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THE ESTIMATION OF HEAT AND DROUGHT TOLERANCE MAIZE LINES USING DNA MARKERS

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ABSTRACT

The main factor which causes to decrease maize grain yield is drought. In most regions where maize is grown, the water stress during the growing period is caused by both lack of soil moisture and high air temperature. The purposes of our study were the estimation and selection of maize lines for drought and heat tolerance based on DNA markers and determination of the correlation between CAPS markers and plant ability to resist the water stress. As the result of study, the significant differences were found between leaf temperature of maize lines which contained favorable alleles by both CAPS markers in 2018 and 2019 (35.72 and 34.41ºC respectively), $LSD_{0.05}$=1.27. The leaf temperature of maize lines which had SNP (A) by dhn C397 (36.95ºC) differenced significantly with lines contained favorable allele by rspC1090 or lines with no favorable alleles in 2018 (33.68 and 34.35ºC respectively). Based on analysis by seeds germinating in sucrose solution the significant differences were observed between the amount of sprouted seeds in lines contained SNP(G) by rspC1090 and lines without any favorable allele (4% and 2.25% respectively), $LSD_{0.05}$= 1.70. As the result of correlation analysis, the positive correlation was determined between SNP(A) by dhnC397 marker in maize lines and leaf temperature in 2018-2019 (r=0.16). The positive correlation was observed between SNP(G)byrspC1090 and the percent of sprouted seeds in sucrose solution (r=0.31). Thus, for complex estimation and maize line selection for drought and heat tolerance it could be recommended to use two CAPS markers dhnC397 and rspC1090.

Keywords: *drought, high temperature, CAPS markers, correlation, maize.*

INTRODUCTION

The maize figures prominently among cereal crops. The need to grow this crop in drought regions is determined by its high productivity in comparison with other crops, as well as a wide range of uses (Panfilova *et al.*, 2020). Therefore, for current breeding, great importance is given to the creation of breeding material and its selection for new maize hybrids. The one of the main stress factors which limits maize yield is drought. In most regions where maize is grown, water stress during the growing season occurs unpredictably. Soil drought is caused by a prolonged absence of rain combined with high air temperature and solar insolation, increased water evaporation from the soil and plants dehydration through transpiration. Atmospheric drought is characterized by high temperature and relatively low humidity (10-20%). Significant atmospheric drought occurs due to the movement of air currents – hot dry winds. It contributes to the disruption of the dynamics of the water flow from the soil into the above-ground organs of plant and its loss by the plant by dramatically increasing the water evaporation from the soil and transpiration (Prodan and Zatula, 2009). Usually, atmospheric and soil droughts occur at the same time. The atmospheric droughts itself often arises in the spring, when the soil is still saturated with water after the snow melts. Soil drought is often observed in the middle or end of summer, when winter water reserves have already been used, and summer precipitation was insufficient.

The selection of drought tolerant maize breeding materials is carried out mainly based on the assessment of yield, changes in the physiological state of plants, assessment of the state of vegetative and generative organs of the plant in the field (Chen *et al.*, 2012; Massino *et al.*, 2013; Krivosheev *et al.*, 2016; Maazou *et al.*, 2016; Satarova *et al.*, 2016; Effendi *et al.*, 2019). The possibilities of molecular biology and plant genetics allow the use of knowledge about the structure of the genome and the genes expression to select drought-tolerant maize lines. Based on Hao *et al.* (2011) studies Liu *et al.* (2015) developed two functional CAPS (Cleaved Amplified Polymorphic Sequences) markers to identify allelic states of *dhn1* and *rsp41* genes associated with drought resistance of maize. In our previous studies (Prysiazhniuk *et al.*, 2018), the assay maize lines by DNA markers to *dhn1* and *rsp41* genes was carried out and it was identified lines with various combinations of favorable alleles. To assess the efficiency of DNA markers selection Hao *et al.* (2011a) proposed criteria for selecting drought tolerant lines based on six indicators: yield, ears per plant, kernel number per row, plant height, anthesis–silking interval, leaf curling.

However, despite the fact that the positive correlation between drought tolerance and the seeds ability to germinate in osmotic solutions (sucrose, mannitol, sorbitol, etc.) has been known, it is interested to determine the efficiency of the maize lines selection by DNA markers and the rapid assessment of drought resistance by germination ability seeds in osmotic solutions. Also, there are studies which confirm that the determination of leaf surface temperature is used for a quick assessment of the plant water status. The water deficit affects leaf temperature by gradually decreasing transpiration as a result of stomata closure (Kögler and Söffker, 2019). Hence, decreased transpiration leads to an increase a leaf temperature. It has been proven that there is a linear correlation between leaf temperature and transpiration (Liu *et al.*, 2011). Therefore, to evaluate maize lines in which favorable alleles were identified by CAPS markers, it is relevant to determine their ability to resist drought and heat using alternative methods for assessing plant response to water stress. The purpose of the study is to evaluate and select drought and heat tolerance maize lines based on DNA markers and to determine the correlation between CAPS markers and the ability of maize plant to resist to water stress.

MATERIAL AND METHODS

Plant material and DNA analysis

In this study 74 inbred maize lines which had been tested the presence favorable alleles by CAPS markers dhnC397 and rspC1090 to genes *dhn1* and *rsp41* respectively were investigated.

DNA extraction, PCR condition, digestion by restriction enzymes and products separation are described in our previous studies (Prysiazhniuk *et al.*, 2018).

Laboratorial experiment

The laboratorial experiment for determination of the maize seeds ability to germinate in osmotic solution was conducted in Laboratory Molecular Genetic Analysis of Ukrainian Institute for Plant Variety Examination (Kyiv, Ukraine) in 2018. For estimation maize inbred lines, the sucrose solution was used with osmotic pressure 10-12 atm (Oleynikova and Osipov, 1976). According to the van't Hoff equation, the concentration of sucrose solution is 0.5 M. It was sown 300 seeds of studied lines and 200 seeds as control (50 seeds to each Petri dish). The 5 ml of osmotic solution was added to each Petri dish for studied lines and 5 ml of water for control. Petri dishes with seeds were incubated in thermostat at 25ºC during 5 days. Then, counting percent of the germinated seed (G) was carried out: the ratio of the average number of germinated seeds in sucrose solution (Awg) to the average number of germinated seeds in the control (N):

$G = Awg/N \times 100\%.$

Field experiment

The field experiment was carried out during 2018-2019 at the experimental sites of Research Institute of Agrarian Business (Dnipro, Ukraine). The temperature of 6-7th leaf of maize lines was measured at the growth stage of development of fruit which came at the first decade of August. It was assayed 21 maize plant of each line with infrared pyrometer Benetech GM550 (China), sensitivity is 0.1ºC. The leaf temperature was measured at the hottest period of day between 1.30 pm and 2.30 pm (Liu *et al.*, 2011; Kögler and Söffker, 2019). For addition estimation of heat and drought tolerance of maize lines which had the different combination of alleles, the yield of studied lines was assayed.

Weather conditions

The weather conditions rates of Research Institute of Agrarian Business in Vesele village (Sinelnykove district, Dnipro region) were provided by Sinelnykove weather station. The air temperature and amounts of precipitation during maize growing cycle in 2018-2019 are shown in Table 1.

Table 1. The air temperature and amounts of precipitation during 2018-2019 maize growing cycle.

Month	Air temperature, ºC			Amounts of precipitation, mm		
	Daily average air temperature	2018	2019	Daily average amounts of precipitation	2018	2019
April	9.2	12.9	10.9	35.0	20.1	14.1
May	15.8	18.6	18.1	50.0	37.0	21.3
June	19.1	21.4	23.9	59.0	43.5	1.0
July	20.9	22.7	20.9	61.0	69.5	33.7
August	20.1	23.6	20.9	35.0	0.0	73.4
September	15.0	18.4	16.1	36.0	23.0	6.4

Statistical analysis

The deviation coefficient of agrometeorological indicators from daily average amounts during studied years was computed using the equation below:

$$Dc = \frac{(X_i - \bar{X})}{\sigma}$$

where *Dc* – deviation coefficient; *Xi* – indicator of current weather; *X* – daily average amounts; σ – mean-square deviation.

The rate of deviation coefficients was determined according to scale: *Dc* =0-1 – close to normal conditions; *Dc* =1-2 – strong different conditions; *Dc* > 2 – close to unique conditions (Yeremenko *et al.*, 2017).

To determine the significant differences of studied indicators the ANOVA is used. The correlation is computed by Spearmen correlation coefficients analysis

RESULTS AND DISCUSSION

As results of inbred maize lines studies by CAPS markers the polymorphism, which indicates drought tolerance and susceptible lines, was determined. According to Liu *et al.* (2015) the polymorphism of CCAAAG(A) type by dhnC397 marker which is associated with drought tolerance is detected by presence only one amplicon of size 164 bp. Two amplicons of size 225 and 61 bp are identified in drought tolerant lines carrying CCGG(G) polymorphism by rspC1090 marker (Liu *et al.*, 2015; Prysiazhniuk *et al.*, 2018) (Fig. 1, 2).

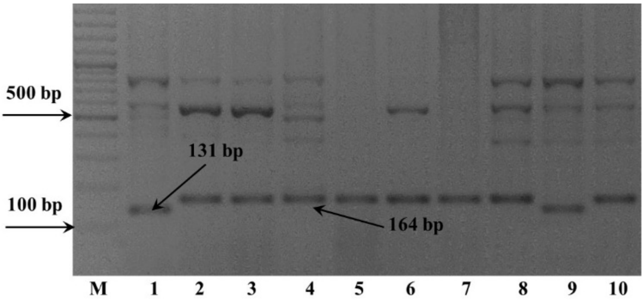

Fig. 1. Electrophoresis of PCR and restriction analysis products (marker dhnC397): 1- RAM 70; 2 – RAM 71; 3 – RAM 73; 4 – RAM 74; 5 – RAM 77; 6 – RAM 86; 7 – RAM 87; 8 – RAM 88; 9 – RAM 89; 10 – RAM 90; M − marker of molecular weight Thermo Scientific O'RangeRuler 100 bp DNA Ladder.

As result of PCR analysis by dhnC397 marker and digestion by StyI restriction enzyme the amplicons of expected size were detected at the electropherogram. The amplicons 164 bp were detected in lines carrying SNP CCAAAG(A) and they are drought tolerant. The polymorphism CCAAAG(G), which is identified by dhnC397 marker in drought susceptible lines, is detected by presence two amplicons of size 131 and 33 bp. In total, of the 74 studied lines, favorable alleles by dhnC397 marker were found in 50 lines, which accounts 68% of the total number of lines.

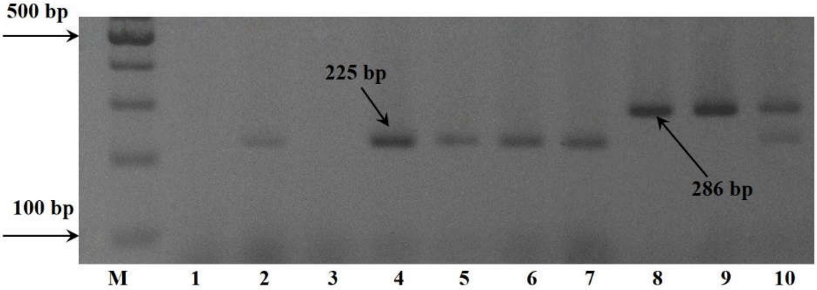

Fig. 2. Electrophoresis of PCR and restriction analysis products (marker rspC1090): 1- RAM 54; 2 – RAM 55; 3 – RAM 56; 4 – RAM 57; 5 – RAM 59; 6 – RAM 60; 7 – RAM 61; 8 – RAM 63; 9 – RAM 64; 10 – RAM 65; M − marker of molecular weight Thermo Scientific O'Range Ruler 100 bp DNA Ladder.

SNP CCGG(G) which is detected by rspC1090 marker and HpaII restriction enzyme was identified in 25 lines (34% of the total number of lines). As result of electrophoresis of PCR and restriction analysis products it was determined two amplicons of size 225 and 61 bp. The maize lines, which the amplicon 286 bp was found in, have the polymorphism CCGG(A). They are susceptible to drought.

Therefore, favorable alleles associated with drought in maize were detected in 14 studied lines by both markers dhnC397 and rspC1090 which accounts 5% of the total number of lines. The amount of lines which had the favorable allele only by dhnC397 marker was 36 (49%); there were 11 lines (15%) which the favorable allele was identified in by rspC1090 marker. No alleles were detected in 13 studied maize lines. Liu *et al.* (2015) studied 210 Chinese maize lines which had a different rates of drought tolerance. As results of their researches, it was determined that 141 lines had favorable alleles by dhnC397 marker and 109 maize lines – by rspC1090 marker.

According to these results the possibility of using dhnC397 and rspC1090 markers as functional markers for lines selection in breeding programs of drought tolerance was proved. For Ukrainian maize lines in our studies the percent of favorable alleles were fewer. However, Liu *et al.* (2015) note that they have already estimated 201 maize lines by morphological characteristics and yield indicators in field. In our experiment the preliminary assessment of maize lines by CAPS markers was conducted, and then its efficiency was verified by alternative approaches using seed germination in sucrose solution and by measuring the leaf surface temperature.In order to assess the ability of maize lines to heat tolerance in field condition the leaf temperature was measured and lines were compared according to presence the favorable alleles by studied CAPS markers.

In accordance with obtained data the average leaf surface temperature of maize lines, which had the favorable alleles by both markers in 2018 was 35.72ºC, in 2019 - 34.41ºC. The average leaf surface temperature of maize lines, which had the favorable alleles by dhnC397 marker was 36.95ºC in 2018 and 35.63ºC in 2019. It was found that in lines carrying the favorable alleles by rspC1090 marker the temperature was 33.68 and 35.30ºC in 2018 and 2019 respectively. The leaf surface temperature of maize lines which had no favorable alleles by studied markers was 34.35 and 34.75ºC in 2018 and 2019 respectively (Fig. 3).

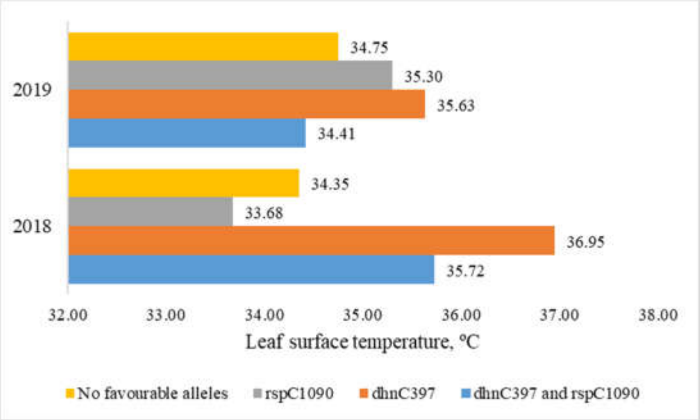

Fig. 3. The average leaf surface temperature of maize inbred lines in 2018 and 2019 ($LSD_{0.05}$=1.27)

As results of studies it was determined the significant differences between average leaf surface temperature of maize lines carrying the favorable alleles by dhnC397 and rspC1090 in 2018. Furthermore, the significant differences were observed between average leaf surface temperature of maize lines with favorable alleles by dhnC397 marker depending on years of experiment. There were not the significant differences between maize lines carrying the favorable alleles by rspC1090 marker and lines with no favorable alleles. It should be noted that average leaf surface temperature of maize lines with favorable alleles by both markers (35.72ºC) and lines carrying the favorable alleles by dhnC397 marker (36.95ºC) in 2018 differ significantly from maize lines with no favorable alleles (34.35ºC) and lines with presence favorable alleles by rspC1090 marker (33.68ºC). There were not any significant differences between the leaf surface temperature of maize lines in 2019.

The significant differences in the leaf surface temperature of maize lines that was observed in 2018 can be explained by a significant variation in air temperature depending on years of research. According to the calculated deviation coefficient by months, it was determined that at the growth stage of development of fruit (August), *Dc* for air temperature is 2 in 2018 and 0 in 2019. This indicates that the air temperature in 2018 was significantly higher than the daily average air temperature during this growing cycle. It was determined that drought tolerance maize lines carrying favorable alleles by both dhnC397 and rspC1090 markers, and only by dhnC397 marker reduced the transpiration. It occurred as response to high air temperature due to that water loss was reduced and reduced transpiration led to an increase the leaf surface temperature of maize lines.

The deviation coefficient for amounts of precipitation in August 2018 and 2019 was -1 and 1 respectively that indicates the weather condition as close to normal conditions. However, the amount of precipitation in 2019, from April to June, was significantly lower compared to the normal amount (*Dc*=-2), which indicates a water deficit in the soil during these growing cycles. Therefore, the determination of the maize lines' ability to germinate in osmotic solutions can be informative for assessing the studied lines in water lack of soil.

To determine the drought tolerance of maize lines by germinating seeds in a sucrose solution, lines with a different combination of favorable alleles were selected according to functional drought tolerance markers: 5 lines with favorable alleles by both markers, 7 lines with favorable alleles by dhnC397 marker, 5 lines with favorable alleles by rspC1090 marker and 3 lines with no favorable alleles.

As a result of the studies, it was determined that the number of seeds that were able to sprout in a sucrose solution ranged from 0 to 7.33%. The average number of seeds for genotypes carrying both favorable SNP(A)(G) is 2.54%, lines with SNP(A) by dhnC397 marker - 2.46%, lines with SNP(G) by rspC1090 marker - 4%, for genotypes with no favorable alleles - 2.25% ($LSD_{0.05}$=1.70). According to ANOVA results, it was found that the percentage of germinated seeds in sucrose solution did not differ significantly depending on the combination of favorable alleles. However, significant differences were found between the number of seeds capable of germination in sucrose solution in lines carrying a single favorable allele and lines that had SNP(G) by rspC1090 marker.

Studies conducted by Rotari *et al.* (2018), found a wide range of variation of the resistance coefficient to seeds physiological dryness on sucrose solution - from 0.00 to 1.05%. Based on the study of indicators complex of the leaf water regime and the developed scale for assessing drought tolerance, the authors proved the effectiveness of seed germination on an osmotic solution for early detection of maize drought-resistant forms (Rotari *et al.*, 2018). The water stress using sorbitol was simulated also in the work of Valentovic *et al.* (2006). The authors evaluated the effect of water deficiency on the antioxidant enzymes activity in two maize hybrids. Based on their data, a significant effect of water stress on 13-day old seedlings of the studied varieties was determined (Valentovic *et al.*, 2006). This confirms the presence of various mechanisms to resist an atmospheric and soil drought.

To determine the correlation between the presence of favorable alleles by CAPS markers and the ability of the studied maize lines to resist high air temperatures and water stress, a Spearmen correlation coefficients analysis was computed. As a result of calculations, the presence of a positive correlation between the presence of favorable allele by dhnC397 marker in maize lines and leaf surface temperature in 2018-2019 was determined (r=0.16). A negative correlation was found between the presence of a favorable allele by rspC1090 marker and leaf surface temperature in 2018-2019 (r=-0.20).

According to the assessment of correlation between the presence of favorable alleles by CAPS markers and the ability of the studied lines to germinate under the osmotic stress on a sucrose solution, a positive correlation was found between the presence of a favorable allele by rspC1090 marker and the percentage of germinated seeds of maize lines (r=0.31). However, a negative correlation was found between the percentage of germinated seeds on a sucrose solution and the presence of a favorable allele by dhnC397 marker (r=-0.32).

Liu *et al.* (2011) studied 187 recombinant maize lines using infrared thermography and evaluated the plants response to water stress. The authors also conducted studies of the correlation between the difference of leaf temperature and shoot biomass. As a result of studies, the authors noted a significant increase of the leaf temperature of the studied lines under water stress. The results obtained by Liu *et al.* (2011) indicate that there is a positive correlation between the difference of leaf temperature and shoot fresh weight and shoot dry weight, the correlation coefficients were 0.160 and 0.163, respectively (Liu *et al.*, 2011).

In our studies, despite some differences in the experiment organization, significant differences were also found between the leaf temperature in drought. However, according to the purpose of our studies, a positive correlation was found between the presence of a favorable allele by dhnC397 marker in maize lines over the studied years and the leaf surface temperature. Significant differences in leaf temperature in maize hybrids due to atmospheric drought were discovered by Coskun *et al.* (2011). The authors investigated the physiological parameters of maize hybrids under high air temperatures in the field. A positive correlation was determined between the leaf temperature and days to flowering, relative injury and chlorophyll content *b* (*Chla/b*) (Coskun *et al.*, 2011).

Kögler and Söffker (2019) studied the features of explorative frequency analysis for measuring the leaf temperature of maize under water stress. The authors conducted an experiment in controlled laboratory conditions studying the leaf temperature under water deficit of the substrate using infrared camera. As a result of the analysis, the authors did not find any correlation between the leaf temperature and water deficit (Kögler and Söffker, 2019). According to our results, a negative correlation was found between ability to germinate under osmotic stress in lines carrying the favorable allele by rspC1090 marker and the leaf surface temperature of studied maize lines. However, a positive correlation was noted between the presence of a favorable allele by rspC1090 marker and the number of germinated seeds in a sucrose solution. Thus, our studies also confirm that the change of leaf temperature of maize does not depend on water deficit in the soil or substrate, but there are significant differences under atmospheric drought.

The *dhn1* gene is known to encode dehydrin, which protects plant vegetative tissue cells from dehydration (Rorat, 2006). Thus, it can be assumed that the presence of the favorable allele of the *dhn1* gene indicates the ability of the maize lines carrying this allele to resist atmospheric drought. Consequently, maize lines with the favorable allele by dhnC397 marker are perspective for producing heat tolerant maize hybrids.

The *rsp41* gene encodes an arginine/serine-rich splicing factor. According to Palusa *et al.* (2007) the strong influence of heat and cold and some hormones on alternative splicing of specific serine/arginine-rich genes suggests that some of the dynamic changes in plant transcriptome in response to abiotic stress can be caused by changes in the serine/arginine-rich proteins structures and their isoforms. In addition, serine/arginine-rich genes that express altered splicing variants in response to stress provide a unique opportunity to use them as a model to analyze the mechanisms by which stress signals modulate alternative splicing and plants respond to water deficit. Thus, the use of rspC1090 marker to the *rsp41* gene can be considered as a means for identifying genotypes that are resistant to water deficit in soil.

The significant differences were noted between lines carrying favorable alleles by both dhnC397 and rspC1090 markers and lines with no favorable alleles by yield of studied lines (Fig. 4). According to obtained data, it was determined that the average yield of maize lines that had favorable alleles by both CAPS markers was 2.70 and 2.50 t ha^{-1} in 2019 and 2018, respectively. The yield of lines carrying favorable alleles by one of the studied markers was 2.10-2.24 t ha^{-1} in 2018 and 2.20-2.31 t ha^{-1} in 2019. The diagram shows that there were no significant differences in yield of studied lines between the years, and also in accordance with the presence of favorable alleles.

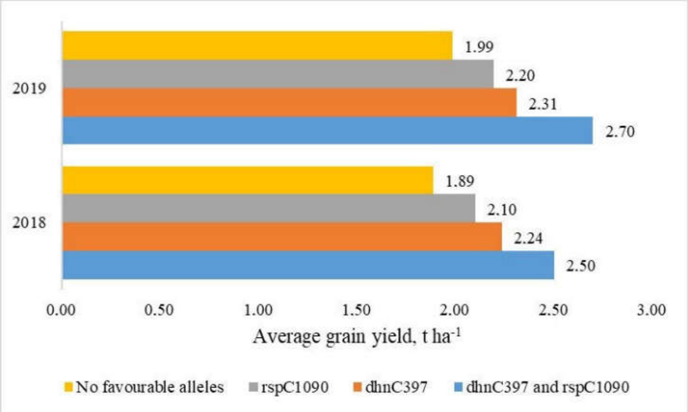

Fig. 4. The average grain yield of maize inbred lines in 2018 and 2019 ($LSD_{0.05}$=0.59)

The grain yield did not differ significantly depending on the years. However, in 2019, a slightly higher yield was observed for all studied lines. This can be explained by the fact that, in accordance with the amount of precipitation and air temperature, 2018 was a more arid during maize growing cycle. Based on climate change analysis and maize yield it was determined that an increase the average air temperature by 1, 2 and 4°C relative to the baseline climate resulted in a yield reduction of 1-21%, 3-34% and 17-67%, respectively (Tesfaye *et al.*, 2018). Authors shown that varieties with combined heat and drought tolerance traits increased maize yield by 3-150%, 4-185% and 7-329% under the hotter and drier climate change scenarios with the average temperature increase of 1, 2, and 4°C, respectively. Therefore, lines, which showed tolerance to heat and drought by studied CAPS markers, can be perspective not only for obtaining resistant hybrids to adverse environmental conditions, but also for obtaining a stable yield under water deficit.

Cairns *et al.* (2013) investigated inbred maize lines ability to resist heat and drought in various arid regions in the field. The authors determined that genetic control of drought and heat tolerance are largely independent of each other (Cairns *et al.*, 2013). Their results are confirmed by our studies very clearly, since the revealed correlations between leaf surface temperature and the presence of a favorable allele by dhnC397 marker associated with the gene encoding dehydrin, as well as a positive correlation between the presence of a favorable allele by rspC1090 marker and the percentage of germinated seeds in osmotic solution indicate different mechanisms for regulating heat resistance and water deficit in soil.

Scientists (Cairns *et al.*, 2013) also noted a significant yield loss of the studied maize lines under the heat. In our studies in 2018, which was characterized by a high air temperature, the yield of the studied maize lines was lower and significantly differed in lines that turned out to be susceptible to heat and drought by both studied CAPS markers.

Chen *et al.* (2012) investigated the effects of heat and soil drought to inbred maize lines in the field based on estimates of plant height, relative water content, leaf firing, leaf blotching, and tassel blasting. According to the results, drought and heat resistant genotypes were selected by authors. Also, the authors proved the inheritance of heat tolerance by hybrids. Therefore, the heat and drought tolerance lines selected as results of our studies can be used in future breeding programs to obtain hybrids resistant to heat and drought. Thus, according to obtained results, dhnC397 and rspC1090 markers can be recommended for selection of maize breeding material of heat and drought tolerance.

CONCLUSIONS

As a result of studies of 74 inbred maize lines using two CAPS markers, it was determined that favorable alleles were identified by both dhnC397 and rspC1090 markers in 14 maize lines, in 36 lines - by dhnC397 marker and in 11 - with rspC1090 marker. The significant differences between leaf surface temperature depending on the year were determined in lines with favorable allele by the dhnC397 marker. As a result of analysis maize lines ability to germinate in the osmotic solution, significant differences were found between the number of germinated seeds in lines with no favorable alleles and lines that had a favorable allele by the rspC1090 marker.

According to the results of the correlation analysis, a positive correlation was determined between the presence of a favorable allele by dhnC397 marker in maize lines and leaf surface temperature in 2018-2019 (r=0.16), a positive correlation was also found between the presence of a favorable allele by rspC1090 marker and the percentage of germinated seeds of maize lines in sucrose solution (r=0.31). Thus, for a comprehensive assessment and selection of maize lines to heat and drought tolerance, the using of two CAPS markers dhnC397 and rspC1090 can be recommended.

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