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ANALYSIS OF DNA SINGLE-NUCLEOTIDE POLYMORPHISM IN MAIZE INBREDS WITH DIFFERENT DEGREES OF SENSITIVITY TO HEAD SMUT (*SPORISORIUM REILIANUM*)

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Aim. Single-nucleotide polymorphism analysis of maize inbreds with different rate of sensitivity to the head smut caused by *Sporisorium reilianum*, assessment of genetic relationships between them, and search for statistically significant associations between SNP markers and phenotypic manifestation of pathogen resistance. **Methods.** The single-nucleotide polymorphism analysis using BDI-III panel with 384 SNP markers, statistical methods. **Results.** The SNP analysis of seven maize inbreds with different sensitivity to *S. reilianum* infection was carried out. The key indicators of single-nucleotide polymorphism markers with BDI-III panel were assessed: the fraction of dimorphic markers was 85.8 %, the average minor allele frequency was 0.2984, the fraction of dimorphic markers with the minor allele frequency >0.2 was 68.2 %, the average polymorphism information content (PIC) was 0.2770. The main indicators of the maize inbreds were determined by SNP markers. It was established that the average homozygosity of the inbreds was 98.5 %, the average gene diversity index was 0.1723, and the average genetic distance was 0.4975. It was found the allelic state of SNP markers of the BDI-III panel — BDI-III-270C and BDI-III-292A, which is characteristic of maize inbreds resistant to head smut, as well as BDI-III-270A and BDI-III-292G for maize inbreds sensitive to head smut. The dendrogram of genetic relationships for the studied maize inbreds was drawn. **Conclusions.** The presence of statistically significant associations between the allelic state of two SNP markers and the phenotypic manifestation of the maize resistance to head smut was established. The random nature of the location of maize inbreds sensitive to head smut on the dendrogram of genetic relationships was revealed. In the future, the identified single-nucleotide polymorphism markers, which were associated with head smut resistance, will be verified on a larger number of maize genotypes of different origin to confirm the possibility of their practical application.

Keywords. *Zea mays* L., head smut, SNP analysis, molecular markers, plant diseases.

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INTRODUCTION

Head smut of maize, which is caused by the biotrophic fungus *Sporisorium reilianum*, results in significant losses of maize grain worldwide, including in Ukraine. Head smut infestation rate in maize genotypes can exceed 70 % (Márquez-Licona G et al., 2021). Annual maize yield loss due to *S. reilianum* averages about 6 %, but can reach 50 % or more (Zhang B et al., 2021). The pathogen infects maize seedlings through the root or mesocotyl, grows through the coleoptile, and reaches the aboveground organs. The pathogen does not kill maize plants, but when it reaches female and male inflorescences, the fungus forms massive fungal sori, turning the tassels into a black powder spore mass, and the ears — into a black cone-shaped galls consisting of fungal fibres and spores. *S. reilianum* spores are spread by dissemination in the soil, on ears and grains. The spores remain viable in soil for several years (Zuo W et al., 2015, Zhang N et al., 2017, Zhao Y et al., 2022, Zhou Y et al., 2022, Zhang Q et al., 2024).

The *S. reilianum* fungus has a genome of 18.7 Mb, containing 23 chromosomes and 467 genes. The functions of 256 proteins of this pathogen are known, in particular, 26 proteins are enzymes, destroying the cellular wall of plants (Zhang B et al., 2021). Eleven pathogen genes encode enzymes hemicellulases (Mercado-Flores Y et al., 2022). Enzyme activity, hormone content and protein expression in maize plants changed in response to the head smut infection (Dittiger LD et al., 2023, Wang Y et al., 2024).

The maize genotypes resistant to head smut are the key to successful breeding process and sustainable yields. In the field, the plant resistance to head smut is assessed at full maturity stage, at harvest, in case of presence of maize tassels and/or ears infected by the pathogen. Sensitivity of maize genotypes to head smut at any stage of plant development can be determined using DNA technologies, particularly molecular genetic markers. The resistance of maize genotypes to *S. reilianum* is a quantitative trait that is inherited stably and controlled by several genes (Zhou Y et al., 2022). Some loci and genes associated with maize resistance to head smut are known. X.W. Lu and J.B. Brewbaker identified quantitative trait loci (QTL), defining maize resistance to head smut and their associated SSR markers on four chromosomes. The most influential region was found on the short arm of chromosome 1 (in bins 1.03, 1.04, and 1.05), which was associated with markers asg75, asg30, and umc167. The resistance locus on chromosome 3 (located in bin 3.04) was associated with marker umc50, on chromosome 9 (in bin 9.03) — phi022, on chromosome 10 (in bin 10.00) — csu25b (Lu XW, Brewbaker JB, 1999). X.H. Li et al. identified five resistance QTLs to head smut: one locus — on chromosomes 1, 3, and 8, and two loci — on chromosome 2, as well as SSR and AFLP markers that were located near the resistance loci (Li XH et al., 2008). Y.X. Li et al. found two resistance QTLs to head smut in maize: *q2.09HR* — on chromosome 2, and *q5.03HR* — on chromosome 5. SNP markers with a significant association with head smut resistance were identified in these loci: PZA00672.8 — in locus *q2.09HR*, and PZE-105072717 — in locus *q5.03HR*, alleles of which (G and A, respectively) ensured the head smut resistance (Li YX, 2015). N. Qiu et al. found two QTLs associated with head smut resistance: locus *qHS1* (located within markers bnlg1014 — umc2224 on chromosome 1) and locus *qHS6* (located within markers umc1006 — umc1857 on chromosome 6) (Qiu H et al., 2021). X. Zhao et al. determined SSR markers flanking locus *qHSR1*: umc1947, umc2202, umc1516, umc1551, umc1525, 2S8, STS661, STS1944, phi101049 and umc2214 (Zhao X et al., 2012).

Based on the maize inbred genotype Mo17, J. Weng et al. determined the haplotype of head smut resistance using SNP markers: PZE-102187486 — C, PZE-102187611 — A, PZA00672.8 — G, PZE-102188421 — A. These markers were located in *qHS2.09* locus (in bin 2.09). The authors also identified two head smut resistance loci *qHS1.03* and *qHS4.03* on chromosomes 1 and 4, associated with SNP markers PZE-101045549 and SYNGENTA6546, respectively (Weng J et al., 2012).

The most investigated head smut resistance gene is *ZmWAK* (*Zea mays WAK*). It is a receptor kinase gene containing a cytoplasmic serine/threonine kinase domain, a calcium-binding epidermal growth factor domain, and an extracellular galacturonan-binding domain. The expression of gene *ZmWAK* depends on the presence/absence of head smut infection. After infection with *S. reilianum*, due to active expression of the *ZmWAK* gene in the infected cells of resistant maize genotypes, apoptosis (cytoplasmic compression, chromatin condensation, nucleus fragmentation and vesicle detachment from the cell membrane) is observed, which allows isolating hyphae in dead cells. Autophagy is observed in the case of infection of sensitive genotypes with a missing functional gene fragment, which cannot prevent the spread of pathogen hyphae throughout a maize plant (Zhang N et al., 2017). In addition, the kinase activity of gene *ZmWAK* allows interaction with *ZmSnRK1α2*, a conserved subunit of α-kinase not related to sucrose fermentation, and its phosphorylation. In turn, the activated *ZmSnRK1α2* phosphorylates and destabilises the transcription factor *ZmWRKY53*, the reduced amount of which leads to downregulation of genes involved in transmembrane transport and carbohydrate metabolism, which results in nutrient starvation for *S. reilianum* in the apoplast (Zhang Q et al., 2024). *ZmWAK* can inhibit the pathogen penetration into maize plants by binding pathogen-secreted proteins (Zhang B et al., 2021). Gene *ZmWAK* is actively expressed in mesocotyls, which are the main region for protection against head smut (Zhang N et al., 2017). Gene *ZmWAK* is located at the *qHSR1* locus on the long arm of chromosome 2 in bin 2.09. Its presence reduces the infection of maize plants by *S. reilianum* by 25 %. The size of *qHSR1* is 152 kb (Zuo W et al., 2015). This locus is restricted by markers SSR148154 and STS661, and closely related to eight SSR markers: umc2214, umc2077, umc2184, phi427434, umc1207, bnlg1893, umc1736, bnlg1520 (Chen Y et al., 2008).

Also, M. Wang and co-authors identified candidate genes associated with head smut resistance. Researchers investigated resistance to *S. reilianum* in maize using Illumina MaizeSNP50 panel. The full genome-associated study using 45,868 SNP markers and 144 maize inbreds determined 19 associations between SNP markers and head smut resistance, and 18 candidate genes related to resistance to pathogen, which covered each of 10 chromosomes (Wang M et al., 2012). Y. Zhou et al. noted 2 candidate genes of head smut resistance in maize (*ZmABP2* and *Zm00001D006403*) in bin 2.07 of chromosome 2. Gene *ZmABP2* is responsible for actin polymerization and depolymerization in response to intracellular and extracellular signals, and in case of head smut infection it controls the determination of tassel meristem development. After infecting with the pathogen, gene *Zm00001D006403* controls the dysorganoplasia of a tassel (Zhou Y et al., 2022).

At the same time, limited data on the practical use of molecular genetic markers associated with maize resistance to head smut, as well as on the clearly defined allelic state of resistance/sensitivity markers to the pathogen, do not allow us to actively involve the available scientific developments in the maize breeding process. Therefore, the relevant field of current research, along with the identification of genes and loci for maize head smut resistance, is the search for and development of new molecular genetic markers associated with resistance to the pathogen, and determination of their allelic state associated with the phenotypic manifestation of resistance traits. Our work was aimed at analysing single-nucleotide polymorphism in maize inbreds with different sensitivity rates to head smut caused by *S. reilianum*, assessing genetic relationships between them and searching for statistically significant associations between SNP markers and phenotypic manifestation of resistance to the pathogen.

MATERIALS AND METHODS

Seven maize inbreds (*Zea mays* L.) with different resistance rates to the pathogen were used as study material. The group of sensitive inbreds was represented by P354 and DK3023 inbreds, the head smut infection rate of which exceeded 20 % against an artificially infectious background. The group of resistant inbreds included DK267, DK315, DK3044, PLS61 and Mo17 with infection rate not exceeding 5 % on the artificial infection background (Denysiuk KV et al., 2024).

Panel BDI-III with 384 SNP markers was used in genotyping the mentioned maize inbreds. This panel was developed by BioDiagnostics, Inc. (USA) based on Illumina VeraCode Bead Plate. In this panel, the markers are biallelic and located on all 10 chromosomes, and have a designability rank score >0.6 (Venkatramana P et al., 2010). SNP genotyping of maize inbreds was conducted using GoldenGate test and the Illumina VeraCode Reader (Fan JB et al., 2006, Venkatramana P et al., 2010) based on Bio Diagnostics, Inc. (USA).

For each sample, 10 seeds were selected using the average sampling method and germinated on filter paper at 26 °C in the dark. DNA was extracted from the 7-day-old seedlings using CTAB method (Murray MG, Thompson WF, 1980). The extracted DNA was applied to the Sentrix Array Matrix (SAM). SNP-genotyping of DNA samples fixed on SAM was conducted with GoldenGate test in completely automated mode on Illumina BeadStation 500G System equipped with BeadXpress Reader for fluorescence detection. The fluorescence results were analysed using Illumina BeadStation GenomeStudio Software. SNP genotyping and fluorescence signal determination for each inbred was performed in 30-fold repetition for each marker. Fluorescence detection with value less than 0.25 was considered as no signal.

The associations between the molecular genetic polymorphism and the phenotypic manifestation of maize genotypes resistance to head smut were determined by the χ^2 method (Ewens WJ, Brumberg K, 2023). The disequilibrium in allele frequencies in two groups of inbreds with alternative pathogen resistance was determined. For this reason, the difference (D) between the major allele frequency in the initial group of sensitive inbreds and the same allele frequency in the derivative group of resistant inbreds was determined. Then, SNP markers were ranked from the highest to the lowest according to the D values. The reliability of the disequilibrium ratio of allele frequencies by markers for two groups of inbreds was confirmed by the Fisher's exact test at the significance rate of 0.05 (Ewens WJ, Brumberg K, 2023). The UPGMA dendrogram of genetic SNP relations was built with using the software TASSEL 3.

RESULTS

The genotyping by single-nucleotide polymorphism markers for two groups of maize inbreds with different head smut resistance allowed to identify nucleotides at 5,376 marker sites. From further analysis, 82 markers with a missing data frequency of >20 % were excluded. For the seven studied inbreds, the share of monomorphic markers studied was 14.2 %, and dimorphic markers — 85.8 %, respectively. The average minor allele frequency was 0.2984±0.0125. The minor allele frequency >0.2 was noted for the prevailing majority of dimorphic markers (68.2 %). The average polymorphism information content of the markers in the studied set of inbreds was high (PIC=0.2770) in the possible range of values 0–0.3750. The average gene diversity shift of the markers was 0.3494±0.0181 with a possible range of values of 0–0.5000. Thus, the key indicators of single-nucleotide polymorphism in the studied inbreds for the used set of SNP markers were within permissible limits, which allowed to proceed directly to the analysis of the data.

The average homozygosity for the seven studied inbreds was 98.5 %, heterozygous SNP sites were 1.5 %, which were registered only in head smut-resistant inbred PLS61. The homozygosity of the inbreds belonging to the resistant group was 98.0 %, and to the sensitive genotype group — 100 % (**Table 1**).

The average genetic diversity index for the two groups of genotypes did not have reliable differences, although there was a tendency to increase the index (0.1761 vs. 0.1714) in the group of inbreds sensitive to head smut. The average genetic diversity index for seven inbreds was 0.1723±0.0026.

The average genetic distance for the seven maize inbreds was at the level of 0.4975±0.0337. The genetic distance between the inbreds P354 and DK3023 of the sensitive group to head smut infection was 0.4861. The average genetic distance in the group of resistant inbreds was 0.5214. In this group, the

Table 1. The characteristics of the groups of maize inbreds with different head smut resistance according to the SNP analysis

Index		Group of sensitive inbreds to head smut infection (DK3023 and P354)	Group of resistant inbreds to head smut infection (DK267, DK315, DK3044, PLS61 and Mo17)
Number of analysed inbreds		2	5
Homozygosity, %		100	98.0
Genetic diversity index of the inbreds, fractions of a unit	average	0.1761±0.0053a	0.1714±0.0022a
	min-max	0.1734–0.1788	0.1679–0.1739
	range	0.0054±0.0401a	0.0060±0.0397a
Genetic distance between the inbreds of the same group, fractions of a unit	average	0.4861	0.5214±0.0565
	min-max	—	0.2222–0.6778
	range	—	0.4556±0.0466
Genetic distance between the inbreds of two different groups, fractions of a unit	average	0.4747±0.0456	
	min-max	0.3806–0.5972	
	range	0.2166±0.0514	

Note. When comparing the two groups, the means with the same letters show insignificant differences at the 0.05 significance level.

minimal genetic distance (0.2222) was observed between inbreds Mo17 and DK3044, and the maximal genetic distance (0.6778) — between inbreds DK315 and PLS61. The range of genetic distances for this group was 0.4556±0.0466.

The average genetic distance between the inbreds of two different groups was 0.4747 and was characterized by a tendency to decrease, compared to the average genetic distance in the group of resistant inbreds (0.5214). Between the inbreds belonging to two different resistance groups, a minimal genetic distance of 0.3806 was observed for Mo17 and DK3023, while a maximal genetic distance of 0.5972 was recorded for P354 and PLS61. The range of genetic distances for the inbreds from two different groups was 0.2166±0.0514, i.e. 2.1 times less than the range of genetic distances for the group of resistant inbreds (0.4556±0.0466).

According to the dendrogram of genetic SNP relationships, the sensitive inbreds to head smut are randomly located, do not form one common remote cluster, and are localised in the middle of separate clusters (**Fig. 1**). Some of the inbred of the different groups show a higher genetic relatedness to each other than to the inbred of their own group. For instance, a sensitive inbred P354 formed one cluster with the resistant inbred DK267, and the sensitive inbred DK3023 formed a cluster with resistant inbreds DK3044 and Mo17, i.e. the inbreds of resistant and sensitive groups had no significant genetic differences.

The disequilibrium in the major allele frequency in the initial group of sensitive inbreds was detected in 241 markers (79.8 %), including 0.5 and higher disequilibrium in 92 markers (30.5 %), compared to the major allele frequency in the derived group of resistant inbreds. So, there is a certain statistical relationship between the allelic state of individual SNP markers of the BDI-III panel and the phenotypic manifestation of maize resistance to head smut.

The most specific nineteen SNP markers associated with pathogen resistance, which were characterised by the highest values of the D index (0.8 and above), are presented in **Table 2**. Statistically significant associations determined using Fisher's exact probability test, which met the significance level of 0.05, were observed for two markers — BDI-III-270 and BDI-III-292. The BDI-III-270 marker is located on chromosome 7, and the BDI-III-292 marker — on chromosome 8.

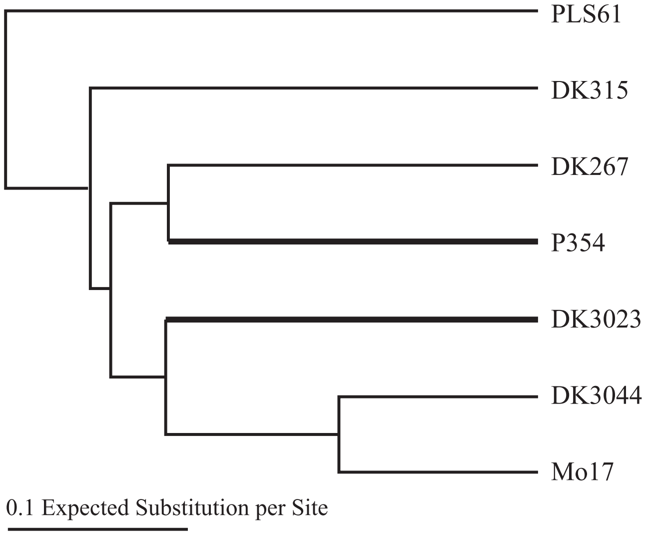

Fig. 1. The dendrogram of genetic SNP relationships built for the seven maize inbreds by the UPGMA method. The sensitive inbreds to head smut are marked with bold lines, the rest are resistant inbreds

Therefore, the following allelic state of SNP markers of the BDI-III panel is characteristic of maize inbreds resistant to head smut infection — BDI-III-270C and BDI-III-292A, whereas for the sensitive inbreds to head smut infection, the following allelic state is characteristic — BDI-III-270A and BDI-III-292G.

DISCUSSION

While assessing the head smut resistance of maize inbreds by SNP markers and looking for associations between the markers and phenotypic manifestation of the trait, it is important to establish whether the inbreds are distinguished by their resistance to the pathogen among the general pool of genotypes or whether such inbreds differ from sensitive genotypes only by certain markers, being related to them no less than to each other. The dendrogram of genetic relationships of the seven compared maize inbreds showed that the sensitive genotypes to the disease did not form a common cluster remote from the cluster of resistant inbreds. Thus, despite head smut resistance, the analysed inbreds were genotypically quite related. This was also confirmed by the average genetic diversity index (0.1723) determined with Illumina BDI-III panel of SNP markers in the studied maize inbreds, and this index was slightly lower for the group of resistant inbreds (0.1714) than for the group of sensitive inbreds (0.1761). A similar genetic diversity index was observed in maize inbreds grown in the

Table 2. The frequencies of SNP alleles in the groups of maize inbreds with different sensitivity to head smut

SNP marker*		Group of sensitive inbreds	Group of resistant inbreds	D	*p*
Number, alternative alleles	Number of chromosome	Major allele frequency	Major allele frequency in the initial group		
1	2	3	4	5	6
270AC	**7.03**	**A=1.0**	**A=0.0**	**1.0**	**0.0476**
292AG	**8.02**	**G=1.0**	**G=0.0**	**1.0**	**0.0476**
65AT	2.03	T=1.0	T=0.0	1.0	0.0667
51AC	2.01	C=1.0	C=0.2	0.8	0.1429
81AG	2.06	G=1.0	G=0.2	0.8	0.1429
82AG	2.06	G=1.0	G=0.2	0.8	0.1429
86AG	2.07	A=1.0	A=0.2	0.8	0.1429
169AG	4.09	A=1.0	A=0.2	0.8	0.1429
202AC	5.03	A=1.0	A=0.2	0.8	0.1429
203AC	5.03	A=1.0	A=0.2	0.8	0.1429
210AG	5.04	A=1.0	A=0.2	0.8	0.1429
370GC	7.09	G=1.0	G=0.2	0.8	0.1429
291AC	8.02	A=1.0	A=0.2	0.8	0.1429
299AC	8.04	C=1.0	C=0.2	0.8	0.1429
300AG	8.04	A=1.0	A=0.2	0.8	0.1429
313AG	8.08	A=1.0	A=0.2	0.8	0.1429
315AG	8.08	A=1.0	A=0.2	0.8	0.1429
319AG	9.01	A=1.0	A=0.2	0.8	0.1429
320GC	9.01	C=1.0	C=0.2	0.8	0.1429
Average	—	1.0	0.2	0.8	0.1288

Note. * The number of a SNP marker of the BDI-III panel is presented; the names of deoxyribonucleotides are given by the abbreviated names of nitrogenous bases: A — adenine; T — thymine; G — guanine; C — cytosine; D — the difference between the major allele frequency for each SNP marker in the initial group of inbreds and the same allele frequency of the same marker in the derived group; *p* — reliability according to Fisher's exact test.

Northern Steppe of Ukraine — 0.1798 and 0.1776 (Denysiuk KV et al., 2024), and in the European genotypes — 0.177 (Arca M et al., 2023), but it was lower than in genotypes from Central America and Mexico — 0.219 (Arca M et al., 2023).

The determination of genotypic characteristics of maize inbreds resistant to head smut and the possibility for their rapid testing are relevant for a successful breeding process. The majority of newly developed high-yielding maize inbreds and hybrids are resistant to *S. reilianum*. However, there are also sensitive genotypes to this infection, which are undesirable for breeding programmes due to the high risk of yield loss and contamination of seeds and soil with *S. reilianum* spores. We identified two SNP markers statistically associated with head smut resistance that can be used for testing maize genotypes to predict their head smut infection.

The head smut resistance of maize is a polygenic trait with complicated mechanisms of protecting from the infection and counteracting the pathogen spreading. The genes and loci associated with head smut resistance were also found on different chromosomes. Our study identified two single-nucleotide polymorphism markers located in non-gene regions on chromosome 7 in bin 7.03 and chromosome 8 in bin 8.02, which are statistically closely related with the resistance to *S. reilianum*. M. Wang et al. found candidate gene *GRMZM2G465226* on chromosome 7 in bin 7.01; it encoded the protein associated with the pathogenesis of head smut. Scientists determined several candidate genes on chromosome 8: *GRMZM2G047152* in bin 8.03, *GRMZM2G075000*, *GRMZM2G164341* and *GRMZM2G017603* in bin 8.05. Genes *GRMZM2G047152* (8.03) and *GRMZM2G017603* (8.05) regulated NB-ARC — the central nucleotide-binding domain, which is characteristic of most plant disease resistance proteins; gene *GRMZM2G075000* (8.05) was responsible for the cross-linked actin; gene *GRMZM2G164341* (8.05) encoded an antifreeze protein (Wang M et al., 2012). X.H. Li et al. identified a maize head smut resistance locus in bin 8.02 and the associated AFLP marker P5055140 (Li XH et al., 2008). The SNP marker BDI-III-292 identified in the same bin 8.02 in the group of maize genotypes resistant to head smut was characterised by the allele of adenine, and in the group of sensitive genotypes it had the allele of guanine. This SNP marker identified by us and the marker found by X. H. Li et al. (2008) are probably located in the same *S. reilianum* resistance locus. We found another SNP marker BDI-III-291 of head smut resistance in bin 8.02 with a difference of 0.8 between the major allele frequency in the initial group of sensitive inbreds and the same allele frequency in the derived group of resistant inbreds, but its disequilibrium was reliable at a significance level higher than 0.05. Further extension of the sampling of the investigated inbreds may allow for extracting a higher number of single-nucleotide polymorphism markers closely associated with head smut resistance in maize.

In the study of maize resistance to head smut, it was found that on chromosome 2 in bin 2.07 there are *ZmABP2* and *Zm00001D006403* genes (Zhou Y et al. 2022), in bin 2. 08 — *GRMZM2G140231* gene (Wang M et al. 2012), in bin 2.09 — *ZmWAK* (Zhang N et al. 2017), *ZmNL* (Di H et al. 2015), *GRMZM2G166566* genes (Wang M et al., 2012), which play an important role in head smut resistance in maize. There is also a known locus of head smut resistance located on chromosome 2 in bin 2.09 — *qHSR1* (Chen Y et al., 2008, Zhao X et al., 2012, Zuo W et al., 2015), known as *qHS2.09* in the authors' papers (Weng J et al., 2012, Di H et al., 2015, Li Y et al., 2015). Our study was unable to identify SNP markers that were closely associated with head smut resistance with reliability of 95 % or higher and were located in bin 2.09. At the same time, the single-nucleotide polymorphism markers BDI-III-51, BDI-III-65, BDI-III-81, BDI-III-82 and BDI-III-86 of chromosome 2 (in bins 2.01, 2.03, 2.06, 2.06, 2.07, respectively) demonstrated a rather high (0.8) disequilibrium of allele frequencies in the different groups of head smut resistance of maize inbreds. The difference between the two studied groups is significant with a reliability of 93.3 % for marker BDI-III-65 (in bin 2.03) and 85.7 % for other markers found by us (in bins 2.01, 2.06, 2.07) of chromosome 2, which is not enough to recommend these markers for testing maize breeding material for resistance to *S. reilianum*.

Literature data on the alleles of molecular genetic markers that determine the sensitivity of genotypes to head smut infection are limited. At the same time, the work on the identification of alleles of SNP markers associated with head smut resistance, which would allow for rapid and accurate testing of breeding material at any development stage of maize and serve as the basis for MAS selection, is relevant. We determined two single-nucleotide polymorphism markers of BDI-III panel– BDI-III-270 and BDI-III-292, which had 95.2 % reliability and can be used for testing maize genotypes for head smut resistance. Alleles BDI-III-270C and BDI-III-292A are characteristic of resistant maize inbreds, while BDI-III-270A and BDI-III-292G alleles are characteristic of sensitive maize inbreds. J. Weng et al. identified alleles for five SNP markers of the MaizeSNP50 BeadChip panel that are characteristic of maize genotypes resistant to head smut: PZE-102187486 — C, PZE-102187611 — A, PZE-102187687 — A, PZA00672.8 — G, PZE-102188421 — A, while the following alleles are characteristic of sensitive genotypes: PZE-102187486 — A, PZE-102187611 — G, PZE-102187687 — C, PZA00672.8 — A, PZE-102188421 — G. The most significantly associated marker was PZE-102187611 (P=1.88E-10) and explained 39.7 to 44.4 % of the phenotypic variance in the 80 inbreds panel (Weng J et al., 2012). Y. Li et al. identified five SNP markers of the panel MaizeSNP50 BeadChip, the alleles PZA00672.8, PZE-102187385, PZE-102187403, PZE-102188421, and PZE-105072717 of which differ in the maize inbreds according to their head smut resistance. The resistant maize inbreds are characterised by alleles G, C, G, A and A, respectively, and the sensitive ones by A, A, A, G and G, respectively. The most significantly associated with head smut resistance were the markers PZA00672.8 on chromosome 2 (P=5.32E-07) and PZE-105072717 on chromosome 5 (P=7.40E-08), which explained 9.9 % of the phenotypic variance (Li Y et al., 2015). H. Di et al. determined the alleles of six dCAPS markers LSdSCAP1, LSdSCAP2, LSdSCAP3, LSdSCAP4, PZE-102188421 (LSdSCAP7-1), PZE-102188421 (LSdSCAP7-2), which are specific for resistant maize inbreds to head smut infection (99, 93, 136, 100, 111 and 99 bp, respectively), alleles characteristic of sensitive inbreds to infection (81, 73, 122, 119, 89 and 130 bp, respectively) (Di H et al., 2015).

CONCLUSIONS

Single-nucleotide polymorphism analysis of maize inbreds with different resistance rate to the head smut *S. reilianum* was conducted; the presence of statistically significant associations between SNP markers and phenotypic manifestation of pathogen resistance was determined. It was found that the average homozygosity for the seven studied maize inbreds was 98.5 %, the average genetic diversity index was 0.1723, the average genetic distance was 0.4975, and the average genetic distance in the group of resistant inbreds was 0.5214 and ranged from 0.2222 to 0.6778. It was found that maize inbreds sensitive to head smut were randomly located on the dendrogram of genetic relationships, did not form one common cluster, and were localised in the middle of separate clusters and showed greater relatedness to some resistant inbreds than to each other. It was determined that the allelic state of the SNP markers of the BDI-III panel — BDI-III-270C and BDI-III-292A — is characteristic of maize inbreds resistant to head smut, and the allelic state of BDI-III-270A and BDI-III-292G is characteristic of sensitive inbreds to head smut. In the future, it is planned to verify the found polymorphisms of SNP markers on a larger number of maize genotypes of different origin to confirm the possibility of their practical application.

Adherence to ethical principles. No experiments involving animals or humans were conducted.

Conflict of interests. There is no conflict of interests.

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АНАЛІЗ ОДНОНУКЛЕОТИДНОГО ПОЛІМОРФІЗМУ ДНК У ЛІНІЙ КУКУРУДЗИ ІЗ РІЗНИМ СТУПЕНЕМ ЧУТЛИВОСТІ ДО ЛЕТЮЧОЇ САЖКИ *SPORISORIUM REILIANUM*

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Мета. Аналіз однонуклеотидного поліморфізму ДНК у ліній кукурудзи із різним ступенем чутливості до летючої сажки *Sporisorium reilianum*, оцінка генетичних взаємовідносин між ними та пошук статистично значущих асоціацій між SNP-маркерами та фенотиповим проявом стійкості до патогену. **Методи.** Метод однонуклеотидного поліморфізму ДНК з використанням панелі BDI-III з 384 SNP маркерами, статистичні методи. **Результати.** Проведено SNP-аналіз семи ліній кукурудзи із різною чутливістю до ураження летючою сажкою *S. reilianum*. Оцінено ключові показники маркерів однонуклеотидного поліморфізму ДНК панелі BDI-III: частка диморфних маркерів склала 85,8 %, середня частота мінорного алеля — 0,2984, частка диморфних маркерів з частотою мінорного алеля >0,2 — 68,2 %, середній індекс інформативності маркерів (PIC) — 0,2770. Визначено основні показники ліній кукурудзи за SNP-маркерами. Встановлено, що середня гомозиготність ліній становила 98,5 %, середнє значення показника генного різноманіття ліній — 0,1723, середня генетична дистанція — 0,4975. Визначено алельний стан SNP-маркерів панелі BDI-III, характерний для стійких до ураження летючою сажкою ліній кукурудзи: BDI-III-270Ц і BDI-III-292A, а також для нестійких до ураження летючою сажкою ліній: BDI-III-270A і BDI-III-292Г. Побудовано дендрограму генетичних взаємовідносин для досліджених ліній кукурудзи. **Висновки.** Встановлено наявність статистично значущих асоціацій між алельним станом двох SNP-маркерів і фенотиповим проявом ознаки стійкості до летючої сажки у кукурудзи. Виявлений рандомний характер розташування нестійких до летючої сажки лінії кукурудзи на дендрограмі генетичних взаємовідносин. Визначені маркери однонуклеотидного поліморфізму ДНК, пов’язані зі стійкістю до летючої сажки, у подальшому будуть верифіковані на більшій кількості генотипів кукурудзи різного походження для підтвердження можливості їхнього практичного застосування.

Ключові слова. *Zea mays* L., летюча сажка, SNP-аналіз, молекулярні маркери, хвороби рослин.