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Adaptability of F₁ sunflower hybrids, created according to an integrated system of line selection for economically valuable traits in various agroclimatic zones

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Purpose. Determine the ecological plasticity and productivity of F, sunflower hybrids created on the basis of maternal and parental lines, selected according to an accelerated selection system of lines resistant to herbicides (imidazoline and sulfonylurea groups) and broomrape (Orobanche cumana Wallr.). Methods. Statistical analysis of F, sunflower hybrids was carried out using the methods of variation statistics, regression and analysis of variance using the Microsoft Office Excel 2016 application package. Molecular biological, biotechnological and classical selection methods were used for the accelerated system of line selection. Thus, for the purpose of targeted selection of sunflower sterility fixers, we used HRG01 molecular SCAR marker to identify the gene for the restoration of pollen fertility (Rf_{c}). To accelerate the creation of parental lines resistant to tribenuron-methyl, we used a culture of immature embryos in vitro. **Results.** The results of testing of F. sunflower hybrids at Kyiv, Chernihiv, Cherkasy (Uman and Shpolianskyi districts), Khmelnytskyi, Kharkiv, Kherson and Odesa regions. The hybrids were created on the basis of selected lines, chosen according to an accelerated selection system for herbicide-resistant lines (imidazoline (IMI-hybrids) and sulfonylurea (SU-hybrids) groups) and broomrape (Orobanche cumana Wall). The standards for comparison with hybrids were: for IMI hybrids – hybrids 'NK Neoma' (Syngenta) and 'ES Genesis' (Euralis), and for SU-hybrids - 'SY Sumiko' (Syngenta) and 'P64LE25' (Pioneer). As a result, it was found that among SU-hybrids, UA 2/106 had a 3.9% higher yield when compared to the standards ('SY Sumiko' and 'P64LE25'). And for IMI-hybrids it was found that hybrids UA 1/67, UA 1/66, UA 1/84 have the same yield of 2.76 t/ha as the 'NK Neoma' standard. IMI hybrids UA 1/92, UA 1/102 have the same yield of 2.91 t/ha as 'ES Genesis'. Conclusions. F, hybrids were created on the basis of the original breeding material selected due to the accelerated system of sunflower lines selection. The hybrids were analyzed according to the yield indicator. The most productive among the tested SU-hybrids was UA 2/106 hybrid, among the IMI hybrids – UA 1/67, UA 1/66, UA 1/84, UA 1/92 and UA 1/102.

Keywords: Helianthus annuus L.; hybrid; yield; test.

Introduction

Sunflower (*Helianthus annuus* L.) is the main oilseed crop in Ukraine; in 2020 it was grown on an area of more than 6 million hectares [1]. In industrial production, high-yiel-

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ding sunflower hybrids characterized by a set of certain economically valuable traits, such as resistance to: herbicides of sulfonylurea and imidazoline groups, diseases, pests, and parasitic weed sunflower broomrape are used. To create sunflower F_1 hybrids, cytoplasmic male sterility (CMS) is used, where the main components of the hybrid are a sterile sunflower pollen maintainer (Nrf_1rf_1) , its sterile analogue (Srf_1rf_1) and a sunflower pollen fertility restorer (N/SRf_1Rf_1) [2]. The selection of each component based on valuable traits [resistance to herbicides and parasitic weed broomrape (Orobanche cumana Wallr.)] is a long selection process that lasts for 6 years, and with testing of hybrids and their subsequent registration lasts 12 years [2, 3]. The

use of molecular biological, biotechnological and immunological methods (testing lines on an artificial infectious background in laboratory conditions to determine the resistance of the starting material to the parasitic plant sunflower broomrape) together with classical breeding methods allows for the accelerated creation and selection of parental lines with economically valuable traits. For example, using molecular markers (RAPD, ALFP, SSR, etc.), it is possible to identify resistance genes: to downy mildew (*Pl* genes) [3–5], parasitic plant sunflower broomrape (Or genes) [3, 6, 7], herbicides (AHAS/ALS genes) [3, 8, 9] and pollen fertility restoration genes (*Rf* genes) [10–14] in paternal sunflower lines. This method allows to carry out targeted selections among the source material of sunflower by the given genes (Pl, Or, Rf, AHAS/ALS, etc.). Among the methods for obtaining paternal components with certain characteristics, the method of culture of immature embryos *in vitro* is effective. This method is also used to study somatic embryogenesis, organogenesis, and regeneration [15-19], to obtain plants with altered traits after their genetic transformation [20], to reproduce seeds with low viability, as well as to obtain distant hybrids [2, 21].

The ultimate goal of selecting the sunflower resulting lines is their further crossing to create hybrids (\mathbf{F}_1), which will have certain economically valuable traits (resistance to herbicides and to a parasitic weed sunflower broomrape, drought, increased yield and oil content, etc.).

A prerequisite for the introduction of new sunflower hybrids into industrial cultivation is testing of hybrids for an objective assessment of their genetic potential, competitiveness and adaptability, in order to determine the cultivation zone to obtain the maximum yield level. Environmental tests make it possible to assess the ecological plasticity in terms of yield, which is one of the methods for studying the reaction rates for this trait and the growing area [22–24].

The aim of the study is to determine the ecological plasticity and yield of F_1 sunflower hybrids in an ecological test, obtained on the basis of maternal and paternal lines, selected according to an accelerated selection system of lines resistant to herbicides (imidazoline and sulfonylurea groups) and broomrape (Orobanche cumana Wallr.).

The hybrids tested in 2020 were selected according to the accelerated selection system for the initial material of sunflower resistant to herbicides (imidazoline and sulfonylurea groups) and a plant-parasitic weed sunflower broomrape, developed during 2016-2020. A feature of the created system of accelerated selection is the phased application of a complex of biotechnological, molecular biological and breeding methods of acceleration and targeted selection of lines with the desired economically valuable traits.

Materials and research methods

Plant material

Sunflower hybrids are created on the basis of maternal and paternal lines resistant to herbicides and broomrape, selected according to an accelerated selection system.

To create hybrids resistant to herbicides of the imidazoline group (the Euro-Lightning herbicide of the Clearfield production system of BASF with the active ingredient imizapyr 15 g/l and imazamox 33 g/l), the following material was used:

- maternal lines - BH320/'NK Neoma' (11/15), BH320/'NK Neoma' (11/103), BH320/'NK Neoma' (11/104), BH039/'EC Artemis' (11/162), BH3978/'Dragan' (12/155) та BH3978/'Dragan' (12/156) [25];

- paternal line - line 3 [26].

For hybrids resistant to sulfonylurea herbicides (herbicide Granstar Gold 75 by Dupont with the active ingredient tribenuron-methyl 750 g/kg), the following was used:

- maternal lines - Ls8A/Lc1093B (9/10), Ls8A/Lc1093B (9/12), Ls8A/Lc1093B (9/117), Zoria FN/Lc1093B (9/138), Zoria FN/Lc1093B (9/166), A12/Lc1093B (10/124) та A12/ Lc1093B (10/216) [25];

- paternal lines - BH0118/SURES-2 (101/1), BH0118/SURES-2 (101/4), BH0118/SURES-2 (101/6), BH0118/SURES-2 (101/7), BH0218/ SURES-2 (101/11, BH0218/SURES-2 (101/12), BH0218/SURES-2 (101/16), BH0218/SURES-2 (101/17), BH0218/SURES-2 (101/18), BH0318/ SURES-2 (101/21), BH0318/SURES-2 (101/24), BH0318/SURES-2 (101/28), BH0318/SURES-2 (101/30) [26].

The system of accelerated selection of paternal lines was carried out according to the scheme shown in Figure 1. Work with the mother lines was conducted in two stages: 1) isolation of sterility maintainers using SCAR marker HRG01; 2) the isolation of broomrape resistant sterility maintainers on an artificial infectious background in laboratory conditions. Work with paternal forms included: 1) study of the regenerative capacity of sunflower pollen fertility restorer lines resistant to imidazolinones, and accelerated creation of fertility restorer lines resistant to tribenuron-methyl when using a culture of immature embryos; 2) isolation of pollen fertility restorer lines resistant to broomrape on an artificial infectious background in laboratory conditions.



Fig. 1. General scheme of the accelerated system of sunflower parental lines selection

Identification of SCAR marker HRG01 was carried out by PCR using a pair of primers flanking the 1.1 cM region between OPK13 454 and E33M61 136 in 13 sunflower linkage group [11]. The nucleotide sequence of the primers to the HRG01 locus was as follows: F primer: 5'-TATGCATAATTAGTTATACCC-3`; R primer: 5'-ACATAAGGATTATGTACGGG-3' [11]. PCR was performed using GenePak PCR Core reagent kits, «Isogen» (Russia). DNA was isolated using the STAB method [27]. The reaction mixture consisted of 0.2 μl of each primer, 2 μl of PCR buffer 10x DreamTagTM GreenBuffer (Thermo Scientific), 0.2 mM of each deoxyribonucleoside triphosphate (dNTP) (Thermo Scientific), 2 units of polymerase 20 ng of genomic DNA. The final volume of the reaction mixture was 20 µL, to which additional 20 µL of mineral oil was added to prevent evaporation of the reaction mixture because thermostat lid is not heated. PCR was carried out in thermal cycler «Tertsik» (Russia) according to the program: initial denaturation for 10 min at 94 °C; 35 cycles – denaturation for 45 s at 94 °C; annealing for 45 seconds at 58 °C; elongation for 60 s at 72 °C; final elongation for 6 min at 72 °C to detect the HRG01 marker.

After the completion of PCR, the amplification products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide. The DNA ladders 50 bp kit (Thermo Scientific) was used to mark the length of the obtained fragments [14].

The studies of the regenerative capacity of sunflower fertility restorer lines resistant to

herbicides of the imidazoline group were carried out on 4 sunflower fertility restorer lines (2, 3, 19, 35) for the induction of organogenesis *in vitro*. To obtain an *in vitro* culture, cotyledons isolated from immature sunflower embryos selected on the 21st day after pollination were used. This work consisted of the following stages: sterilization of seeds, isolation of explants (cotyledons), induction of adventitious shoots and their elongation, rooting of regenerated plants, and adaptation of regenerated plants in a greenhouse.

21-day-old immature seeds were soaked for one day in distilled water to soften the shell, then the husks were separated from the immature seeds and the immature seeds were sterilized in 70% ethyl alcohol (1-2 min), a solution of household bleach «Bilyzna» (dilution in water in ratio 1 : 2) for 20 min, followed by washing with sterile distilled water (three times).

For the induction of adventitious buds, 5 modifications of the Murashige-Skoog medium (MS) [28] were used, supplemented with vitamins B5 [29], 3% sucrose, 5 mg/L AgNO3 and the following growth regulators:

1) 2 mg/L N-isopentenylaminopurine (2-iP), 0.5 mg/L indole-3-acetic acid (IAA), 0.1 mg/L thidiazuron (TDZ) [26];

2) 2 mg/L N-isopentenylaminopurine (2-iP), 0.5 mg/L picloram, 0.1 mg/L thidiazuron (TDZ);

3) 1 mg/L 6-benzylaminopurine (BAP), 1 mg/L 1-Naphthaleneacetic acid (NAA), 0.1 mg/L gibberellic acid (GA₂) [18]; 4) 1 mg/l 6-benzylaminopurine (BAP), 0.25 mg/L indole-3-acetic acid (IAA), 0.1 mg/L gibberellic acid (GA₃);

5) 2 mg/L kinetin (Kn), 0.5 mg/L 1-Naph-thaleneacetic acid (NAA).

The pH of the medium was adjusted to 5.7 ± 0.1 using 1M KOH or HCl solution and autoclaved at 120 °C for 20 minutes.

The proliferation of adventitious buds was carried out on medium 1 and on medium supplemented with 3 mg/L 6-benzylaminopurine (BAP) and 2 mg/L N-isopentenyl aminopurine (2-iP).

For induction of morphogenesis, part of the explants were cultured for 21 days at a 16-hour photoperiod at a temperature of 25 °C, the rest of the explants were cultured for 14 days in darkness and 7 days at a 16-hour photoperiod at a temperature of 25 °C.

Adventitious shoots elongation was performed on MS media [28] with vitamins B5 [29], 3% sucrose, 5 mg/l AgNO3, supplemented with: 1) 0.1 mg/L 6-benzylaminopurine (BAP) [20]; 2) 1 mg/L N-isopentenyl aminopurine (2-iP), 0.5 mg/L 6-benzylaminopurine (BAP) [30]; 3) 0.2 mg/L gibberellic acid (GA₃) [18]. Regenerated plants, which formed a well-developed root system, were adapted in a greenhouse with photoperiodic lighting (16 hours of light: 8 hours of dark) and a temperature of 25 °C.

As a result of these experiments, the optimal cultivation conditions were established to obtain the maximum proportion of sunflower regenerants, and an effective rooting system of adventitious shoots was developed, which allows the regenerant plants to be adapted to the greenhouse conditions [17].

Using an immature embryo culture of sunflower for the accelerated isolation of tribenuron-methyl resistant lines. The study carried out during 2017–2019, began with the crossing of fertility restorer lines BH0118, BH0218, and BH0318, which do not contain the tribenuron methyl resistance donor SURES-2 (TBM gene-resistance AHASL1-2) [19].

As a result of crossing the fertility line restorers BH0118, BH0218, and BH0318 with the tribenuron methyl resistance donor SURES-2, the genotypes SURES-2/BH0118, SURES-2/BH0218, SURES-2/BH were obtained. On the 21st day after flowering, 30 immature seeds were isolated from each basket and introduced into *in vitro* culture. For introduction into *in vitro* culture, immature seeds were sterilized in 70% ethyl alcohol (1-2 min), a solution of household bleach «Bilyzna» (dilution in water in a ratio of 1 : 2)

for 20 min, followed by washing with sterile distilled water (three times). After sterilization of immature seeds, the embryo with endosperm was peeled off. Then it was placed in Petri dishes with a basic MC medium [28] with the addition of 0.1 mg/L 6-benzylaminopurine (BAP). On 10–14 days of *in vitro* cultivation, sunflower seedlings with formed roots were obtained; they were subsequently planted in the soil, where they were adapted to greenhouse conditions and self-pollinated to produce I₁ seeds. On days 10–14 of *in vitro* cultivation, sunflower seedlings with formed roots were obtained; they were subsequently planted in the soil, where they were adapted to greenhouse conditions and self-pollinated for obtaining I_1 seeds.

In the spring of 2018, I, seeds obtained from self-pollinated regenerant plants that underwent adaptation after cultivation in vitro were sown at the breeding base of the All-Ukrainian Scientific Institute of Breeding (VNIS) located in the Obukhiv district of the Kyiv region in the village of Bezimenne. The plants were treated with the herbicide Granstar Gold 75 with the active ingredient tribenuron-methyl at a dose of 100 g/ha. For spraying, a selection sprayer created by the engineers of the VNIS company according to their technology was used, which made it possible to uniformly apply the herbicide to the leaf plate and the growth point of sunflower plants. Plants that showed no signs of herbicidal stress were forced to self-pollination. In July of the same year, immature embryos were selected from self-pollinated plants resistant to tribenuronmethyl on the 21st day after flowering and reintroduced into *in vitro* culture to carry out another cycle of self-pollination and obtain I_3 seeds.

In 2019, I_3 seeds were sown in a breeding field (Obukhiv district of Kyiv region, Bezimenne village) and treated with herbicide. Plants noted to be resistant to the herbicide were forced to self-pollinate again [19].

Testing for resistance to broomrape of maternal and paternal lines. Testing of these lines was carried out in the department of plant immunity to diseases and pests of Ukrainian Scientific Institute of Plant Breeding (VNIS).

Seeds of the parasite weed broomrape were collected from the host plant in the phase of physiological ripeness to carry out such testing. Seeds were collected in the Zaporizhzhia, Kharkiv, Kirovohrad, Odesa, Donetsk, Luhansk and Kherson regions on the fields of sunflower hybrids resistant to the E, F and G broomrape races (information on the resistance of hybrids was used from the catalogs of Limagrain, Syngenta, Pioneer companies with detailed information on sunflower hybrids), as well as from the demonstration fields of sunflower seed producing companies located near the central roads in each area, which were subsequently sieved to separate dry plant residues.

Seeds of sterility maintainer and fertility restorer lines were sown in pots with an infected peat mixture, which included 5 L of peat (= 1 kg 300 g), 2 kg of sand and 2 g of broomrape seeds.

After 30-35 days, the sunflower plants were carefully removed from the peat mixture and recorded the presence of broomrape tubercles. The count was carried out visually – the presence or absence of broomrape tubercles was determined on each of the studied plants.

Limagrain hybrids, namely 'LG 50505' (resistant to the G race of broomrape) – resistance standard (St R «resistance») and 'LG 5665' (resistant to the E race of broomrape) – susceptibility standard (St S «susceptible») were used as standards (St), for comparison the level of plant damage by bloomrape [26].

Method for environmental testing of F_1 hybrids and statistical processing of the results. Testing of sunflower hybrids was carried out in accordance with the method generally accepted for the culture [31, 32]. In accordance with the methods [33, 34], the parameters of ecological plasticity and stability of sunflower hybrids were calculated. When calculating the coefficient of linear regression (bi), the level of ecological plasticity of hybrids was established. When using the standard deviation from the regression line (S_i^2) , the stability of the hybrid to various growing conditions was revealed, where X_i is the mean value of the trait of the I genotype under points, I, is the environmental index. According to the coefficient of ecological plasticity (bi), hybrids are divided into three groups:

1) high plasticity bi > $(1 + \sigma)$ – under favorable conditions (under conditions with the maximum manifestation of the trait), the manifestation of the trait increases;

2) medium plasticity bi = $(1 \pm \sigma)$ – the manifestation of the trait is at the level of medium sensitivity in the sample of hybrids under study;

3) low plasticity bi = $(1 - \sigma)$ – the manifestation of the trait decreases under favorable conditions.

Hybrids were created in a winter nursery located in South America (Chile), the city of Rancagua, during 2019–2020. The line used in the creation of sunflower hybrids was previously selected according to the accelerated complex selection system described above.

Depending on the resistance to certain herbicides, the hybrids were divided into sulfonylurea herbicide resistant (SU hybrids) and imidazoline herbicide resistant (IMI hybrids). The standards against which yields were compared were hybrids: for IMI hybrids, a hybrid of Syngenta 'NK Neoma' and Euralis 'ES Genesis', and for SU hybrids, a hybrid of Syngenta 'SY Sumiko' and Pioneer 'P64LE25', as these hybrids are among the most productive in Ukraine.

 F_1 hybrids were tested during 2020 at 8 sites in the Obukhiv district of Kyiv region, Borozna district of Chernihiv region, Shpola district of Cherkasy region, Uman district of Cherkasy region, Teofipol district of the Khmelnytskyi region, Pervomaisk district of Kharkiv region, Novotroinske district of Kharkiv region, Kalievskyi district of Odesa region. The hybrids were sowed according to a randomized scheme in two repetitions. The hybrids were divided into blocks, 40 hybrids per block, where 4 hybrids were standards.

The total size of the plot was 20 m², the size of the accounting plot was 10 m². The density of plant standing before harvesting corresponded to the recommended number for the zone -60-65 thousand plants per hectare in the zone of sufficient moisture and 50-55thousand plants in the zone with moisture deficiency. So, the zone of sufficient moisture includes Khmelnytskyi, Kyiv and Chernihiv regions, the zone of insufficient moisture – Cherkasy and Kharkiv, the zone of deficient moisture – Kherson and Odesa regions.

Research results

The creation of a high-yielding sunflower hybrid takes about 12 years, of which it takes from 6 to 8 years to create maternal and paternal lines, therefore various methods are increasingly being used in sunflower breeding programs to speed up the creation of initial sunflower breeding material. Methods that allow targeted selection for certain characteristics include molecular biology methods, biotechnological methods (immature embryo culture, in vitro cell and tissue culture), assessment of material resistance to pathogens using an artificial infectious background, etc.

Thus, currently there are works that are separately aimed at the use of molecular markers to determine the presence of certain genes responsible for the manifestation of a trait [4-7].

Among the various biotechnological methods used to improve sunflower lines are immature embryo culture, culture of protoplasts and haploids [35]. However, work with sunflower is limited by its regenerative capacity in vitro [36, 37]. Although methods for studying sunflower regeneration by direct organogenesis were described [16, 30, 38], it has been established that sunflower regenerative capacity depends on a number of factors, such as: genotype, nutrient medium components, explant type and age, and in vitro cultivation methods. Therefore, a critical moment in the development of an effective sunflower regeneration protocol is the selection of cultivation conditions, the choice of an explant and a genotype that will be marked by a high regenerative capacity.

The proposed system for selecting maternal and paternal sunflower lines with economically valuable traits is based on a phased combination of biotechnological, molecular biological and breeding methods combined into one complex system for accelerated selection of lines (Fig. 2 and Fig. 3). The system of accelerated selection of paternal lines was carried out according to the scheme shown in Figure 1. The molecular SCAR marker HRG01 was used on maternal lines to identify the fertility restorer gene (Rf1). Using this method, we carried out a targeted selection of sterility maintainers among maternal lines, with genotype Nrf_1rf_1 [14]. This accelerated the selection of maternal lines, which were later tested on an artificial infectious background in the laboratory for resistance to broomrape [25].

The work with paternal lines was carried out in two directions: 1) the regenerative capacity was studied by direct organogenesis on pollen fertility restorer lines resistant to imidazoline group herbicides [17]; 2) accelerated creation of sunflower pollen fertility restorer lines resistant to sulfonylurea group herbicides using an immature embryo culture of [19]. As a result of the studies performed with fertility restorer lines, the selected material was tested on an artificial infectious background in order to isolate the lines resistant to broomrape [26].





Fig. 2. Scheme of research when working with maternal forms

So, we have shown that when using SCAR marker HRG01, it is possible to carry out a targeted selection of sunflower sterility maintainers. 477 lines resistant to herbicides of the imidazoline group were tested, including 130 lines BH320/'NK Neoma', 156 lines BH039/ 'ES Artimis', 191 lines BH3978/'Dragan'. As a result, it was found that the sterility maintainers (Nrfrf) [samples in which fertility restorer gene (Rf_1) was not detected] in the BH320/'NK Neoma' maternal lines were all tested samples, 107 among the BH039/'ES Artimis' line (4) and 128 samples in the BH3978/ 'Dragan' combination. In total, out of 477



Fig. 3. Scheme of research when working with paternal forms

imidazoline lines, 365 were sunflower pollen sterility maintainers.

When testing 344 samples of lines resistant to herbicides of the sulfonylurea group, where 105 samples of lines of the Ls8A/

Lc1093B combination, 120 samples of the 'Zoria FN'/Lc1093B combination, and 119 samples of the A12/Lc109B combination, it was found that all samples are sterility maintainers [14].



Fig. 4. Electrophoregram of amplification products using SCAR marker HRG01 of BH039/ 'ES Artimis' maternal line

M50 – 50 bp DNA Ladder molecular weight marker. Lanes: 113, 115, 117, 122, 124, 127, 129, 131, 132 – individual plants of the studied lines (no 426 bp amplicon); 114, 116, 119–121, 126, 128, 130, 133–137 – amplicon is observed in plants.

In the study of sunflower pollen fertility restorer lines resistant to imidazolinones, according to the regenerative ability, which consisted of the induction and elongation of adventitious shoots, rooting and adaptation of regenerative plants to greenhouse conditions, line 35 was selected for high regenerative ability. As a result of the study, optimal cultivation conditions were selected to obtain the maximum share of sunflower regenerants and an effective system for adventitious shoots rooting was developed, which allowed to adapt regenerated plants to aseptic conditions [17].

As a result of crossing the fertility restorer lines BH0118, BH0218 and BH0318 with the tribenuron-methyl resistance donor SURES-2 (TBM gene-resistance *AHASL1-2*), these combinations were obtained: BH02 2, BH0318/ SURES-2. As a result of the staged cultivation of 21-day immature sunflower embryos and with the selection of tribenuron-methyl-resistant plants (in the field), during 2017–2019 ten lines homozygous for tribenuron-methyl resistance were isolated from each combination of BH0118/SURES-2, BH0218/SURES-2, BH0318/SURES-2 [19].

Selected maternal (709 sterility maintainers, of which 365 were resistant to imidazolinones and 344 were resistant to tribenuron-methyl) and paternal lines (4 lines with resistance to imidazolinones and 30 lines from each combination of BH0118/SURES-2, BH3/SURES-2) were tested on an artificial infectious background in laboratory conditions in order to isolate the lines resistant to broomrape.

Testing on an artificial infectious background in the laboratory was carried out by visual assessment of the presence of broomrape (Fig. 5.) during the winter period of 2019.



Fig. 5. Visual assessment of the presence of broomrape on sunflower lines, where 1 is a resistant plant (no broomrape was found) and 2, 3 is a susceptible to broomrape plant (tubercle of a parasitic weed were found)

When evaluating maternal lines resistant to imidazolinones on an artificial infectious background, it was found that three lines from BH320/'NK Neoma' (11/15, 11/103, 11/104), one line (11/162) from BH039/'ES Artimis' and two lines from the combination BH3978/'Dragan' (12/155, 12/156) were highly resistant to G-race of broomrape. Among lines resistant to tribenuronmethyl three lines from Ls8A/Lc1093B (9/10, 9/12, 9/117) and two lines from 'Zoria FN'/Lc1093B (9/138, 9/166) and A12/ Lc1093B (10/124, 10/216), as highly resistant to broomrape were chosen [25]. The results of the visual assessment are presented in Table 1.

When assessing the parental lines, it was found that among the imidazoline lines (2, 3, 19, 35) on an artificial infectious background under laboratory conditions, line 35 was isolated as highly resistant, since no signs of damage by broomrape were found in 100% of the plants. Among those resistant to tribenuron-methyl, four lines highly resistant to G-race of broomrape were distinguished from the combinations BH0118/SURES-2 (101/1, 101/4, 101/6, 101/7) and BH0318/SURES-2 (101/21, 101/24, 101/28, 101/30, and five lines (101/11, 101/12, 101/16, 101/17, 101/18) from the BH0218/SURES-2 combination [26] (Table 2).

Therefore, using the accelerated selection system, we chose maternal and paternal lines resistant to herbicides (imidazoline and sulfonylurea groups) and sunflower broomrape in a short period of time (2016–2020) [14, 17, 19, 25, 26].

The selected lines were used to create hybrids of the first generation of sunflower used in tests in various agroecological zones of Ukraine.

During the testing of F_1 sunflower hybrids, it was observed how environmental conditions affect yield. Therefore, the adaptability of hybrids to different agro-climatic conditions was assessed by the coefficient of ecological plasticity (b_i) and the indicator of reproduction of this trait under different growing conditions (S_i^2) [33, 34].

It was revealed that for SU-hybrids the most comfortable growing conditions and obtaining high yields were observed in Chernihiv ($I_i = 1.29$) and Cherkasy (Shpolianskyi district) ($I_i = 1.00$) regions. The least comfortable growing

Table	1
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Lines	Comple number	Total number of plants,	Number of resistant plants			
Lines	Sample number	pcs.	pcs.	%		
	Lines resistar	t to imidazoline herbicide	es			
	11/15	20	20	100		
BH320/'NK Neoma'	11/103	20	20	100		
	11/104	20	20	100		
BH039/'ES Artemis'	11/162	17	17	100		
	12/155	20	20	100		
DESTRY DIAYAII	12/156	20	20	100		
	Lines resistan	t to sulfonylurea herbicide	es			
	11/10	20	20	100		
Ls8A/Lc1093B	11/12	20	20	100		
	11/117	20	20	100		
'7 aria EN' /1 a1002D	11/138	20	20	100		
ZUIIA FIN/LC1095D	11/166	19	19	100		
A12/Lc1003P	12/124	20	20	100		
MIC/ LUIU93D	12/216	17	17	100		
		Standards				
LG 50505 (St R)	St1	20	20	100		
LG 5665 (St S)	St2	20	20	0		

Resistance to broomrape of lines maintainers of sterility

Table 2

Resistance to bloomrape in fertility restorer lines

Linos	Sampla number	Total number	Number of ur	stable plants	Number of resistant plants		
Lilles	Sample number	of plants, pcs.	pcs.	%	pcs.	%	
	Line	s resistant to sul	fonylurea herb.	icides			
	101/1	20	0	0.0	20	100	
	101/2	15	15	100	0	0.0	
- ²	101/3	13	13	100	0	0.0	
ES	101/4	20	0	0.0	20	100	
UR I	101/5	16	2	12.5	14	87.5	
3/5	101/6	20	0	0.0	20	100	
118	101/7	18	0	0.0	18	100	
) 우	101/8	19	19	100	0	0.0	
B	101/9	14	14	100	0	0.0	
	101/10	20	3	15.0	17	85.0	
	Total number	175	66	37.7	109	62.3	
	101/11	12	0	0.0	12	100	
	101/12	12	0	0.0	12	100	
2-	101/13	19	19	100	0	0.0	
(ES	101/14	19	19	100	0	0.0	
L H	101/15	15	15	100	0	0.0	
3/S	101/16	8	0	0.0	8	100	
218	101/17	20	0	0.0	20	100	
P P	101/18	20	0	0.0	20	100	
8	101/19	21	19	90.5	2	9.5	
	101/20	15	15	100	0	0.0	
	Total number	161	87	54.0	74	46.0	
	101/21	20	0	0.0	20	100	
5-2	101/22	13	2	15.4	11	84.6	
RE 03	101/23	18	5	27.8	13	72.2	
SU	101/24	15	0	0.0	15	100	
	101/25	14	13	92.9	1	7.1	
	101/26	13	10	76.9	3	23.1	
-2 %	101/27	19	2	10.5	17	89.5	
ES-	101/28	13	0	0.0	13	100	
UR	101/29	19	5	26.3	14	73.7	
	101/30	18	0	0.0	18	100	
	Total number	162	37	22.8	125	77.2	

Continue table 2

Lines	Sample number	Total number	Number of ur	istable plants	Number of resistant plants			
	Sample number	of plants, pcs.	pcs.	%	pcs.	%		
Lines resistant to imidazoline herbicides								
2	l1/1	20	3	15.0	17	85.0		
3	l1/2	17	13	76.5	4	23.5		
35	l1/3	20	0	0.0	20	100		
19	l1/4	19	5	26.3	14	73.7		
		Stand	dards					
LG 50505 (St R)	St1	20	0	0.0	20	100		
LG 5665 (St S)	St2	20	20	100.0	0	0.0		

Table 3

Yield and adaptability of SU hybrids											
	Yield of hybrids, t/ha						Adapt parar	ability neters			
Number	Odesa region	Kherson region	Cherkasy region (Shpoliansky district)	Kyiv region	Cherkasy region (Uman district)	Khmelnytskyi region	Kharkiv region	Chernihiv region	Average yield	Coefficient of ecological plasticity, b _i	Stability, S _i ²
			Hig	h plastic	ity						
UA 2/205 UA 2/206 UA 2/186 UA 2/117 UA 2/235 UA 2/207 UA 2/136 UA 2/189	1.49 1.06 1.38 1.16 2.03 1.29 1.50 1.06	0.56 0.85 0.99 0.44 1.15 0.48 0.63 0.80	3.67 4.09 4.05 3.80 4.56 3.66 4.11 3.49	3.34 2.95 3.19 3.37 2.69 3.18 3.85 2.99	3.18 3.14 2.70 2.55 2.22 3.35 2.75 2.96	2.58 3.61 2.87 3.07 2.50 3.18 2.81 3.98	1.72 1.24 1.61 2.24 1.32 1.80 1.82 1.94	4.53 4.24 4.41 4.58 4.79 4.33 4.08 4.49	2.63 2.65 2.65 2.65 2.66 2.66 2.66 2.69 2.71	1.26 1.30 1.20 1.30 1.18 1.27 1.24 1.21	13.43 14.43 12.15 14.32 12.21 13.66 13.11 12.62
UA 2/162	1.22	0.86	4.29	3.13	2.39	3.27	2.10	4.52	2.72	1.25	13.27
UA 2/114	1.95	0.75	3.70	3.39	2.94	3.13	1.93	4.64	2.80	1.17	11.62
0A 2/204	1.02	0.00	Medi	um plast	icitv	5.07	2.14	4.00	2.01	1.50	10.12
UA 2/123 UA 2/192 UA 2/184 UA 2/109 UA 2/106 UA 2/131 UA 2/166 UA 2/143 UA 2/118 UA 2/170	2.72 2.05 1.07 1.86 2.13 1.97 1.20 1.84 1.93 1.71	1.06 1.23 1.03 0.86 1.30 0.67 1.23 0.96 0.82 0.94	3.55 3.59 3.68 3.48 4.01 3.15 3.64 3.64 3.32 3.70	3.42 2.88 2.93 3.94 3.96 3.75 2.98 3.44 3.04 3.28	3.03 2.77 3.00 2.81 3.31 2.99 2.95 2.67 2.84 3.18	2.16 2.86 3.56 2.59 2.37 2.15 2.44 2.74 3.10 2.89	1.59 1.73 2.22 2.16 2.21 2.16 2.30 1.81 1.64 1.76	4.03 4.10 3.38 3.62 3.96 3.94 4.21 3.95 4.12 3.76	2.69 2.65 2.61 2.67 2.91 2.60 2.62 2.63 2.60 2.65	0.89 0.91 0.94 0.95 0.97 0.99 1.00 1.01 1.01	6.91 6.97 7.74 7.67 7.83 8.08 8.35 8.45 8.45 8.70 8.67
UA 2/177	1.71	0.95	3.99	2.64	3.23	2.66	1.98	3.98	2.64	1.01	8.78
UA 2/210 UA 2/130 UA 2/187 UA 2/209 UA 2/115	1.50 1.50 2.13 1.82 1.98	0.63 0.84 0.75 0.60 0.53	3.52 3.45 3.28 3.35 4.20	2.05 3.54 2.92 2.90 3.44	3.23 2.94 2.85 2.96 2.74	3.96 1.98 3.21 3.52 3.25	1.76 2.25 1.42 1.75 1.71	3.63 4.29 4.51 4.40 3.61	2.61 2.60 2.64 2.66 2.68	1.04 1.06 1.08 1.12 1.12	9.47 9.71 10.05 10.76 10.79
			Lov	v plastic	ity		=				
UA 2/110	3.30	0.94	3.13	3.25	1.90	3.41	1.38	3.68	2.62	0.72	5.01
Mean Environment index (I _i) LCD _{0,05} σ	1.6 -0.97 0.08 -	0.9 -1.69 0.05 -	3.5 1.00 0.08 -	3.1 0.52 0.08 -	2.8 0.24 0.07 –	2.7 0.15 0.11 -	1.8 -0.74 0.08 -	3.8 1.29 0.11 -	2.5 _ 0.04 _	1.0 - - 0.2	8.8 - - 2.77

conditions were in Kharkiv (I $_{\rm i}=-0.74$), Odesa (I $_{\rm i}=-0.97$) and Kherson (I $_{\rm i}=-1.69$) regions.

It was found that among hybrids resistant to sulfonylurea herbicides, 50.5% of hybrids have a high yield level (2.55-2.91 t/ha). It was established that among the SU hybrids, the most productive hybrid is UA 2/106(2.91 t/ha), since in terms of yield the hybrid had an excess of 3.9% compared to standard hybrids.

Among the hybrids with a yield in the range of 2.55-2.91 t/ha, the hybrid RU 2/110 was less sensitive to growing conditions with an average yield of 2.62 t/ha, with an ecological plasticity coefficient $b_i =$ 0.72 and stability index $S_{12} = 5.01$. Medium sensitive hybrids included: UA 2/130, UA 2/131, UA 2/118, UA 2/184, UA 2/210, UA 2/166, UA 2/143, UA 2/187, UA 2/177, RU 2/192, RU 2/170, RU 2/209, RU 2/109, RU 2/115, RU 2/123, RU 2/106 with a yield of 2.60-2.69 t/ha and an ecological plasticity coefficient $b_i = 0.89-1.12$. The hybrids with the maximum manifestation of traits with the coefficient of ecological plasticity $b_{i} = 1.17 - 1.32$ were hybrids UA 2/114, ÚA 2/235, UA 2/186, UA 2/189, UA 2/136, UA 2/162, RU 2/205, RU 2/207, RU 2/117,

RU 2/206, RU 2/204 with a yield of 2.63-2.81 t/ha (Table 3).

For IMI hybrids, the most favorable conditions were noted in Cherkasy region (Shpolianskyi district) ($I_i = 1.09$), and unfavorable conditions were observed in Kharkiv ($I_i = -0.39$), Odesa ($I_i = -0.07$) and Kherson regions ($I_i = -1.82$).

The share of IMI hybrids with a yield in the range of 2.55–2.91 t/ha, was 46.2%. Among them with high plasticity ($b_i = 1.18-1.29$) were hybrids UA 1/92, UA 1/102, UA 1/94, UA 1/62, UA 1/76 with a yield of 2.61–2, 91 t/ha. And the middle plasticity was noted in hybrids UA 1/67, UA 1/66, UA 1/84, UA 1/23, UA 1/61, UA 1/59, UA 1/60, UA 1/55, UA 1/89, UA 1/101, RU 1/86, RU 1/87, RU 1/83, RU 1/100 with a yield of 2.60–2.76 t/ha (Table 4).

In addition, it was found that among the IMI hybrids, three hybrids – UA 1/67, UA 1/66, UA 1/84 with averaged in 8 locations of Ukraine yield indicators (2.76 t/ha)

Table 4

	Yield of hybrids, t/ha								Adapt paran	ability 1eters	
Number	Odesa region	Kherson region	Cherkasy region (Shpolianskyi district)	Kyiv region	Cherkasy region (Uman district)	Khmelnytskyi region	Kharkiv region	Chernihiv region	Average yield	Coefficient of ecological plasticity, b _i	Stability, S _i ²
	-		-	High r	lasticity			-		-	
UA 1/92 UA 1/102	1.84 1.84	0.81	4.23 4.23	3.73	2.75	3.21 3.21	2.11 2.11	4.58 4.58	2.91 2.91	1.21 1.21	7.45 7.45
UA 1/94 UA 1/62 UA 1/76	0.98	0.40 0.66 0.66	4.10 4.16 4.16	3.06 3.06	2.83 2.83 2.83	3.28 3.28	1.57 1.57	4.31 4.31	2.61 2.61 2.61	1.18 1.29 1.29	8.39 8.39
				Medium	ı plasticit	y					
UA 1/67 UA 1/66 UA 1/84	2.53 2.76 1.18	0.60 0.39 0.96	3.31 3.69 4.05	3.89 4.19 3.11	2.53 2.51 2.91	2.89 2.83 2.75	2.21 2.28 3.24	4.13 3.40 3.85	2.76 2.76 2.76	0.97 0.97 1.01	5.35 5.63 5.66
UA 1/23 UA 1/61 UA 1/59	1.84 1.46 1.71	0.63	4.04 3.73 3.38	3.88 3.50 3.97	2.71 3.44 2.69	2.89 3.06 2.66	2.27 2.06 2.64	3.74 3.74 3.65	2.75 2.72 2.72 2.72	1.11 1.06 0.89	6.53 6.17 4.32
UA 1/55 UA 1/89	1.74	0.76	3.74 4.56	3.89	3.22	3.03 2.50	1.59	3.72 3.73 4.79	2.71	1.10	6.43 5.58
UA 1/86 UA 1/87	1.71 1.50	0.95	3.99 3.52	2.69	3.23	2.50 2.66 3.96	1.98	3.98 3.63	2.60	0.96	4.89 6.21
UA 1/83 UA 1/100	1.34 1.34	0.40	3.33 3.33 3.61	3.35 3.35 3.31	2.62	3.18 3.18 2.82	2.49	4.13 4.13	2.60	1.13 1.13	7.15 7.15
Environment index	-1.07	-1.82	1.09	0.79	0.11	0.31 0.09	-0.39	0.98	0.03	-	_ _ _
σ	_	_	_	_	_	_	_	_	_	0.17	_

Yield and adaptability of IMI hybrids

are at a yield level with the standard 'NK Neoma'. And the hybrids UA 1/92 and UA 1/102 with an average yield of 2.91 t/ha correspond to the yield level of 'ES Genesis' standard.

The study was carried out in the Department of Genetic Engineering of Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine in the framework of scientific projects III-1-15 «Study of physiological, biochemical and molecular biological features of the functioning and inheritance of heterological genes in plant systems» and III-1-20 «Targeted changes in the genome and pleiotropic effects in genetically transformed plant systems» during 2016–2020.

Conclusions

As a result of the accelerated system of maternal and paternal lines selection, material resistant to herbicides and sunflower broomrape was selected; on its basis sunflower F_1 hybrids were created.

As a result of ecological tests conducted in Kyiv, Chernihiv, Cherkasy (Uman and Shpola districts), Khmelnytskyi, Kharkiv, Kherson and Odesa regions, the yield of the obtained sunflower hybrids was studied. Based on the findings, it was revealed that among hybrids resistant to sulfonylurea herbicides, the highyielding hybrid was UA 2/106, which showed a 3.9% increase in yield compared to 'SY Sumiko' and 'P64LE25' standard hybrids. Among hybrids resistant to imidazoline herbicides, the yields at the level of the 'NK Neoma' standard were hybrids UA 1/67, UA 1/66, UA 1/84, with a yield of 2.76 t/ha. Yield at the level of the hybrid standard 'ES Genesis' (2.91 t/ha) was for hybrids UA 1/92, UA 1/102.

Thus, it was determined that with the use of an accelerated system of source material selection, it is possible to create high-yielding sunflower hybrids in a short period of time (4 years).

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Бабич В. О.^{1,2*}, Боровська І. Ю.², Шарипіна Я. Ю.², Парій Я. Ф.², Симоненко Ю. В.^{1,2} Адаптивність гібридів F₁ соняшника, створених за комплексною системою добору ліній з господарсько-цінними ознаками, у різних агрокліматичних зонах. *Plant Varieties Studying and Protection*. 2021. Т. 17, № 4. С. 290–304. https://doi.org/10.21498/2518-1017.17.4.2021.249004

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Мета. Визначити екологічну пластичність та урожайність гібридів F_1 соняшника, створених на основі материнських та батьківських ліній, що були відібрані за прискореною системою добору ліній, стійких до гербіцидів (імідазолінової та сульфонілсечовинної груп) та вовчка соняшникового (*Orobanche cumana* Wallr.). Методи. Статистичний аналіз гібридів F_1 соняшника проведено за допомогою методів варіаційної статистики, регресійного та дисперсійного аналізу за використання пакету прикладних програм Microsoft Office Excel 2016. Для прискореної системи добору ліній використовували молекулярно-біологічні, біотехнологічні та класичні методи селекції. Так, з метою цілеспрямованого відбору закріплювачів стерильності соняшника нами було використано молекулярний SCAR-маркер HRG01 для ідентифікації гену відновлення фертильності пилку (Rf_1). Для прискореного створення батьківських ліній, стійких до трибенурон-метилу, нами використано культуру незрілих зародків. **Результати.** Наведено результати тестування гібридів F_1 соняшника у Київській, Чернігівській, Черкаській (Уманський та Шполянський р-н), Хмельницькій, Харківській, Херсонській та Одеській областях. Гібриди створено на основі відібраних ліній, добір яких проводили за прискореною системою добору ліній, стійких до гербіцидів [імідазолінової (ІМІ-гібриди) та сульфонілсечовинної (SU-гібриди) груп] і до вовчка соняшникового. Стандартами, з якими проводили порівняння гібридів, виступали: для IMI-гібридів – гібриди 'NK Neoma' (Syngenta) та 'ES Genesis' (Euralis), а для SU-гібридів – 'SY Sumiko' (Syngenta) та 'P64LE25' (Pioneer). В результаті встановлено, що серед SU-гібридів UA 2/106 мав більшу на 3,9% урожайність у порівнянні зі стандартами ('SY Sumiko' та 'P64LE25'). А для IMI-гібридів встановлено, що гібриди UA 1/67, UA 1/66, UA 1/84 мають таку ж врожайність у 2,76 т/га, що й стандарт 'NK Neoma'. IMI-гібриди UA 1/92, UA 1/102 мають таку ж врожайність у 2,91 т/га, що й стандарт 'ES Genesis'. Висновки. Завдяки прискореній системі добору ліній соняшника було відібрано вихідний селекційний матеріал, на основі якого було створено гібриди F₁. Гібриди аналізували за показником урожайності. Найурожайнішими серед протестованих SU-гібридів був гібрид UA 2/106, серед IMI-гібридів – UA 1/67, UA 1/66, UA 1/84, UA 1/92 та UA 1/102.

Ключові слова: Helianthus annuus L.; гібрид; урожайність; випробування.

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