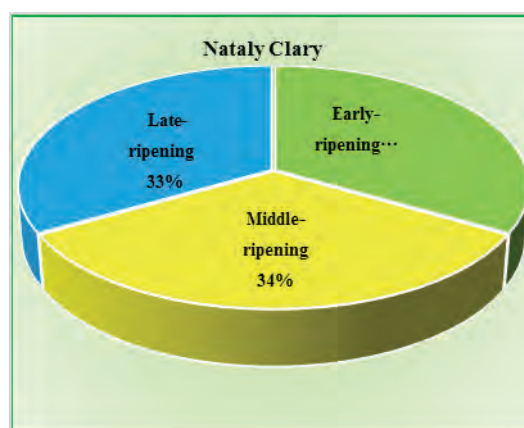


Figure 2. Inbred lines of *S. sclarea* derived from the early-ripening variety Ambra Plus with a different period of vegetation.

It should be mentioned that the number of late-ripening inbred lines originated from the early-ripening variety Ambra Plus is the highest (48%), while early- and mid-ripening varieties constitute 24 and 28%, respectively (Fig. 1). The inbred lines derived from the variety Nataly Clary are divided into early-, mid-, and late ripening equally for the duration of vegetation (Fig. 2).

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, the height of the variety Ambra Plus is 120.6 cm (Table 1). The values for plant height in the early-ripening lines derived from this variety range from 105.3 (AP114-11S2) to 137.7 cm (AP37-11S2). The inbred lines with the lowest values for height were recorded in the set of lines with a medium period of maturation (AP103-11S2) – 98.1 cm and late-ripening ones (AP30-11S2) – 98.2 cm (Table 1).

The other important quantitative trait is inflorescence length. It makes 63 cm in Ambra Plus, while the lines derived from this variety have developed inflorescences with a length ranging from 47.9 cm to 74.3 cm. These lines also differ for the number of inflorescence ramifications, especially of the second degree. The number of oleiferous glands placed on their epidermis is known to be more significant and their density reaches that of the oleiferous glands on the flower calyx.

All these traits influence the synthesis and accumulation of essential oil, the content of which in the inbred lines derived from Ambra Plus makes 0.180-0.404% at a standard humidity (70%). Recalculated for dry matter, it varies between 0.458 in the early-ripening AP62-11S2 and 1.665% (d.m.) in the line with a medium period of vegetation

AP49-11S2 (Table 1). The oil content in Ambra Plus is 0.424% (70% of humidity) and 1.194% (d.m.). Thus, inbreeding allowed us to also obtain variability for the content of essential oil; thus, it was much higher in some lines, and much lower in others in comparison with the initial Ambra Plus variety. We conventionally divided the lines in four groups for the essential oil content: with a low (0.458-0.700% (d.m.)), medium (0.701-1.000% (d.m.)), high (1.001-1.350% (d.m.)), and very high (1.351-1.958% (d.m.)) content of oil.

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 31% (Fig. 3a). The variety Ambra Plus belongs to this group with 1.192% (dry matter). Thus, by using inbreeding we have obtained lines with a very high content of essential oil, making 11% of the total lines derived from this variety.

Table 1. Diversification of quantitative trait values in the inbred lines of *Salvia sclarea* L. derived from early-ripening Ambra Plus, the 2nd year of vegetation, 2015.

Inbred line	Plant height, cm	Inflorescence length, cm	Number of ramifications:		Essential oil content, %	
			primary	secondary		
	X sX	X sX	X sX	X sX	Humidity 70%	Dry matter
Early-ripening 58-64 days						
AP 10-11 S ₂	121.3±2.9	59.4±12.4	13.8±1.7	19.2±4.5	0.336	1.367
AP 37-11 S ₂	137.7 ±11.4	74.3 ±1.3	14.8 ±3.5	24.7 ±8.6	0.359	1.142
AP 62-11 S ₂	115.0 ±5.6	47.9 ±5.0	12.2 ±1.4	19.9 ±6.2	0.224	0.458
AP 77-11 S ₂	118.2 ±12.0	67.8 ±11.5	15.1 ±2.3	22.2 ±6.1	0.180	0.547
AP 97-11 S ₂	120.5 ±8.0	57.7 ±7.6	12.2 ±1.9	16.8 ±4.3	0.337	1.224
AP 114-11S ₂	105.3 ±6.8	56.0 ±6.2	13.2 ±2.1	20.6 ±7.3	0.336	1.032
AP 115-11S ₂	106.8 ±7.1	53.3 ±4.3	12.6 ±1.8	23.4 ±8.5	0.404	1.333
middle-ripening 65-70 days						
AP 34-11 S ₂	118.6± 11.7	63.9± 0.7	14.2± 2.5	18.4 ±8.0	0.404	1.398
AP 49-11 S ₂	102.1 ±5.2	51.0 ±6.3	12.0 ±1.8	16.0 ±5.5	0.494	1.665
AP 54-11 S ₂	116.4 ±5.5	57.3 ±4.0	13.6 ±1.5	21.9 ±5.4	0.589	1.367
AP 66-11 S ₂	99.4 ±5.3	51.7 ±4.2	13.6 ±0.8	19.7 ±3.1	0.404	1.158
AP103-11 S ₂	98.1 ±4.7	49.3± 3.0	12.2 ±1.1	16.2 ±4.9	0.449	1.351
late-ripening, 71-82 days						
AP 28-11 S ₂	138.1±9.2	73.4±5.3	16.0±2.1	29.8±10.1	0.359	1.279
AP 30-11 S ₂	98.2± 5.3	52.8 ±5.0	11.8 ±1.4	14.4± 3.8	0.449	1.958
AP 52-11 S ₂	105.7 ±6.5	50.1 ±4.2	12.6 ±1.3	17.8 ±3.8	0.426	1.397
AP 60-11 S ₂	111.8± 6.5	51.7 ±4.7	11.4 ±1.3	18.2 ±4.1	0.359	1.310
AP 115-11S ₂	106.8 ±7.1	53.3 ±4.3	12.6 ±1.8	23.4 ±8.5	0.404	1.333
Ambra Plus. st.	120.6±6.9	63.0±6.5	14.8±2.2	20.8±6.3	0.424	1.192

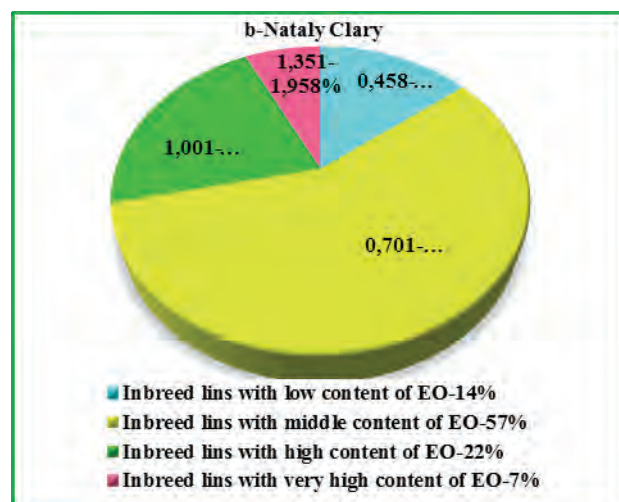
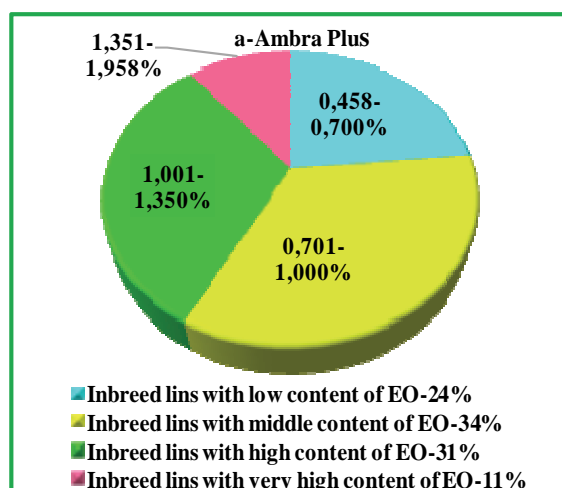


Figure 3. The content of essential oil in the inbred lines of *Salvia sclarea* derived from (a) Ambra Plus and (b) Nataly Clary.

As shown above, phenological and phenotypic traits, including quantitative traits that determine the line phenotype are subject to diversification.

The inbred lines derived from the late-ripening variety Nataly Clary, as well as those derived from early-ripening Ambra Plus differ phenologically providing early- and mid-ripening genotypes, the other lines remain late-ripening as the initial variety (Table 2, Fig. 2). The segregation of the quantitative trait indices is obvious. Only two early-ripening lines (NC4-11S2 and NC26-11S2) and two late-ripening ones (NC99-11S2 and NC21011S2) have provided plants with a higher length than that of the variety Nataly Clary from which they come from, this trait varying between 93.3 cm (NC60-11S2) and 132.6 cm in the line NC26-11S2 (Table 2). Among the early- and mid-ripening

lines, seven developed longer inflorescences (58.6-60.8 cm) than those of the original variety. More marked differences were observed in the number of ramifications (the 2nd degree) of inflorescences, eight lines exceeding (22.0-28.3 cm) the indices recorded in the variety Nataly Clary (14.6-21.5 cm) for this trait.

Table 2. Diversity of the quantitative traits in S_2 inbred lines of *Salvia sclarea* L. derived from the late-ripening variety Nataly Clary, the 2nd year of vegetation, 2015.

Inbred line	Plant height, cm	Inflorescence length, cm	Number of ramifications:		Essential oil content, %	
			primary	secondary	Humidity, 70%	Dry matter
	$\bar{X} \pm s\bar{X}$	$\bar{X} \pm s\bar{X}$	$\bar{X} \pm s\bar{X}$	$\bar{X} \pm s\bar{X}$		
Early, 59-64 days						
NC 6-11 S_2	116.2±6.8	60.8±6.7	14.8±2.5	23.6±11.0	0.359	1.605
NC 8 -11 S_2	95.5±5.3	47.4±5.3	10.4±0.8	11.4±2.5	0.359	1.069
NC 4 -11 S_2	125.8±2.9	62.4±6.7	15.0±2.3	28.3±9.3	0.359	1.161
NC 10 -11 S_2	110.8±7.3	54.0±6.2	13.6±1.8	22.3±7.6	0.337	1.075
NC 20 -11 S_2	110.8±5.6	58.7±4.5	14.0±1.8	22.6±4.3	0.449	1.064
NC 19 -11 S_2	110.4±6.2	52.4±3.8	14.0±1.6	24.4±5.3	0.584	1.767
NC 26 -11 S_2	132.6±6.3	60.3±4.5	13.4±1.6	19.6±5.1	0.269	0.696
NC 104-11 S_2	115.4±4.2	55.9±4.3	13.0±1.4	18.6±5.3	0.314	1.027
Middle, 65-70 days						
NC 61-11 S_2	106.1±7.5	49.8±3.1	10.8±1.3	14.2±2.8	0.494	1.428
NC 34 -11 S_2	118.7±5.4	56.1±6.3	14.4±2.2	30.6±9.5	0.224	0.567
NC 55-11 S_2	109.4±7.3	56.3±6.3	14.2±1.9	22.0±8.3	0.404	1.162
NC 96-11 S_2	116.4±5.3	54.9±3.9	13.2±1.9	23.0±8.3	0.337	1.198
Late, 71-80 days						
NC 13-11 S_2	108.0±5.5	53.9±7.5	14.6±1.6	21.4±7.1	0.202	0.725
NC 21 -11 S_2	124.8±3.6	61.4±6.6	15.0±1.9	20.0±6.6	0.180	0.619
NC 60-11 S_2	93.3±6.6	47.1±4.4	12.2±1.4	16.0±4.1	0.337	1.188
NC 75-11 S_2	117.7±5.3	57.1±5.3	13.6±2.0	22.6±7.3	0.404	1.153
NC 77-11 S_2	93.8±6.0	49.0±4.6	12.4±1.5	16.4±6.5	0.224	0.555
NC 99-11 S_2	131.7±3.9	52.2±3.1	12.6±2.3	26.6±10.0	0.359	0.978
NC 100-11 S_2	126.4±7.1	62.5±8.5	16.4±2.9	29.2±8.5	0.292	0.779
Nataly-Clary, st	118.6±4.0	57.7±6.9	14.6±6.4	21.6±6.4	0.402	1.154

Inbred lines with enhanced and very enhanced content (1.161-1.767%, dry matter) of essential oil in comparison with 1.154% (dry matter) 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 (Table 2; Fig. 3b). As for the late-ripening inbred lines, the differences from the initial variety are less spectacular (Table 2). Differences were recorded in the trait of plant height, two inbred lines being higher (125.8 cm and 132.6 cm), the other lines developing lower plants. Other two inbred lines (NC21011S₂ and NC100-11S₂) formed longer inflorescences by 2.7 and 4.8 cm, respectively, than the variety Nataly Clary. Though the plants developed a higher number of ramification of the second degree of inflorescences, all these differences failed to provide a higher content of essential oil, on the contrary, the majority of the late-ripening inbred lines have a lower content of essential oil (0.555-0.725%, dry matter) with the exception of the lines NC75-11S₂ (1.153%, dry matter) and NC60-11S₂ (1.188%, dry matter). 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: 14% of lines with low content, 57% of medium content, 22% of high content, and 7% of very high content (Fig. 3b).

Taking into consideration that the studies were carried out and the findings were obtained in the conditions of drought and intense heat of the year 2015, the value of the genetic material is indisputable.

The studies carried out to appreciate the uniformity/variability of the inbred lines derived from the early-ripening variety Ambra Plus have demonstrated that the variability of the trait plant length becomes low as early as in S_2 making $V=2.3-10.1\%$, while the uniformity of inflorescence length varies from low values ($V = 1.7-8.8$) to medium ones ($V = 10.4-19.6\%$).

All the inbred lines with increased and very high content of essential oil derived from 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. The improvement of the quality and the development of new genotypes with valuable quantitative traits, including a very high content of essential oil through inbreeding of *Salvia sclarea* are efficient, the inbreeding provoking

- phenotypic and genotypic segregation of complex hybrid populations into a wide range of genotypes, some of them promising.
- The phenotype of the inbred lines derived from the varieties Ambra Plus and Nataly Clary is diversified, attesting genetic segregations expressed in diversity of the values of quantitative trait indices such as plant height, inflorescences length and their structure and in the content of essential oil supported by these traits.
 - Along with the inbred lines in which the content of essential oil is lower than that in the lines they originate from, lines have been produced with enhanced (1.001-1.350% d.m.) and very enhanced (1.351-1.958% d.m.) content, the latter ones making 11% of the lines derived from the variety Ambra Plus and 7% of the total number of the lines derived from Nataly Clary.
 - Inbreeding results in phenological changes: the S₂ 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.
 - The inbred lines with enhanced and very enhanced content of essential oil are valuable genotypes in view of the improvement of raw material quality and the increase of the productivity of *Salvia sclarea* L. cultivars.

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