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GENETIC DIVERSITY OF SOYBEAN VARIETIES AND THEIR BIOLOGICAL POTENTIAL AS AFFECTED BY AGRONOMICAL PRACTICES

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ABSTRACT
The purpose of study was asses the genetic diversity of soybean varieties by SSR markers and investigate their productivity formation as affected by organic fertilizer, growth regulators and moisture retainer treatments in the Forest-Steppe zone of Ukraine. The effects of agronomical practices were assessed based on yield, protein and oil content. The study was carried out during 2016-2018.
As result it was found that the most similar varieties based on 4 SSR markers were varieties with genetic distances 1.73. The most different was Aliaska variety with genetic distances 3.16-3.87. Hieba, Kano and Ustia varieties, which are early-season varieties, were distributed in different clusters. It was determined that maximum of yield was obtained for Kano variety with combining moisture retainer, organic fertilizer and growth regulator “Vermystym D”. The highest protein content in studied varieties was determined in case of combining organic fertilizer and growth regulators. The maximum of oil content was noted in Ustia and Kano varieties with application moisture retainer, organic fertilizer, growth regulators “Vermystym D”. Thus, the biological features and applied nutrition affected studied indicators.

Keywords: molecular genetic polymorphism, DNA analysis, yield, protein and oil contained

INTRODUCTION

Every year all over the world, soybean (*Glycine max* (L.) Merr.) hybridization breeding programs include many lines and elite varieties of soybean (HUDCOVICOVA & KRAIC, 2003; ABUGALIEVA, 2013; RAMAZANOVA, 2016; LI *ET AL.*, 2017). During the breeding, developing varieties can be improved by new sources of genetic resources. Soybeans, in comparison with many types of agricultural plants, have a relatively low level of genetic variability (KUCHARIK & SERBIN, 2008; BABYCH & BABYCH-POBEREZHNA, 2012; IVANYUK, 2012). Therefore, the development of approaches for genetic diversity assessment of soybean varieties is relevant for breeding. To date, specific SSR markers have been developed, which allow to assess genetic diversity of soybean varieties by PCR method for control the transfer of genetic material (PRYSIAZHNIUK *ET AL.*, 2017). A significant increase in the cultivated area of soybeans testifies to its extremely important role in the agrarian complex of Ukraine. The yield and product quality of soybeans is determined by a complex of factors and are formed with an optimal ratio of all elements (PRYSIAZHNIUK *ET AL.*, 2018A). The study of new agricultural practices is actual issue, because over the past decades there has been a significant breakthrough in the formation of ideas and the practical application of new micronutrient fertilizers and growth regulators. Thus, the study of growth regulators’ and micronutrient fertilizers’ effect was carried out by many scientists on various agricultural crops. In particular, majority of these researches proved the positive effect of these treatments on productivity formation of various agricultural crops, including soybeans (AZIZI *ET AL.*, 2012; DHAKNE *ET AL.*, 2015). In

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addition, scientists note the positive effect of moisture retainer application (BAJAJ *ET AL.*, 2008; DEMIRTAS *ET AL.*, 2010). Thus, the purpose of study was to assess the genetic diversity of soybean varieties by SSR markers and investigate their productivity formation as affected by organic fertilizer, growth regulators and moisture retainer treatments in the Forest-Steppe zone of Ukraine.

MATERIALS AND METHODS

Twenty-three soybean varieties (Abelina, Alinda, Arnika, Berkana, DH 530, Kano, Hieba, DH 618, Monarkh, OAC Kalipso, OAC Lakeview, OAC Madok, Perlyna, Furio, Karra, Alaska, Arisa, Nordika, Amadeus, SG Eider, SG SR Picor, ASUKA, Ustia) were investigated by 4 SSR markers (Satt726, Satt063, Satt114 and Satt228) for genetic diversity assessment. The sample within each studied variety consisted of thirty individual DNA samples, combined in mixtures, six in each, which constituted five DNA mixtures for each variety, and PCR was performed (PRYSIAZHNIUK *ET AL.*, 2017). Molecular genetic analysis was carried out in Laboratory Molecular Genetic Analysis of Ukrainian Institute for Plant Variety Examination (Kyiv, Ukraine). Field experiment was performed at the experimental sites of Ltd "Research Institute of soybean" (Hlobyno, Poltava region).
Kano, Hieba and Ustia were studied as affected by moisture retainer Aquasorb, organic fertilizer Parostok, growth regulators Vermystym D and Ahrostymulin. The treatments were carried out as described in our previous study (PRYSIAZHNIUK *ET AL.*, 2018B). The sown area was 54 m^2, and the record area was 35 m^2 with 3 replications. The weather conditions in 2016 and 2018 differed from daily average values, however, in general, they were favorable for soybean cultivation. During the growing season of 2017, amount of precipitation was 202 mm which was half as compared to 2016 (412 mm).
The effects of agronomical practices were assessed based on yield, protein and oil content. The oil and protein content were determined using an Infraneo infrared grain analyzer (CHOPIN Technologies, France), calibrated with standard samples provided by Ukrmetrteststandard (TKACHYK, 2017). Statistical analysis of experimental data was performed using STATISTICA 12.0 software (trial version) (ERMANTRAUT *ET AL.*, 2007).

RESULTS AND DISCUSSION

As result of 23 soybean varieties DNA analysis by four SSR markers alleles of expected size were identified. It was found that from 3 to 7 varieties were polymorphic by studied SSR markers. Alinda variety had two alleles by all studied markers. Alaska variety had three alleles by Satt726 marker, ASUKA variety had 3 alleles and Hieba variety had 2 alleles by Satt114 marker. Thus, intra-variety polymorphism should be taken into account while the authenticity of soybean varieties is determined. It should be noted that majority of the studied varieties had one locus per marker. The number of identified alleles varied from 10 to 16 per marker. It was shown that the most polymorphic marker was Satt726 (PIC 0.92). For other markers PIC was 0.82-0.91 (*Table 1*).

Table 1. Characteristics of the studied loci

SSR marker	Number of alleles	Allele size (bp)	PIC
Satt228	14	207-269	0.91
Satt726	16	188-275	0.92
Satt063	14	105-201	0.90
Satt114	10	77-125	0.82

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Thus, the obtained high PIC values (0.89 on average), indicate that the identified alleles are evenly represented among soybean varieties.

In order to analyze the polymorphism of 23 soya varieties by SSR markers traits, a cluster analysis was carried out and genetic distances between the varieties were calculated (*Figure 1*).

As results of cluster analysis eight clusters were obtained by Satt063, Satt114, Satt228 and Satt726 markers. It was found that clusters were formed by Abelina and Berkana; DH 618 and OAC Kalipso; Kano, Karra and SG SR Picor; ASUKA and Ustia; Furio and Amadeus; DH 530 and Monarkh; OAC Lakeview and SG Eider; Alaska and Arisa varieties.

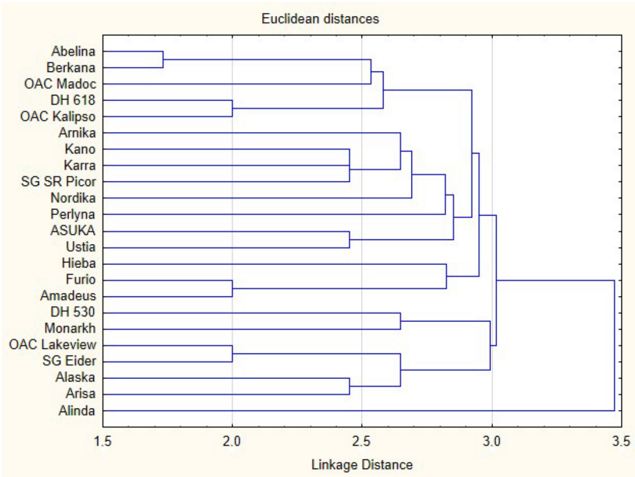

Figure 1. Dendrogram of soybean varieties by SSR markers

Analysis of genetic distances of the studied soybean varieties showed that the largest distance was between Alaska and Alinda (3.87) varieties. With the increase of the affinity of the varieties, their genetic distances shorten. In this study, the most similar varieties were Abelina and Berkana with the genetic distance 1.73. It should be noted that the most distinct variety by studied SSR markers was Alinda variety with genetic distances 3.16-3.87. Alaska variety was rather distinct with genetic distances 2.65-3.87. Even though this variety was included in cluster with Arisa variety it turned out to be the closer to OAC Lakeview variety. The distances between the majority of varieties varied from 2.00 to 3.46. Hieba, Kano and Ustia varieties, which are early-season varieties, were distributed in different clusters.

The molecular genetic polymorphism of soybean varieties with SSR markers was assessed by PAGAR *ET AL*. (2017). Authors studied thirteen soybean genotypes which were screened using 31 polymorphic SSR markers. The pair wise coefficient of genetic similarity between all soybean genotypes ranged from 0.792 to 0.929. It was determined that the unique bands can be used for the identification of specific soybean genotype. SONG *ET AL*. (2004) developed 391 SSR markers for soybean. Authors showed that SSRs were not uniformly spaced over a linkage group, clusters of SSRs with very limited recombination were frequently present. The part of this markers set was used in this study. Thus, SSR markers Satt063, Satt114, Satt228 and Satt726 were effective for genetic diversity assessment of studied varieties. They allowed to differ 23 soybean varieties according to genetic distances and shown the most similar and distinct varieties.

The biological potential of soybean varieties which belonged to the same maturity group was assessed by combining moisture retainer, organic fertilizer, growth regulators treatments. The affects were estimated by yield, protein and oil content of three soybean varieties.

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It was determined that the average yield for 2016-2018 of studied varieties was: Kano variety - 4.87, Hieba variety – 2.76 and Ustia variety – 3.10 t/ha (*Figure 2*).
The studied agronomical practices influenced the soybean yield in different ways. The lowest yield of all studied varieties was obtained without any treatments: Kano - 4.43, Hieba - 1.91, Ustia - 2.70 t/ha. It was found that yield of all studied varieties was increased with organic fertilizer treatment: Kano – 4.99, Hieba – 2.43, Ustia – 3.21 t/ha which significantly differed from control variant without any treatments. It should be noted that treatments by growth regulators Vermystym D or Ahrostymulin did not affect significantly yield of Kano and Ustia varieties.

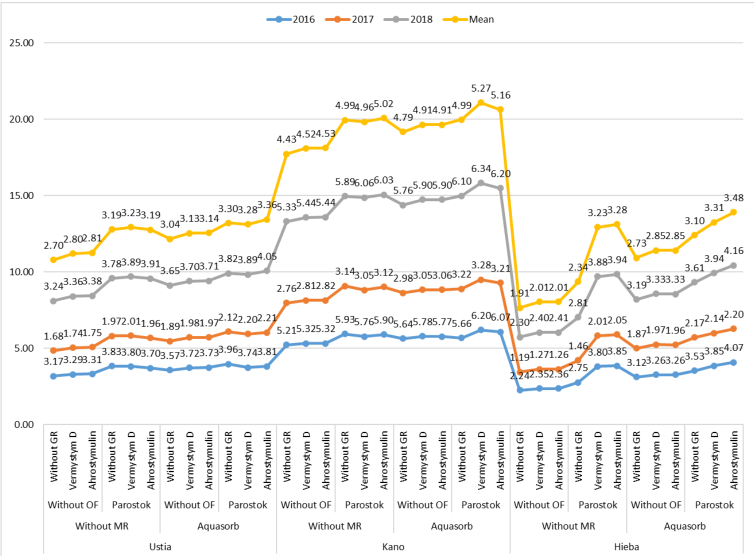

Figure 2. Yield of soybean varieties for 2016-2018, t/ha (LSD$_{0.5}$: 2016 – 0.23, 2017 – 0.20, 2018 – 0.24 t/ha, mean – 0.27 h/ha)

For Hieba variety the treatment by growth regulators Vermystym D and Ahrostymulin increased the yield significantly. The yields of Hieba variety in this variant were 3.23 and 3.28 t/ha, respectively. In variant of complex treatment by organic fertilizer and moisture retainer, the yield of Kano variety was 5.14 t/ha. Additionally, combining of growth regulators treatment with organic fertilizer and moisture retainer was most efficiency for Kano variety yield increasing by 0.28 t/ha with Vermystym D and by 0.17 t/ha with Ahrostymulin. The combining organic fertilizer and moisture retainer treatment allowed to obtain higher yield of Hieba variety – 3.10 t/ha. The most efficient was treatment by growth regulators Vermystym D or Ahrostymulin, the yield of Hieba variety increased by 0.21 and 0.37 t/ha respectively. Thus, proposed agronomical practices allow to significantly increase the yield of soybeans even under favorable conditions for plant development. In particular, for Kano variety, the minimum yield was obtained in control variant without any treatments - 4.43 t/ha, and the maximum - 5.27 t/ha - with combined treatments, for Hieba variety - 1.91 and 3.48 t/ha, and for Ustia variety - 2.70 and 3.36 t/ha, respectively.
The qualitative characteristics of soybean seeds are an important complex feature for obtaining a high-quality yield. It is known that foliar application of fertilizers and growth regulators has a positive effect both on plants productivity and the quality characteristics of the soybean yield. The protein content is major characteristic of soybean seeds. The soybean varieties with high protein content have more essential amino acids – 22.5% while essential amino acids content in varieties with low protein content is 17.8%. The protein

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content of studied soybean varieties according to moisture retainer, organic fertilizer, growth regulators treatments is represented in *Figure 3*.

As result of this study, it was determined that different combination of moisture retainer, organic fertilizer, growth regulators treatments affected protein content in different ways. In particular, in control variant, Kano variety contained 43.1% of protein in seeds, Hieba and Ustia varieties - 39.8 and 41.1%, respectively. It was found that moisture retainer application did not affect protein content of soybean varieties significantly. The organic fertilizer treatment allowed to increase protein content in seeds of Kano variety by 0.81%, Hieba variety – by 1.24 and Ustia variety – by 0.76%. The highest values of this characteristic were obtained in variant of combining organic fertilizer and growth regulators treatments. In particular, the protein content in Hieba variety in variant of organic fertilizer and growth regulator Vermystym D application was 41.9%, in variant of organic fertilizer and growth regulator Ahrostymulin – 44.8%; in Kano variety – 45.0%, in Ustia variety – 42.0% in both variants, respectively.

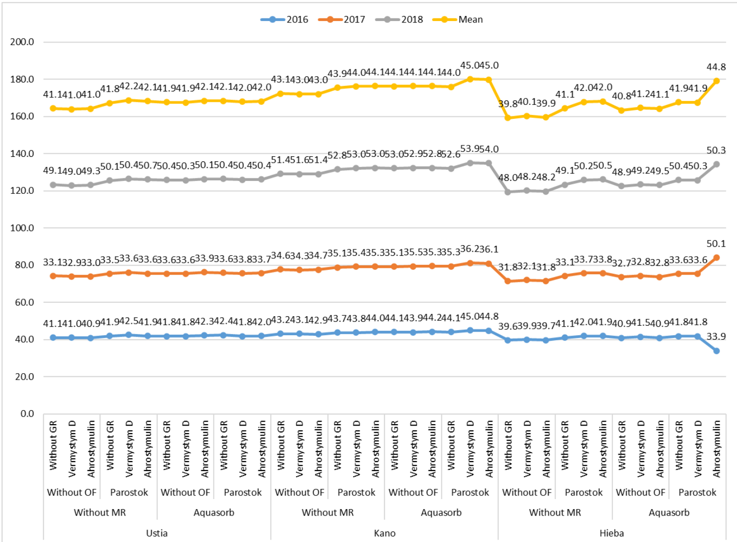

Figure 3. The protein content of soybean varieties for 2016-2018, % ($LSD_{0.5}$: 2016 – 1.10, 2017 – 1.00, 2018 – 1.20%, mean – 1.70%)

According to obtained results, it can be concluded that organic fertilizer followed by growth regulators treatments can significantly improve soybean products quality due to increased content of protein.

The major of researchers proves that an increase in the protein content occurs under conditions of insufficient precipitation and high air temperature during yield formation (PETRYCHENKO, 2006; ASSEFA *ET AL*., 2018; MERTZ-HENNING *ET AL*., 2018; ALSAJRI *ET AL*., 2020). It should be pointed that during the studied years the variability of weather conditions was noted during pod formation and ripening period of soybean. In particular, the air temperature in August 2016, 2017 and 2018 was higher than daily average value by 4.0, 3.9 and 3.1°C, respectively. At the same time amounts of precipitation was by 7.0; 40.0 and 46.3 mm less than daily average amounts of precipitation, respectively.

On average, according to obtained results, the oil content in seeds of Ustia variety was 19.8%, Kano variety - 21.8, and Hieba - 21.8% (*Figure 4*).

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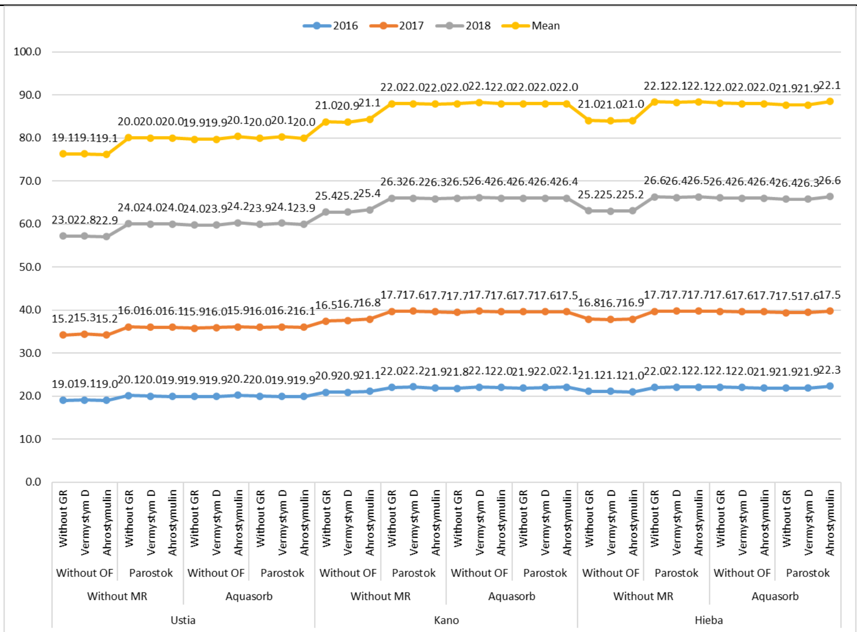

Figure 4. The oil content of soybean varieties for 2016-2018, % (LSD$_{0.5}$: 2016 – 0.40, 2017 – 0.30, 2018 – 0.50%, mean – 0.40%)

The maximum values of oil content were in varieties Ustia and Kano in the variant of combining moisture retainer, organic fertilizer and growth regulators Vermystym D and Ahrostymulin - 20.1 and 22.0%, respectively. In Hieba variety, the highest value of oil content in seeds was obtained as affected by hydrogel and organic fertilizer combined with the growth regulator Ahrostymulin - 22.1%. This means that the oil content in soybean seeds was largely influenced by the biological characteristics of plants and agronomical practices.

Temperatures that vary spatially and temporally over the soybean growing areas affect soybean seed yield and quality (ALSAJRI *ET AL.*, 2020). Authors proved that the cultivar × temperature interaction was significant for total biomass, seed yield, protein and oil content. This study showed that the influence of air temperature increasing during growing season of soybean can be minimized by moisture retainer, organic fertilizer and growth regulator treatments. ASSEFA *ET AL.* (2018) found out that variability in genotype × management × environment influencing affected soybean yield, protein and oil content. The similar study was carried out by DHAKNE *ET AL.* (2015). They showed that application of growth regulator recorded significantly higher soybean yield.

Therefore, for obtaining high yield and good seed quality of soybean, it is necessary to use a set of additional treatments that contribute to optimization of nutrition and regulation of growth and development processes of plants.

CONCLUSIONS

As the results of this study, it was determined that the most polymorphic SSR marker was Satt726. There were detected 16 alleles in studied soybean varieties (PIC 0.92). It was found out that the identified alleles of microsatellite loci are fairly evenly represented in soybean varieties, as evidenced by the high PIC values (0.82-0.92). According to cluster analysis, the most similar varieties based on 4 SSR markers were varieties with genetic distances 1.73. The most different was Aliaska variety with genetic distances 3.16-3.87. All studied varieties were distributed to 8 clusters. This indicates the high level of differential ability of marker system.

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The highest yield in the experiment was obtained in Kano variety with complex treatments of Aquasorb moisture retainer, Parostok organic fertilizer and the plant growth regulator Vermystym D - 5.27 t/ha. The maximum of protein content was obtained in variants of the complex treatments of organic fertilizers and growth regulators for all of studied varieties. The maximum values of oil content were noted in Ustia and Kano varieties combined effect of factors - Aquasorb, organic fertilizer Parostok and growth regulator Vermystym D - 20.1 and 22.0%, respectively.

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