Genotypic differences of soybean (*Glycine max* (L.) Merrill) as a factor of biological intensification of agroecosystems

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Abstract— *Unfavorable environmental conditions limit the continued yield increases of modern commercial cultivars and hybrids of agricultural plants in the intensive agroecosystems. Therefore, the genotypic differences in resistance/tolerance to biotic and abiotic stresses and the yield of soybean (Glycine max (L.) Merrill) are the focus of our long-term studies (2010 – 2018). The soybean breeding lines, collection varieties and commercial cultivars are investigated. The pathogens of viral diseases, namely, the Soybean mosaic virus (SMV) and the Alfalfa mosaic virus (AMV) have been identified. The soybean genotypes having one of such dominant genes as Rsv1, Rsv1t or Rsv1y (locus Rsv1) proved to be resistant to local strains of SMV. The genotypes with a relatively high level of the yield and resistance to viral diseases and downy mildew (Peronospora manshurica (Naum.) Syd.) are detected. Artificial selection of soybean genotypes for cold tolerance during the seed germination and seedling development period should be carried out taking into account the effect of early planting onto yield components and other plant morphological traits. Soybean yields, as a result of genotype-environment interactions, and the addressed introduction of commercial cultivars into specific agroecosystems are discussed. Selected genotypes can be used in agronomic practice and also as germplasm in breeding of the new high-yielding soybean cultivars with a good adaptability to soil and climatic conditions of Ukraine.*

Keywords— addressed cultivars, cold tolerance, downy mildew, soybean viruses, yields.

I. INTRODUCTION

Photosynthesis of agrophytocenoses is a basis of agroecosystems primary bioproductivity. An average radiation-use efficiency (RUE, dry matter produced per unit of intercepted solar radiation) of soybean plants is 1.02 g MJ^{-1} [1]. It should be noted, that starting from the moment of assimilates production by photosynthesis and till the accumulation of dry matter in the seeds there are many complex interconnected processes on the molecular, cellular, organismal and population levels which all taken together have a decisive influence on the yield [2, 3, 4, *et al.*].

1.1 The importance of soybean genotypic differences in agrophytocenoses

Genotypic differences among soybean plants exist in ability to capture solar radiation by the leaf surface and, as a result, the yield of cultivars may significantly change [5]. Soybean genotypes with reduced chlorophyll content and relatively high photosynthetic activity are promising for the crop sown with an increased plant density [6]. In addition, the ability of agrophytocenosis to intercept and use solar radiation rises in intercropping, compared with monocropping [7]. Therefore, it is possible to increase the use efficiency of soybean agrophytocenoses by selecting genotypes with higher photosynthetic capacity.

Soybean as a legume plant spends up to 20% of photosynthesis products to provide symbiotic nitrogen fixation. In its turn, high nitrogen-fixing activity of root nodules leads to an increase in the photosynthesis intensity and raise in the yield [8]. Genotypic variability in the symbiotic ability of soybean [9] and differences in the nitrogen-fixing activity of rhizobia [10] contribute to the effective combination of high values of the biological nitrogen fixation, photosynthetic capacity and yield of commercial soybean cultivars. For instance, in Brazil, where the average annual soybean yields level is relatively high (over 3 t ha^{-1}), soybean plants receive about 80% of their required nitrogen by symbiosis with nitrogen-fixing bacteria [11, 12]. In this way, the existing genotypic variability of soybean makes it possible to improve naturally nitrogen balance of soil-biotic complex and hence the ecological safety of intensive agroecosystems [13, 11, 14].

1.2 Unfavorable environmental factors and soybean yield

Soybean plants are highly sensitive to unfavorable environmental factors, including temperature regime, conditions of moisture supply and diseases. These factors have a negative influence on the yield formation and ultimately lead to significant reduction in its quantitative and qualitative characteristics [15, 16].

In Ukraine, the soybean is represented in agroecosystems by the commercial cultivars with high yield potential and valuable morphological and biological traits. On this basis, they are listed in the State Register of cultivars suitable for introduction and dissemination. However, under conditions of the separate field, farm or group of farms (at the level of the specific agroecosystem) the environmental stress factors can cause yield instability of soybean cultivars. Due to the differences in the level of yield components stability of soybean genotypes [16] it is important to select cultivars that would be able to realize the yield potential under changing environmental conditions.

It's needed to highlight that the breeding programs in the world leading countries at present are increasingly oriented towards creating cultivars and hybrids of agricultural plants characterized not by the maximal yield but the optimally high and stable yield level [17, 18]. Along with the state breeding programs there are private breeding programs in Ukraine. However, it is important to keep the state breeding priorities with new content, since they may not have commercial attractiveness today but are potentially significant in the future.

1.3 Research aim

Our long-term main tasks were to search for new approaches, develop and improve the methods of adaptive breeding for optimal combination of the yield, resistance, adaptability and quality in the soybean cultivars. In the breeding process were used the wild-type genotypes (*Glycine soja* Sieb. and Zucc.) which can adequately respond to changing environmental conditions.

II. MATERIAL AND METHOD

2.1 Field experiment description and plant material

Different genotypes of soybean (*Glycine max* (L.) Merrill) including the breeding lines (F8 – F10), collection varieties and commercial cultivars were studied during 2010 – 2018. The breeding lines were created on the basis of hybridization (*G. max* x *G. max*; *G. max* x *G. soja*) and artificial selection for yield and its components, biotic and abiotic resistance /tolerance, early maturity, plant height and other valuable plant traits. Among the collection soybean varieties, 20 varieties with known virus resistance genes were obtained from the US Department of Agriculture (National Plant Germplasm System). Field studies with soybean genotypic diversity were carried out under soil and climatic conditions of Vinnytsia region on the experimental fields of the Vinnytsia National Agrarian University and the Scientific-Production Center "Soybean" of the National Academy of Agrarian Sciences of Ukraine.

In the experimental field soybean seeds were planted manually in 3 rows of plots with a row length of 2.5 m and row spacing of 0.45 m, with four replications. The seeds were planted in soil at a depth of 2 to 4 cm (depending on soil moisture) and seed spacing 5 cm. Two seeds per hole (using a manual planter) were planted in the middle row of each plot. After seedling establishment, the plants were thinned in the middle row with plant spacing 5 cm. Two outer rows were left as borders in addition to the outer 0.25 m at the end of each plot. Phenological observations and biometrics of quantitative traits in the plants were carried out according to methodical recommendations [19]. In the R8 stage (full maturity), 5 plants (in a row) and the plants in a 0.9 m^2 area were collected from the middle row of each plot to evaluate yield and useful economic traits. After measurements, the collected plants were threshed with a threshing machine. Also, the yield components and yield of breeding lines and commercial soybean cultivars were evaluated in another field experiment. The plot consisted of four 10-m sowing rows spaced 0.45 m apart and with seed spacing 3 cm, with three replications. The small farm machinery (seeding machine, rotary hoe and combine harvester) was used in this experiment.

The soybean was sown after winter wheat or barley in the crop rotation. The tillage consisted of the plowing (in autumn) and two or three cultivations (spring seedbed preparation). The fertilizer system included the application of inorganic fertilizers at a ratio of 10 kg N, 20 kg P_2O_5 and 20 kg K_2O ha^{-1} and seed treatment by rhizobial inoculants. The herbicides were used considering technological recommendations of soybean growing.

2.2 Methods for testing biotic and abiotic resistance/tolerance

The genotypic differences of soybean plant resistance to downy mildew and viral diseases were studied in the conditions of natural infection at the R2 stage (full bloom). The disease severity was determined by estimation of 25 to 30 plants of each genotype on a 5-point scale in accordance with the methodical requirements [20]. In virological studies were used methods of the extraction and purification of the local virus isolates (*Soybean mosaic virus and Alfalfa mosaic virus*), enzyme-linked immunosorbent assay (ELISA), reverse transcription polymerase chain reaction (RT-PCR), DNA sequencing and electron microscopy [21].

Cold tolerance degree of each genotype in the stage of seed germination was assessed on the basis of seed germinability values at cold (7°C, on the 21st day of germination) and at optimal (23°C, on the 7th day of germination) temperatures (using refrigeration thermostat KBW 720 Binder). Soybean seeds were germinated in the paper, wetted with distilled water in four replications (40 seeds per one). The experiment was conducted in accordance with the ISTA seed germination rules [22]. In the field experiment seedlings were counted on the 28th day after sowing under conditions of early planting (April 1 – 5) and on the 14th day under conditions of optimal planting (late April – early May).

2.3 Statistical analysis

Mathematical processing of experimental data was performed by using of Microsoft Excel 2013 and Biostat software packages.

III. RESULTS AND DISCUSSION

3.1 Downy mildew damage of soybean and yield

The frequency analysis of the distribution of soybean breeding lines (on average 100 genotypes per year during 2012 – 2016) according to their yield and plant damage caused by downy mildew infection (*Peronospora manshurica* (Naum.) Syd.) demonstrated differences in phenotypic expression of these characteristics as a result of genotype-environment interactions (Fig. 1). The relatively high level of resistance to downy mildew (disease severity from 0 to 10% on average per plant) was observed in 16.7% of all investigated breeding lines. However, only individual breeding lines (C25712, C80213) differed by the absence of downy mildew symptoms and yielded above 3 t ha^{-1}. In most breeding lines with the yield higher than 3 t ha^{-1} the disease severity was in the range 1 – 7%. The breeding lines with the disease severity from 30 to 50% were characterized by a low yield (1.5 – 2.0 t ha^{-1}) in most cases. At the same time were also found some breeding lines (C36112, C51413) without symptoms of downy mildew lesions but with the yield that did not exceed 2 t ha^{-1}.

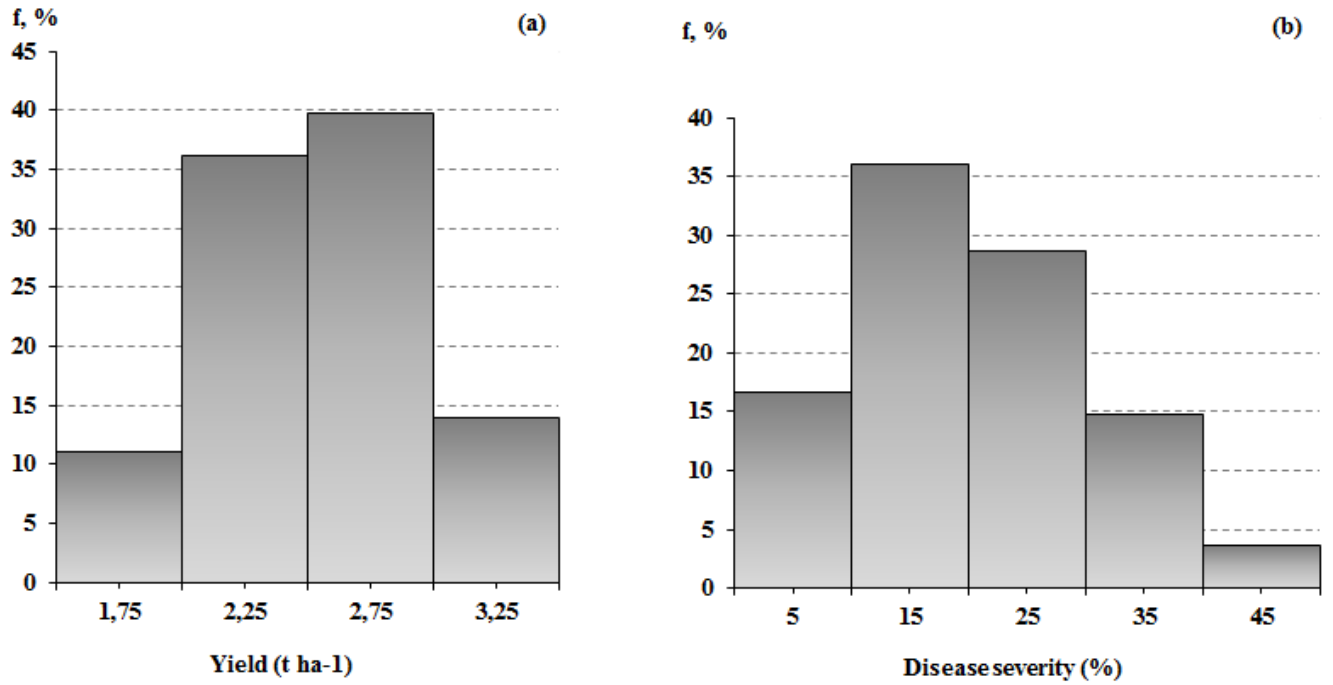

FIG. 1. Distribution of the soybean breeding lines according to their yield (a) and plant damage caused by downy mildew (b); f – relative frequency; (2012 – 2016)

In addition, the pathogen resistance and the yield of soybean genotypes were studied on the basis of classical understanding of the "plant–pathogen–environment" interaction system [23, 24]. When environmental factors are more favorable for a plant it can reduce the disease severity and not lead to significant negative pathogen influence onto yield. If environmental factors are more favorable for pathogen the disease severity will increase and it will lead to yield decrease. In case when the vectors of environmental influences onto pathogen and plant coincide, their balanced coexistence would be reasonable. Thus, it is possible to explain the low yield and absence of downy mildew symptoms on the plants of individual soybean breeding lines. Therefore, environmental factors can have a significant modifying effect on both the pathogen resistance and the yield of soybean genotypes, that substantially complicates the artificial selection for combination of these traits.

3.2 Viral infection and soybean virus resistance

Significant differentiation of soybean genotypes referring to viral diseases damage severity was found. More than 1000 soybean genotypes were investigated in the field experiments from 2010 to 2018. Such pathogens of viral diseases as *Soybean mosaic virus* (SMV) and *Alfalfa mosaic virus* (AMV) have been identified by the ELISA and RT-PCR methods. As it turned out, the SMV infection was most common among the breeding and collection genotypes of soybean in the conditions of the experimental field of the Vinnytsia National Agrarian University. This infection was identified in more than 70% of the tested genotypes. SMV disease severity on average per plant did not exceed 5% in some collection varieties (Swift, Ajma) only. Whereas the values of disease severity were in the range of 20 – 55% in most other varieties (Aurora, Gamma 85, MON 04, Chernovitska 6, Olima etc.). The AMV infection was identified in less than 10% of soybean genotypes, but the plants infected with this virus could reduce their yield (weight of seeds per plant) by more than 3 times. Besides, the viral infection (detected by the methods of ELISA and electron microscopy) in the plant organism did not always have its phenotypic expression (the infection was in the latent state).

The collection varieties of soybean with known SMV resistance genes (loci Rsv1, Rsv3, Rsv4) [25] were investigated under conditions of natural infection in relation to their ability to resist local SMV strains. Among these varieties, both resistant (plants without symptoms of viral infection) and susceptible (plants with symptoms of systemic viral infection, including leafnecrosis and stem-tip necrosis) genotypes were detected (Fig. 2). The soybean genotypes (York, Ogden, Suzumaru, PI96983, Hourei, PI 398476, VIR 2980, PI 486355 varieties) which have one of the dominant genes Rsv1, Rsv1t or Rsv1y (each alone or in combination with genes Rsv3 and Rsv4) were resistant to local SMV strains. Severally, Rsv3 and Rsv4 were ineffective genetic determinants of resistance to local SMV strains. Although according to the studies [26], the Rsv4 gene can provide resistance to a wide range of SMV strains. Ukrainian isolate of SMV named UA1Gr was identified as belonging to the G4 strain group [27].

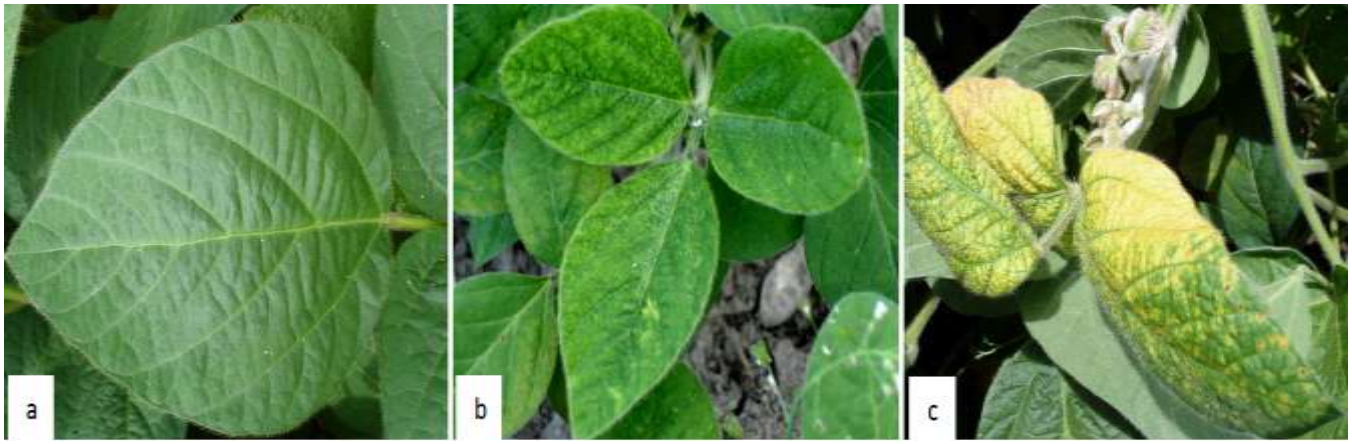

FIG. 2: Soybean leaf without symptoms of viral infection (a); Soybean leafs with systemic mosaic symptoms (b) and stem-tip necrosis (c)

The *Rsv1*-f/-r gene-specific to the *Rsv1* locus molecular marker [28] and the Satt542 and Satt634 microsatellite markers linked with the locus *Rsv4* [26] were used for the genotypic identification of commercial soybean cultivars for resistance to SMV infection. It was determined that the high-yielding cultivars Vinnychanka and Gorlytsia (the cultivars created by the adaptive breeding methods) have the effective genetic determinants of resistance to SMV infection. These cultivars can be used in agronomic practice and also as germplasm in the selective breeding of new soybean cultivars with virus resistance and good adaptability to soil and climatic conditions of Ukraine.

3.3 Soybean genotypic differences in low-temperature sensitivity

Formation of high-yielding soybean agrophytocenoses largely depends on the abiotic factors. In particular, soybean requirements to warmth appear in the stages of seed germination and seedling development. Soybean seedlings appear 5 – 7 days after sowing under favorable temperature conditions (20 – 25°C). But a decrease in temperature to 7 – 10°C slows the seed germination and delays the emergence of seedlings for up to 25 days or more. Therefore, an increase in cold tolerance of

commercial soybean cultivars during the seed germination and seedling development period would contribute to the utilization of spring soil moisture by plants more efficiently. That would lead to the activation of growth and photosynthetic processes, and consequently to the increase of quantitative and qualitative characteristics of the yield.

Significant differences in cold tolerance at the seed germination and seedling emergence period were found among the soybean breeding lines. The genotypes (C88812, C11512, C84912, C23112) with comparatively high values of seed germinability (above 90%) under low-temperature conditions were identified in the laboratory and field experiments (Table 1). The genotypes with the inferior ability of seeds to germinate under conditions of low temperatures were detected too. Seed germinability of the investigated genotypes was above 90% under the optimal temperature regime (23°C).

TABLE 1.
COLD TOLERANCE ESTIMATION OF THE SOYBEAN BREEDING LINES AT THE SEED GERMINATION AND SEEDLING EMERGENCE PERIOD (ON AVERAGE DURING 2014 – 2017)

Breeding line	Seed germinability (mean ± SE), %		
	laboratory conditions		early planting under field conditions
	23°C	7°C	
C88812	96.9	94.29± 1,96	91.88± 2,16
C11512	94.4	93.57 ±2,07	91.25± 2,23
C89712-2	92.5	92.86 ±2,18	83.13± 2,96
C84912	96.3	92.14 ±2,27	90.63 ±2,30
C23112	91.9	91.43 ±2,37	93.12± 2,01
C39811	96.3	86.43± 2,89	73.38 ±3,49
C16112	93.8	81.42± 3,29	91.25 ±2,23
C83012	94.4	70.71 ±3,85	68.13± 3,68
C84212	95.0	72.14 ±3,79	72.50 ±3,53
C23911	93.1	62.86 ±4,08	66.88± 3,72
Negrutsa*	96.3	92.86 ±2,18	90.63± 2,30
$t_{01} = 2.63$			

SE – standard error; t_{01} – value of Student's t-test at 99% confidence level; * – collection variety, cold-tolerance check indicator

Early planting contributed to the differentiation of breeding lines with respect to the yield and other important agronomic traits. In particular, the plant height at maturation (on average during 2014 – 2017) in more than 80% of breeding lines was decreased within 10-25 cm depending on the genotype under early planting (April 1 – 5) compared to optimal planting (late April – early May). A significant decrease in yield (more than 0.5 t ha^{-1} on average during 2014 – 2017) was observed in most breeding lines under early planting conditions compared to optimal planting. The genotypes (C84912, C23112) with the yield of more than 3 t ha^{-1} by planting early and the yield of less than 2.5 t ha^{-1} under optimal planting were also found. In addition, the genotypes (C88812, C11512) whose yields remained relatively high (2.9 – 3.4 t ha^{-1}) at both planting dates were detected.

Previous studies of the genetic control of soybean cold tolerance have shown the relative independence of genetic factors which define the cold tolerance in the stages of seed germination, seedling development, and bloom and pod formation [29]. Therefore, the evaluation and selection of soybean genotypes for cold tolerance should be conducted differentially considering the ontogenesis stage. Besides, the artificial selection of soybean genotypes for cold tolerance during the seed germination and seedling development period should be carried out taking into account the effect of early planting onto yields and other important agronomic traits.

3.4 Yield of soybean in Ukraine

The analysis of soybean world production indicators (Table 2) shows that more than 55% of the sown areas and more than 65% of the grain production of soybean belongs to the USA and Brazil. The yield potential of soybean cultivars is effectively realized (3.3 – 3.5 t ha−1) in these countries. Therefore, the average yield of this crop in the world is relatively high and is over 2.7 t ha^{-1}.

In Ukraine, the average soybean yield during 2016 – 2018 varied from 2.0 to 2.6 t ha^{-1}. It is significantly lower than the average yield achieved by the leading soybean-producing countries in the world (tab. 2). Though, it should be noted that there is a sufficient potential of cultivars in Ukraine to increase the average yield of soybean. Currently, the State Register includes more than 200 high-yielding commercial soybean cultivars of the domestic and foreign breeding which are able to achieve the yield higher than 3 t ha^{-1} in favorable growing conditions. However, the unfavorable environmental factors (pathogens, low and high temperatures, moisture shortages and others) cause the significant differences in yields among cultivars depending on their resistance/tolerance and adaptability to environmental factors. The changes in yields among cultivars under unfavorable environmental conditions have a negative effect on the average soybean yield. Furthermore, the cultivation factors contributing to the high yield realization of cultivars in optimal conditions can be completely ineffective under stress [12].

TABLE 2.
THE MAIN INDICATORS OF MODERN WORLD SOYBEAN PRODUCTION (USDA, DECEMBER 2018) [30]

Year	World	USA	Brazil	Ukraine
Area (million hectares)				
2016	119.76	33.47	33.90	1.86
2017	124.69	36.23	35.15	1.98
2018	128.31	35.75	36.20	1.70
Production (million metric tons)				
2016	349.30	116.92	114.60	4.29
2017	339.47	120.04	120.30	3.89
2018	369.20	125.18	125.18	4.40
Yield (t ha^{-1})				
2016	2.92	3.49	3.38	2.31
2017	2.72	3.31	3.42	1.97
2018	2.88	3.50	3.37	2.59

The addressed introduction of cultivars into specific agroecosystems is one of the effective ways to solve the problem of the average soybean yield raising and stabilization. This is carried out on the basis of cultivars with high adaptability to the soil-climatic and technogenic conditions of specific agroecosystems. Cultivars with high adaptability to specific agroecosystems might be called the "addressed cultivars". The addressed introduction of commercial soybean cultivars is important for Ukrainian farmers who aim to stabilize the average yield level of this crop above 3 t ha^{-1} [31].

IV. CONCLUSION

Soybean genotypic differences in resistance/tolerance to biotic and abiotic stresses, in yield components and other valuable morphological traits constitute the biological basis for a formation of the effective agrophytocenoses. It is necessary to evaluate breeding lines and commercial cultivars under conditions of specific agroecosystems where their addressed introduction is expected. Selected soybean genotypes (the best recombinant inbred lines and cultivars) can be used in agronomic practice and also as germplasm in breeding of the new high-yielding cultivars with good adaptability to soil and climatic conditions of Ukraine.

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