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The selection of maize parent lines within marker assisted selection (MAS) by crtRB1-3'TE marker for Steppe zone of Ukraine

L. Prysiazhniuk[1,*], Y. Honcharov[2], S. Melnyk[1] and O. Kliachenko[3]

[1]Ukrainian Institute for Plant Variety Examination, Henerala Rodimtseva Str. 15, UA03041 Kyiv, Ukraine
[2]Research Institute of Agrarian Business, Tokova Str. 2A, UA52502 Vesele village Dnipro region, Ukraine
[3]National University of Life and Environmental Sciences of Ukraine, Faculty of Plant Protection, Biotechnology and Ecology, Heroiv Oborony Str. 15, UA03041 Kyiv, Ukraine
[*]Correspondence: prysiazhniuk_l@ukr.net

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Abstract. Maize has a large genotypic diversity and a broad scale of economically important traits. Therefore, it is extremely important for breeding to obtain hybrids which can ensure high yield even under severe growing conditions, such as in Steppe zone of Ukraine. This study aimed to determine the optimal allele ratio by crtRB1-3'TE marker in parental components of maize modified hybrids. There were investigated four hundred sixteen maize hybrids which are modified hybrids of heterotic model (Iodent × Iodent) × Lancaster germplasm. SCA (specific combining ability) effects for grain yield and grain moisture content were calculated in maize hybrids with different allele combinations of crtRB1 gene. As results, the stable positive SCA effects were calculated for hybrids with allele combination (296 bp + 875 bp) × 543 bp × 296+875 bp during both 2019 and 2020 (1.23 and 0.99 t ha^{-1}, respectively). The lowest SCA effects for grain moisture content were obtained for modified hybrids with allele combination (543 bp × 543) × 296 + 875 bp both in 2019 and 2020 (-0.54 and -0.36%, respectively). The greatest influence SCA effects for grain yield had the interaction of allele combinations and year weather conditions (39%), the impact the allele combinations was 36%. The year weather conditions had the greatest impact on SCA effects for grain moisture content (44%), the allele combination (36%). Thus, it was determined that SCA effects for studied indicators of heterotic model (Iodent × Iodent) × Lancaster under contrasting weather condition are resulted from both genotypes of hybrid parents and favourable allele presence.

Key words: *Zea mays* L., carotenoids, crtRB1 gene, favourable allele, SCA effects.

INTRODUCTION

Maize (*Zea mays* L.) is currently one of the most important crops in the world. Maximizing its productivity and yield while maintaining the quality is one of the primary goals of corn producers (Bojtor et al., 2021). Maize is a crop with enormous diversity of economically important traits. Maize, being a C4 plant, can be considered a potential source of bioenergy as it possesses all the essential traits like wide adaptation, superior carbon sequestration, and efficient nitrogen utilization (Choudhary et al., 2020; Bojtor et al., 2021). Meanwhile, maize can be used directly for human food, processed into various types of food products, such as flour, cornmeal, grits, starch, snacks, tortillas, and breakfast cereals, or used for animal feed (Gayosso-Barragán et al., 2020; Sun et al., 2022).

Maize has the potential to meet the vitamin A requirements by providing precursors for vitamin A biosynthesis. Carotenoids are an important source of vitamin A. Maize kernel has considerable variation for the levels of carotenoids content and breeding for these economically important compounds is feasible. An understanding of the plant carotenoid biosynthesis pathway has opened an avenue for the deployment of functional markers to improve carotenoid accumulation in maize grain (Gebremeskel et al., 2018). The main genes related to the accumulation of carotenoids in maize grain include the lycopene ε-cyclase (lcyε) and β-carotene hydroxylase (crtRB1). The crtRB1 gene polymorphism is connected with increased β-carotene rate in maize grain and revealed by crtRB1-3'TE marker. It is possible to identify three allele variants: 543 bp (favorable allele), 296 bp and 296 + 875 bp (Muthusamy et al., 2015). The efficiency of using this marker in MAS for maize line with high carotenoids level selection and strong correlation between the presence of favourable allele and carotenoids content in kernel was demonstrated by our previous study and many other authors (Senete et al., 2011; Messias et al., 2014; Muthusamy et al., 2015; Zunjare et al., 2018; Prysiazhniuk et al., 2022a). However, introgression of favorable alleles of crtRB1 gene can have dramatically different effects depending on the genetic background (Diepenbrock et al., 2021).

According to the State Statistics Service of Ukraine, the sown area of maize over the past three years increased to 5.5 million hectares (Prysiazhniuk et al., 2022a). The Steppe zone of Ukraine has the longest growing season, but receives the lowest precipitation and often suffers from drought. Therefore, it is extremely important for plant breeding to obtain hybrids which can ensure high yield under such growing conditions. The one of important agronomical traits is also harvest grain moisture content. High grain moisture contents at harvest necessitate grain drying prior to the transport and storage of ears and requires the extra expenses for the grain basic moisture content (Petkevičius et al., 2008; Li et al., 2021).

Combining ability may be considered as the potential of an individual inbred line to contribute better fitness-related traits to hybrid progeny. In maize breeding programs, knowledge of specific combining ability (SCA) of hybrid combinations as well as the identification and exploitation of heterotic groups, are crucial for successful hybrid production (Gami et al., 2018; Iseghohi et al., 2020). Thus, we aimed to determine the optimal allele ratio by crtRB1-3'TE marker in parental components of maize modified hybrids by assessing SCA effects for grain yield and grain moisture content.

MATERIALS AND METHODS

Plant material and laboratory measurements

Four hundred sixteen maize modified hybrids of heterosis model (Iodent × Iodent) × Lancaster germplasm (three-way cross hybrids) were used in this study. The effects of specific combining ability (SCA) were assessed by the top-crossing method. Test crosses were made with using sister sterile hybrids (Lancaster germplasm) as testers. All breeding materials were provided by Research Institute of Agrarian Business (Dnipro, Ukraine).

The laboratory studies were carried out in Laboratory of molecular genetic analysis, Ukrainian Institute of Plant Variety Examination (Kyiv, Ukraine) in 2019. The DNA marker crtRB1-3'TE was used to identify the allele state of crtRB1 gene (Muthusamy et al., 2015). DNA extraction procedure, PCR parameters and amplicons visualisation were described in our previous study (Prysiazhniuk et al., 2022a). To assess the impact of alleles combinations on grain yield and grain moisture content, genotypes of modified hybrids with all combinations of detected alleles of crtRB1 gene were used.

Field measurements and environments

The field experiment was carried on during 2019-2020 on pilot plots of Research Institute of Agrarian Business (Vesele village, Dnipro region, Ukraine). The field experiment was designed according to classical crosspollinated plant breeding methods (Dospekhov, 1985). The grain moisture content was estimated during harvesting using the plot combine (Wintersteiger, Germany). The weather conditions rates were provided by Sinelnykove weather station (Table 1).

Table 1. The amounts of precipitation and air temperature during 2019–2020 maize growing season

Month	Amounts of precipitation, mm			Air temperature, °C		
	Normal daily average	2019	2020	Normal daily average	2019	2020
May	50.0	21.3	2.9	15.8	18.1	13.7
June	59.0	1.0	0.0	19.1	23.9	22.1
July	61.0	33.7	1.3	20.9	20.9	23.2
August	35.0	73.4	14.0	20.1	20.9	21.5
September	36.0	6.4	0.0	15.0	16.1	19.5

Statistical analysis

The coefficient of agrometeorological indicators from normal daily average amounts during 2019–2020 was computed using the equation below:

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

where Dc – deviation coefficient; X_i – indicator of current weather; X – normal 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).

The significant differences of studied indicators and the rate of factors impact on SCA for grain moisture content were determined by ANOVA using STATISTICA 12.0 software (trial version).

The correlation between the allele state of crtRB1 gene and SCA effects for grain yield and grain moisture content was evaluated with Mantel test (Pearson corelation) using XLSTAT software (trial version) (Prysiazhniuk et al., 2022b). The distances matrices were obtained based on allele combination and SCA effects values. The unweighted pair-group average amalgamation rule was used for genetic distances calculation based on allele state, single linkage rule - for distances based on SCA effects.

RESULTS AND DISCUSSION

For the crtRB1-3'TE marker, all three possible allele variants were identified among the lines which were parental components of studied modified hybrids accept three lines variants. There were not identified any expecting alleles. For each type of allele combinations of modified hybrids, SCA effects for grain yield was calculated (Table 2).

Table 2. SCA of modified hybrids with different allele combinations for grain yield and grain moisture content 2019–2020

Allele combinations of crtRB1 gene			Average SCA for grain yield, t ha^{-1}		Average SCA for grain moisture content, %	
♀	♂	pollinator	2019	2020	2019	2020
296	296	543	-2.08	-0.52	-0.26	-0.13
296	296	296	0.63	-0.35	0.21	-0.15
296	296	296 + 875	3.16	0.03	0.61	0.36
296	543	543	0.38	0.07	0.16	0.03
296	543	296	0.29	0.06	-0.15	-0.03
296	543	296 + 875	0.36	0.09	-0.36	-0.07
296	296 + 875	543	-0.35	0.60	-0.05	-0.12
296	296 + 875	296	-0.06	0.43	0.02	-0.05
296	296 + 875	296 + 875	0.99	-0.55	0.07	0.20
543	296	543	0.18	-0.77	0.20	-0.03
543	296	296	0.38	0.05	-0.17	-0.04
543	296	296 + 875	0.53	1.26	-0.25	0.15
543	543	543	-1.36	-0.96	0.11	0.24
543	543	296	0.70	-0.03	-0.15	0.14
543	543	296 + 875	0.84	2.58	-0.54	-0.36
543	296 + 875	543	0.24	0.22	-0.03	0.04
543	296 + 875	296	0.14	-0.18	-0.02	0.06
543	296 + 875	296 + 875	-0.68	-0.32	0.08	-0.07
296 + 875	543	543	2.17	-1.17	0.33	0.14
296 + 875	543	296	-0.01	0.34	-0.04	0.00
296 + 875	543	296 + 875	1.23	0.99	-0.79	0.17
296 + 875	296 + 875	543	1.04	-0.89	-0.18	0.07
296 + 875	296 + 875	296	-1.26	0.58	0.13	-0.07
296 + 875	296 + 875	296 + 875	-1.93	1.21	0.03	-0.05
296 + 875	-	543	0.94	-0.56	-0.28	-0.07
296 + 875	-	296	0.35	-0.05	0.21	-0.09
296 + 875	-	296 + 875	0.35	-0.66	0.20	0.27

According to obtained results, SCA effects for grain yield of modified hybrids were characterised by diversity depending on studied year. It was shown that in general, hybrids which had high positive SCA effect in 2019 or 2020, demonstrated low SCA in another year. The highest SCA effect for grain yield was observed in hybrids with allele combination (296 bp ×2 96 bp) × 296 + 875 bp in 2019 p. (3.16 t ha^{-1}). Meanwhile, the SCA effect in hybrids with this combination in 2020 was low (0.03 t ha^{-1}). The same situation was observed for hybrids with allele combination (543 bp × 543 bp) × 296 + 875 bp. These hybrids demonstrated low SCA in 2019 (0.84 t ha^{-1}), but the high SCA effects were observed in 2020 (2.58 t ha^{-1}). Hence, the stable positive SCA effects were calculated for hybrids with allele combination (296 bp + 875 bp) × 543 bp × 296+875 bp during both 2019 and 2020 (1.23 and 0.99 t ha^{-1}, respectively). Hybrids with allele combination (296 bp × 543 bp) × 543 bp had also low positive SCA effects during 2019–2020 (0.38 and 0.07 t ha^{-1}, respectively).

The lowest SCA effects both in 2019 and 2020 were obtained for hybrids with allele combination (296 bp × 296 bp) × 543 bp. They were -2.08 and -0.52 t ha^{-1}, respectively. The hybrids with allele combination (543 bp × 543 bp) × 543 bp demonstrated low SCA effects during 2019–2020 (-1.36 and -0.96 t ha^{-1}). However, hybrids with allele combination (296 + 875 bp × 296 + 875 bp) × 296 + 875 bp shown low SCA effects in 2019 (-1.93 t ha^{-1}), meanwhile in 2020, the SCA was positive (1.21 t ha^{-1}).

It was determined that the lowest SCA effects on grain moisture content were obtained for modified hybrids with allele combination (543 bp × 543) × 296 + 875 bp both in 2019 and 2020. They were -0.54 and -0.36%, respectively. In 2019, the lowest SCA effects were calculated for hybrids with allele combination (296 + 875 bp × 543 bp) × 296+875 bp (-0.79%). In 2020, the hybrids with this allele combination showed low positive SCA effects (0.17%). The positive SCA effects for grain moisture content during the experiment were noticed for hybrids with allele combination (296 bp × 296) × 296 + 875 bp (0.61 and 0.36% in 2019 and 2020, respectively). The hybrids with allele combination (296 bp × 296 bp) × 543 bp demonstrated low SCA effects for grain moisture content during 2019-2020 (-0.26 and -0.13%, respectively). However, these hybrids have shown negative SCA effects for grain yield as it was described.

Considering the weather conditions during the maize pollination stage in 2019 the weather conditions were close to normal, *Dc* for air temperature and the amount of precipitation were 0 and -1, respectively. In 2020 during this period the weather conditions characterized by the high air temperature and the lack of precipitation, *Dc* for weather condition indicators were 2 and -2 respectively. On the other hand, the weather conditions during physiological maturity period in 2019 were close to normal in contrast to 2020. The air temperature during August-September 2019 was close to normal temperature (*Dc* was from 0 to 1). In comparison to 2019, in 2020 *Dc* was 2 which indicates strong different conditions. The deviation coefficients for amounts of precipitation during August-September 2019 were from 1 to -2. In 2020, during this period *Dc* has negatiive values (from -1 to -2).

To assess the rate of factors impact on SCA effects for grain yield and grain moisture content of maize modified hybrids, the ANOVA was used. Partitioning mean squares into its components revealed significant influence of allele conbination, year wheather condition and their interaction on SCA effects (Table 3).

Table 3. Mean squares from the analysis of variance of grain yield and grain moisture content of maize modified hybrids evaluated during 2019-2020[1]

Effect	DF[2]	Grain yield	Grain moisture content
Intercept	1	4.37	0.02
Alleles combination	26	2.69	0.20
Year	1	1.87	0.25
Alleles combination*Year	26	2.97	0.11
Errors	108	0.007	0.008

[1] Significant at 0.05 probability levels; [2] DF - Degree of freedom.

According to the obtained results, it was determined that the greatest influence of SCA effects for grain yield had the interaction of allele combinations and year weather conditions (39%). Partitioning mean squares into its components revealed that the impact of the allele combinations on SCA effect for grain yield was 36%. Furthermore, the year weather conditions had the lowest impact on SCA effects (25%) (Fig. 1).

Menkir et al. (2014) reported that environments, hybrids and hybrid × environment interactions had significant effects on carotenoid content in their study which was examined the effect of crossing parental lines from two AFLP-based groups on carotenoid accumulation and agronomic performance in hybrids. It was determined that environments, hybrids and hybrid × environment interactions had significant effects on grain yield. In this study, similar results were observed with the greatest influence of allele combination and the interaction between allele combination and year weather conditions.

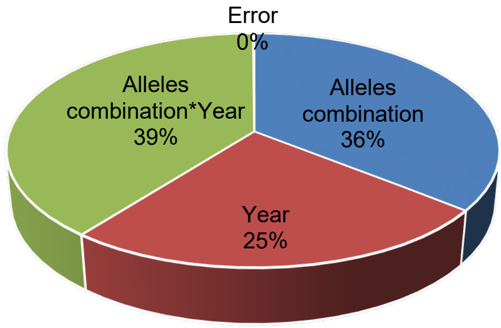

Figure 1. The rate of factors impact (mean squares) on SCA for grain yield of maize modified hybrids during 2019–2020.

It was reported that the one of the critical stages for the formation of main yield components is the pollination. Maize plants are sensitive to high air temperature and moisture deficit. Stress can cause kernel abortion at the cob tip, and wilted leaves from moisture stress in the morning can lead to a yield loss of up to 7% per day (Baum et al., 2019). Moreover, the critical stage of grain yield is physiological maturity. Stress at this point can reduce the number, size and weight of the harvestable kernels.

Muthusamy et al. (2016) assessed the combining ability and nature of gene action for kernel carotenoids and identified superior hybrid combinations for specific carotenoids over commercially available hybrids. As obtained results, investigated maize genotypes were stable across environments.

According to the obtained data in this study, 2020, during critical stages of maize growing season, characterized of high air temperature and precipitation deficit. These factors could cause modified hybrids grain yield decrease. Meanwhile, in 2019, there was the softer weather condition which was the reason of higher rate of grain yield. It should be noted that the high impact rate of interaction of year weather conditions with allele combinations on SCA effects for grain yield could be expressed as the adaptation of modified hybrids to different weather conditions. On the other hand, genotypes which were shown the positive SCA effects both in 2019 and 2020 could be considered as stable hybrids with ability to enable stable grain yield rate under limited environmental conditions (Bonea & Dunăreanu, 2022). There was no corelation between the presence of the favourable alleles by crtRB1-3'TE marker and SCA effects for grain yield for studied modified hybrids. Muthusamy's et al. (2016) investigation demonstrated the similar conclusion that the grain yield did not show association with carotenoids.

As a result of analysis, the year weather conditions had the greatest impact on SCA effects for grain moisture content of modified hybrids (44%). The allele combination revealed a weaker influence (36%). It was shown that the interaction of allele combination and year weather conditions impacted on SCA effects for grain moisture content significantly. The rate of impact was 19% (Fig. 2).

Researchers have previously reported that the physiological maturity is also the critical stage to determine grain moisture content at harvest. Water loss from kernels occurs in two phases. Before physiological maturity, the decrease in grain moisture content is due to successive accumulation of dry matter via grain filling and the water loss rate is constant and highly dependent on genetic factors. After physiological maturity, the accumulation of dry matter ceases, and the reduction in grain moisture content is primarily due to water evaporation from kernels and thus can be greatly affected by environmental factors (Liu et al., 2020).

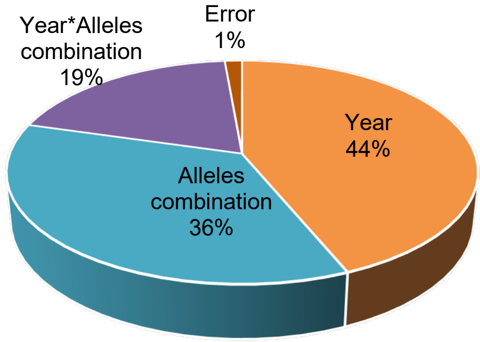

Figure 2. The rate of factors impact (mean squares) on SCA for grain moisture content of maize modified hybrids during 2019–2020.

On the other hand, the grain dehydration rate before and after physiological maturity is also closely related to grain moisture content at harvest. Maize genotypes with a fast dry-down rate generally have low ear moisture at harvest. The grain dehydration rate of maize is affected by many factors, such as variety, endosperm type, planting density, temperature, and humidity.

Taking into account that during maturity stage of studied maize modified hybrids the dry period occured, the lowest SCA effects for grain moisture content were observed in 2020. However, it is more attractive to breeding to consider hybrids with allele combinations which demonstrated low SCA effects under mild weather condition as 2019 was characterized. It is because this weather conditions allows to assess the genetic potential of studied genotypes excluding the weather impact. Kang & Zuber (1989) reported that white hybrids had slightly higher grain moisture content than yellow hybrids because white maize lacks phytoene synthase, an enzyme involved in both carotenoid and abscisic acid biosynthesis. This study shows that majority of modified hybrids, which demonstrated low SCA effects for grain moisture content, had at least one favorable allele connected with high carotenoids content.

In order to assess relationship between the presence of favorable alleles by crtRB1-3'TE marker and SCA effects for grain moisture content in modified hybrids and testers, the Pearson's correlation coefficient was estimated. It was determined that there was no correlation between the presence of favorable alleles and SCA effects of modified hybrids. However, the analysis revealed the weak correlation between the presence of favorable alleles and SCA effects of testers. The coefficient of correlation $r = 0.31$ at 0.05 probability levels. It could be explained by the fact that three-way maize hybrids possess broader adaptation than single-cross hybrids (Makinde et al., 2022). Meanwhile, the SCA of sister sterile hybrids used in this study were estimated through characteristics obtained for modified single cross hybrids during top-crossing. Thus, testers demonstrated higher genotype impact on SCA effects for grain moisture content than modified hybrids. Hence, as a result of this study, it was shown that factors as genotypes with different allele combination by crtRB1-3'TE marker and year weather conditions impacted significantly on grain yield and grain moisture content of maize heterotic model (Iodent × Iodent) × Lancaster.

CONCLUSIONS

In the study, SCA effects for grain yield and grain moisture content of maize modified hybrids were found to be significant. By assessment of the rate of factors impact on SCA effects for grain yield it was found that the interaction of allele combinations and year weather conditions had the greatest influence on modified hybrids (39%). This can indicate of the modified hybrids' ability to adapt to different weather conditions. The high rate of influence SCA effects for grain moisture content revealed by the year weather conditions and allele combination (44 and 36%, respectively). It is concluded that taking into account the different weather condition in Steppe zone of Ukraine during studied year, there were selected genotypes with allele combinations of crtRB1 gene (296 bp × 543 bp) × 296 + 875 bp, (543 bp × 296 bp) × 296 bp, (543 bp × 296 bp) ×296 + 875 bp, (543 bp × 543 bp) × 296 + 875 bp, (296 + 875 bp × 543 bp)× 296 + 875 bp with provided positive SCA effects for grain yield and low negative SCA effects for grain moisture content. These newly identified cross combinations hold promise in breeding programme to enhance carotenoids in maize with combination important agronomical traits such as high grain yield and low grain moisture content.

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