BIOTECH STUDIES

Development of homozygous maize lines differing in oil and zein content using *in-vivo* maternal haploid technique

Fatih Kahrıman[1,2]* ID, Asude Kahrıman[2] ID, Abdurrahman Muhammed Güz[1] ID, Nebahat Nur Yüksel[1] ID

[1]Department of Field Crops, Faculty of Agriculture, Çanakkale Onsekiz Mart University, 17100, Çanakkale, Türkiye.
[2]BAF Elektronik Yazılım Tarım San. ve Tic. A.Ş. 17100, Çanakkale, Türkiye.

How to cite

Abstract

This study was carried out in order to develop homozygous lines that differ in grain quality from the local maize population. Twelve different local maize landraces were used as donor materials in the study. These populations were subjected to induction crossing under greenhouse conditions in September 2020 with the ADAIL-I inducer line. In September 2021, a total of twelve haploid lines were grown in greenhouse conditions. Some plant traits and some grain quality characteristics were examined. Zein protein fractions were also analyzed with SDS-PAGE analysis. The haploid induction rates (HIR) of donor materials ranged from 6.08% to 11.71%. The average HIR value of the ADAIL-I inducer line was determined as 8.20%. The average value of plant height of developed lines varied between 123 cm and 250 cm; first ear height between 54 cm and 120 cm; stem diameter between 0.7 cm and 1.2 cm; crude oil content between 2.39% and 7.54%; oleic acid content between 15.34% and %30.98; linoleic acid content between 50.4% and 67.8%; protein content between 6.75% and 13.74%; and zein content between 4.58% and 5.04%. Some the homozygous lines carry the desired protein bands in terms of zein fractions.

Introduction

Maize is one of the most common cereal species, and its cultivation is becoming more widespread every year. Among the staple cereal species, maize has reached the second place in the world in terms of cultivation areas and the first place in terms of the amount of production (Erenstein et al., 2021). The use of this type of grain used in human and animal nutrition in different industries has also become widespread. Considering the cereal cultivation in our country, the maize has had a significant increase in the planting area and production amount in the last 10 years (TUIK, 2020). However, one of the biggest shortcomings in terms of marketing and converting, maize products to commercial value is that the production of high-value-added raw materials from maize has not become widespread enough. Raw materials that are used in the production of products with high added value are imported raw or processed.

Zein proteins and crude oil are important products with high added values obtained from maize kernels. Of the raw materials required in the production of maize oil are mostly imported items in Turkey. The primary reason for this situation is due to the lack of Turkish maize varieties suitable for oil production. The oil content varies between 3-5.5% in normal maize genotypes (Lambert, 2001). Although it is not included in the oil plants, maize is used as a source of vegetable oil both in the world and in Turkey in this regard. The most important advantage of maize in comparison with the species classified as oil plants is that it is a high-

yielding plant (TUIK, 2020). In addition, maize oil has desirable properties both in terms of both health and nutrition. For this reason, a significant effort is being made worldwide to develop high-oil maize lines and hybrids (Singh et al., 2014). There is more than 6% oil in the genotypes described as "high-oil maize" (Lambert, 2001). The development of high oil maize lines has reached such an advanced point that today there are lines that contain about 20% oil in their seed, have been developed with the support of tissue culture methods and are the subject of patents (Foley, 2009; Patent no: US007495155B2). Development of high oil maize studies is important for seed breeding targets in Turkey and it is an issue that lags behind other countries. Raw zein extracts, which are used as protein-based film raw materials in the textile, food and pharmaceutical industries in recent years, are among the raw materials with high added value that can be obtained from maize. The price per kilogram of raw zein obtained from maize is about 10-40 US dollars (Anderson & Lamsal, 2011). There are decisions taken at the international level to focus on the production of biodegradable plastics to reduce environmental pollution. These decisions will effect on the increasing the production of raw materials used in biodegradable plastics or films in the near future It is expected that the sectors for maize-derived products are developing around the world and will have significant commercial potential in the coming years (Anonymous, 2019). To take part in the zein market, it is important to increase the activities aimed at this market and to meet the needs of raw materials. The most important aspect in this regard is that maize lines and varieties suitable for the production of high added value raw materials have not been developed yet in our country. It is possible to say that there is a significant gap in this area in Turkey.

An important time is allocated for the work of developing pure lines by classical methods in breeding programs, and this is undesirable for breeders (Eder & Chalyk, 2002). In this regard, it becomes possible to develop 100% homozygous lines in a short time with the help of *in vivo* or *in vitro* methods as an alternative to traditional breeding methods (Chalyk, 1994, Chidzanga et al., 2017). The *in vivo* doubled haploid method is widely preferred in practice by researchers in maize breeding studies due to its high success rate and ease of use (Ren et al., 2017). In this context, various studies have been conducted in different countries on the development of homozygous lines from maize germplasms and the use of these lines in variety of development programs such as Germany, (Schmidt, 2003), USA (Seitz, 2005), Croatia (Mazur et al., 2019), Turkey (Cerit et al., 2016; Erdal et al., 2019). The *in vivo* doubled haploid technique has proven its validity as a practical method of developing parental lines in maize and has become a widely used technique in practice. The *in vivo* doubled haploid technique can also be used for the development of parental lines in terms of grain quality characteristics. So far, it is known that there is no study on the development of homozygous lines for the oil and zein content in Turkey.

The aims of this study are, to develop homozygous maize lines from 12 different donor materials, mostly consisting of local maize populations, ii) and to study the agronomic characteristics of the developed lines, in addition to zein and oil contents, as well as the variations in zein fractions. With the current study, it was aimed to give a new direction to launch seed breeding programs suitable for the production of high added value raw materials from the seeds of developed varieties.

Materials and Methods

Plant Material

Twelve local maize landraces were used as donor material which were collected from different regions of Turkey and previously screened for zein and oil content. These populations are obtained from the Faculty of Agriculture of Ordu University. The local populations have no restrictions on their commercial and research use. The ADAIL-1 inducer line was used as a male parent to obtain haploid seeds from donor materials. This inducer was developed by the Sakarya Maize Research Institute in Türkiye.

Field-Greenhouse Experiments and Laboratory Studies

The study was carried out within an 18-month plan covering field and greenhouse trials between 2020-2022 (Figure 1). First of all, the donor materials and the inducer line were germinated in the growth chamber. Once the plants reached the third to fourth leaf stage, they were transplanted into 30-cm-diameter plastic pots and grown under greenhouse conditions in the winter period of 2018 for induction crossing. In this context, donor materials were pollinated by the ADAIL-I inducer line according to the method proposed by Prasanna et al. (2012). Controlled pollination method was used and the pollen collected from different plants belonging to the inducer line were brought together and transferred to the previously protected ears of the donor materials. Heating and lighting conditions under the greenhouse were provided at 20°C and 16/8-h photoperiod (day/night), respectively. Irrigation was carried out according to the water requirements of the plants with the drip irrigation method. Fertigation was

Figure 1. The diagram showing the steps of current study.

applied using drip irrigation fertilizer containing NPK and an equal amount of fertilizer was made with irrigation. At harvest, the ears were collected by hand and transported to the laboratory for the separation of haploid/diploid seed samples. Kernels from each ear sample were seperated according to the classification method, taking into account R1-nj (Navajo) gene expression, proposed by Prassana et al. (2012). The haploid induction ratios according to the donors were determined by the formula below, based on the number of seeds total and putative haploid kernels. These calculations were made at the cob level and then the average values were calculated according to the populations and the haploid induction rate (HIR) values of the donor materials were determined.

HIR(%) = (putative haploid/ total number of seeds) × 100

Colchicine treatment was applied as a stem injection method proposed by Zabirova et al. (1996) according to the method. For this purpose, haploid seeds were planted into the germination trays and moved to a controlled environment. When the plants reached the 3-4 leaf stage, 100 µL colchicine solution (0.125% colchicine 0.5% DMSO) was injected into the stem of haploid plants using a sterile injector (Figure 2). The plants were not watered for one day after the injection to ensure the expected effect of colchicine treatment. After several days, double haploid seedlings were transplanted to the field with 70 × 20 cm row spacing in May 2021 (Figure 2). This trial was set up in a single block and side by side rows and irrigated with a drip irrigation system. Fertilization was carried out with the drip irrigation system with the account of 8 kg of pure nitrogen and 4 kg of pure phosphorus per decare. When the plants reached the generative stage, they were pollinated in a controlled pollination method recommended by Kahriman (2016). For this purpose, field controls were made every morning between 8:00 and 10:00 A.M and pollen samples collected from doubled haploid lines were transferred to the ear of the same plant. Pollen collection was performed using a special vacuum system (Kahriman, 2021), due to the fact that doubled haploid plants could have limited pollen production. When the plants reached harvest maturity, ears were harvested by hand and DH_0 seed samples were obtained by the threshing of the ear samples.

Twelve doubled haploid lines were obtained from twenty donor materials. Some of the donors had sterility and no emergence of doubled haploid seeds. Therefore, the rest of the study was conducted using twelve doubled haploid lines. In September 2021, DH_0 seeds of twelve doubled haploid lines from the previous field trial were planted in germination viols. Emerging plants were grown in greenhouse conditions by transplanting in the 18 liter pots containing field soil and manure with 3:1 ratio (Figure 2). Applications and growth conditions in this step were kept the same as those specified in the induction crossing experiment. Each plant was selfed with the controlled pollination method recommended

by Kahriman (2016) and kernel samples were obtained for further analyses. In the greenhouse experiment where double haploids were tested, observations and measurements were made regarding the following traits.

Figure 2. Colchicine treatment, transplanting DH0 plants into the field and growing DH1 plants in the greenhouse.

Agronomic Traits: Plant height, first ear height, stem diameter measurements were carried out according to the maize technical instruction of the Seed Registration Certification Directorate (TTSM, 2018).

Crude Oil Content: It was determined using a Near Infrared Spectroscopy (NIR) device with a previously developed local calibration model (Kahriman et al., 2021).

Oleic and Linoleic Acid Content: The oleic and linoleic acid contents of the lines was determined using a local calibration model (Kahriman et al., 2021) developed on the Near Infrared Spectroscopy (NIR) device.

Zein Content: Extraction of zein fractions was carried out using 70% ethanol and 2% ß-mercaptoethanol (Yau et al., 1999). The defeated flour samples were weighed 100 mg into the eppendorf tube. One mL extraction solution was added and the tubes were incubated at 22°C for 1 hour after vortexing. Then, the samples were centrifuged at 10,000 x g for 10 min. The supernatants were taken into a new tube for further steps and preserved at + 4°C. The Bradford method was used for the quantitative content of zein proteins from the obtained extracts. In this context, 200 µL of Bradford solution was added on 50 µL samples within a 96-well microplate, and the absorbance values were recorded at 595 nm with a microplate reader (Biotek Instruments) after waiting for 45 min at 10°C. Using the same method, the standard curve was created with the BSA (Bovine Serum Albumin) standard and the zein content of the samples was determined with the help of this curve (Bradford, 1976).

Separation of Zein Fractions: Zein fractions were separated using the SDS-polyacrylamide gel electrophoresis technique in a vertical electrophoresis device. SDS-PAGE gel by 12% concentration (12.35 mL distilled water, 14.1 mL 30% stock acrylamide solution, 18.8 mL 4X Tris solution, 350 µL 10% ammonium persulfate, 35 µL TEMED) was prepared (Yau et al., 1999). The prepared gel solution was filled between the gel plates. To load samples onto the gel, the samples were kept in a hot water bath at 95°C for 5 min. Subsequently, it was centrifuged at 3000×g. After

centrifugation, 8 µL was taken from each sample and molecular standards were loaded with the samples. To complete separation, the samples were run at 100 V until blue dye reached the bottom of the gel. After the samples were separated, the gels taken from the gel tank were left in the shaker overnight with a mixture solution of 60 g TCA, 1 g Coomassie Brilliant Blue and 25 mL of ethanol made up to 500 mL with distilled water. The gel image was taken and scored according to the presence and absence of bands. The molecular weights of the bands were determined using the MW (Molecular Weights) standard (Catalog number: 26617, Sigma, USA).

Statistical Analysis

The data obtained from the study were analyzed in the R statistical package program (R Core Team, 2019). The data for the examined features were carried out with one-way analysis of variance. The Predictive PCA-Biplot graphical method was used to show the differences of genotypes in terms of the investigated traits (La Grange et al., 2009). While the vectors on these graphs show the numerical values for the examined traits, the angles between the vectors and the direction of the values are used to evaluate the correlations between the related traits. If the angle between the vectors is small and the numerical values on the vector change in the same direction, it is understood that there is a positive correlation between the traits related to these vectors, and if the vector values are in the opposite direction, there is a negative correlation. The gel images obtained from SDS-PAGE analysis were transferred to the GelAnalyzer program to encode the band values appearing in gel analyze as present/absent (1/0), and the molecular weights of the bands appearing in the samples were determined according to the band positions of the molecular standard. Genotype profiles were scored according to molecular weights and genotypic evaluation was made according to variation of zein band fractions.

Results and Discussion

Development of Homozygous Lines by In-vivo Maternal Haploid Technique

The HIR values of donor materials according to the number of seeds obtained from induction crossing and number of putative haploid seeds are presented in Table 1. According to the donor materials, haploid induction rates varied between 6.08% and 11.61%. Considering these values of the donors, the average HIR of the ADAIL-1 inducer line was calculated as 8.20%. In different studies, it is seen that HIR values vary in a wide range in the induction crossing of different inducer lines and different donors. HIR values were found between 7.1% and 12.8% in 7 donor materials by Zararsız et al. (2019). In another study, five donor materials and two inducers were used and HIR values were found between 9.20% and 16.10%. Although there is no study on the change in HIR value of the same inducer line in different locations or conditions, it has been emphasized in previous studies that the HIR values of the inducer lines may change depending on environmental conditions (Rotarenco et al., 2010). If the donor materials carry some inhibitory genes that prevent the effect of the Navajo genes, some unexpected situations may be encountered in the calculations of the HIR value (Chaikam et al., 2015) observed that HIR value of some donor materials (population 9) were significantly low, while others were found to be high, which may be a result of this situation. On the other hand, the ADAIL-1 inducer line was never tested under greenhouse conditions during its breeding process. It was stated in the paper on the development that this inducer had an HIR value of 11-12% under field conditions (Cengiz & Esmeray, 2021). The HIR value of the ADAIL-1 inducer in greenhouse conditions was tested for the first time in our study. The fact that this inducer line has a lower HIR value under greenhouse than field conditions could be attributed to the fact that it was not tested for HIR values under greenhouse during the development process.

Table 1. The number of seeds obtained induction crossing, the number of putative haploids and HIR values by source materials in this study

Code	Number of Seeds of Induction Crossing	Putative Haploids according to R1-nj Marker	Haploid Induction Rate (%)
Pop 1	616	43	7.00
Pop 2	220	21	9.43
Pop 3	331	25	7.51
Pop 6	286	21	7.28
Pop 8	301	30	9.85
Pop 9	507	41	8.09
Pop 10	430	50	11.61
Pop 15	305	23	7.69
Pop 16	362	22	6.08
Pop 17	510	42	8.16
Pop 18	217	20	9.07
Pop 19	469	31	6.64
	Total:4554	Total:368	Mean:8.20

Table 2. Descriptive statistics and results of analysis of variance about the investigated traits

Trait	Descriptive Statistics				Analysis of Variance
	Mean	Minimum	Maximum	Std. Dev.	Mean of Squares
Plant Height (cm)	179.0	124.0	250.0	29.9	9720.7**
First Ear Height (cm)	77.9	54.3	120.0	17.8	3453.5**
Stem Diameter (cm)	1.58	0.70	2.10	0.421	1.93**
Protein (%)	9.74	6.75	13.70	1.94	41.03**
Oil Content (%)	4.15	2.39	7.54	1.4	21.27**
Oleic Acid (%)	22.2	15.3	31.0	4.8	251.04**
Linoleic Acid (%)	60.7	50.4	67.8	5.43	321.7**
Zein (%)	4.77	4.58	5.04	0.172	0.32**

** statistically significant at the $p<0.01$ level.

Agronomic and Kernel Quality Characteristics of the Doubled Haploid Lines

Descriptive statistics and variance analysis results related to the traits examined in the study are shown in Table 2. Among the agronomic traits examined, the plant height was found between 124 cm and 250 cm, the first ear heights were between 54 cm and 120 cm, and the stem diameter was between 0.70 and 2.10 cm. In terms of kernel quality characteristics, the oil contents of the lines were found between 2.39% and 7.54%, oleic acid contents were found between 15.34% and 30.98%, and linoleic acid contents were found between 40-50% and 67.8%. While the protein contents of the lines were varied between 6.75% and 13.70%, the minimum and maximum values of the lines in terms of zein content were determined as 4.58% and 5.04%, respectively. The results of the analysis of variance showed that there were significant differences between the lines for all the traits examined (Table 2).

The results of some agronomic traits and kernel quality characteristics of the lines in the experiment carried out with these seeds under greenhouse conditions are shown in Figure 3a and Figure 3b. According to the Predictive Principal Component Analysis-Biplot (P-PCA-Biplot) method, it is seen that the lines have a remarkable variation in terms of agronomic

and kernel quality characteristics. The lines numbered 15, 16, 18, and 19 were above the average of lines in terms of plant height (PH) and first ear height (FEH). In terms of stem diameter, it was determined that lines 1, 10, 15, 16, 17, 18, 19 had values above the average (Figure 3a). In the study by Bayhan et al. (2021), in which homozygous pure lines were tested under greenhouse conditions, the plant height was found between 103.75 cm-190.00 cm, the first ear height was between 29.00 cm and 63.00 cm, and the stem diameter between 6.54 mm and 8.45 mm. Although the results obtained in our study were within the limits specified values, it was observed that some genotypes were out of the limits than the reported ranges. It has been emphasized that environmental factors such as lighting and temperature, may affect plant growth in studies carried out under controlled conditions and plant growth differed from field conditions (Poorter et al., 2016). Although greenhouse conditions are controlled environments, differences may arise between greenhouse trials carried out in different regions due to the climatic characteristics of the region where the greenhouse is located and the characteristics of the light source used (LED, Halogen, etc.). On the other hand, the materials used in these studies are homozygous lines obtained from different donor materials. There may be
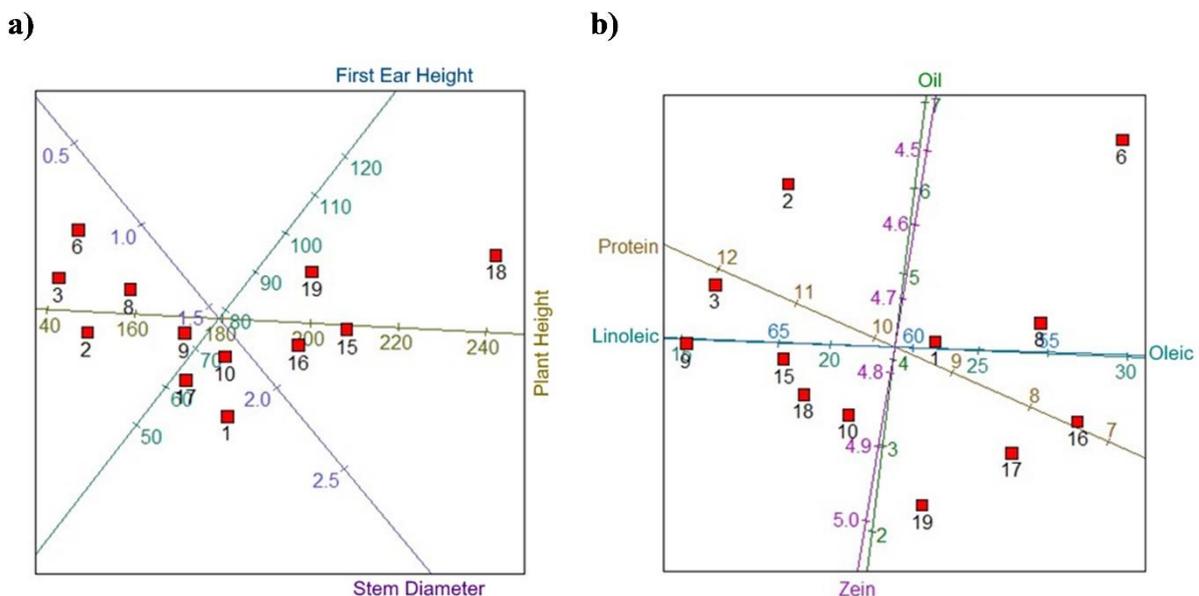
a)

b)

Figure 3. The results of Predictive PCA-Biplot Analysis for agronomic (a) and grain quality (b) traits.

differences in the results obtained between studies due to genotypic effects.

It was determined that genotypes 2 and 6 were genotypes with higher oil content compared to the others. They could be considered "High Oil Maize" given the fact that their average for oil contents was higher than the previously mentioned limit (>6%). In terms of zein content, lines 16, 17 and 19 were found to have values above the average. In terms of protein content, lines 2, 3 and 9 had higher protein content than the other lines. It was determined that the lines with high oleic acid content were lines 6, 8 and 16, while lines 3 and 9 were prominent in terms of linoleic acid content (Figure 3b). The number of studies examining kernel quality traits in studies conducted under greenhouse conditions is limited. On the other hand, it was determined that there was a significant variation for kernel quality characteristics in maize inbred lines in studies carried out under field conditions. In a study conducted by Kahriman et al. (2016), the inbred lines with different genetic backgrounds showed a considerable variation for protein (9.6% to 21.7%), oil contents (3.33% to 14.5%), oleic acid contents (22.1% to 47.1%), and linoleic acid contents (40.7% to 63.8%). While the results obtained in our research were between the values specified for protein and oil, it was noted that there were genotypes outside the specified limits in terms of other characteristics. As in the agronomic traits, these differences could be attributed to the growing conditions and genotypic effects.

When the values on the vectors of agronomic traits and the angles between the vectors are taken into account in PPCA-Biplot graphics, it can be interpreted as there is a positive correlation between stem diameter, first ear height and plant height (Figure 3a). In addition to studies reporting a positive correlation between plant height and stem diameter in maize (Mousavi & Nagi, 2021), positive correlations are also reported between plant height and first ear height (Sadek et al. 2006). According to the PPCA-Biplot for quality traits (Figure 3b), it was observed that there was a negative correlation between zein and oil contents. Ray et al. (2019) attributed the negative correlation between oil and protein content in maize to the differences in the proportional weights of embryo and endosperm in the maize kernel. They suggested that the increase in embryo size increases the oil content, and this situation decreases the protein content because it decreases the proportional share of the endosperm. Zein proteins are the predominant type of protein in maize and constitute 50-60% of the total protein. Therefore, the negative correlation between oil and zein content in our study was also found and it could be related to the situation described by Ray et al. (2019). There was a negative correlation between oleic and linoleic acid (Figure 3b). High and negative correlations between two fatty acids in maize were also determined in previous studies (Baldin et al., 2018, Ray et al., 2019). This relationship has been associated with the activity of the n-6

desaturase enzyme and is based on the fact that linoleic acid synthesis occurs from oleic acid.

Protein Band Analysis

According to the results of protein band analysis, it was determined that there were some differences in zein fractions in doubled haploid lines. SDS-PAGE gel analysis showed that 19 kDa and 22 kDa bands are present in all lines (Figure 4) In addition to these bands, 27 kDa zein fractions and 15 kDa fractions were also observed in some lines. On the other hand, 27 kDa zein bands were not found in lines 2, 6 and 8. These lines are genotypes with higher values in terms of oil content than the others. Although zein band analyzes were performed for qualitative discrimination in current study, it was noted that 27 kDa band intensities of some genotypes (3 and 9) were higher than the others despite the same amount of sample loaded to SDS-PAGE gel.
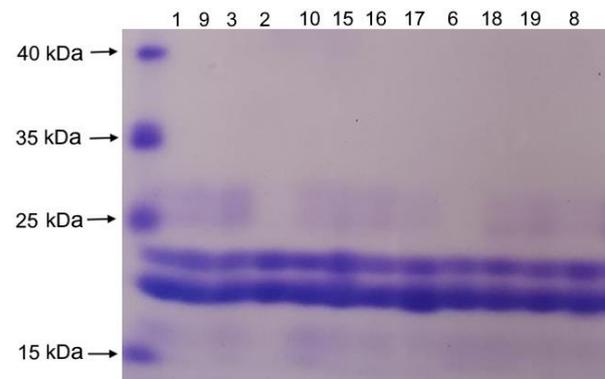

Figure 4. Distribution of zein fractions in SDS-PAGE gel analysis.

Although zeins are the dominant fraction in maize proteins, band variation may vary according to different genotypes. Zein proteins are divided into two subgroups, prolamins and prolamin-like proteins. These groups are mainly separated depending on the differences in the disulfide bonds. Zeins classified as prolamin group are 19 and 22 kDa α-zeins, while prolamin-like zein differences include γ (50, 27 and 16 kDa), β (15 kDa) and δ (18, 10 kDa) zein fractions (Feng et al., 2009). The densities of the sub-fractions vary according to the formation stages of the zein structures in the maize kernel. While zein protein structures consist of γ and β fractions in the early stage, α and δ fractions begin to form in the middle developmental stage, and α-zein fractions become dominant in the last stage (Holding, 2014). While α-zein fractions with a size of 19-22 kDa are located in the inner part of the zein structure, whose formation is completed, β and γ fractions are localized in the outer layers. The higher amount of α-zein in maize genotypes compared to other fractions is related to this formation process. In most of the lines developed in our study, bands belonging to the prolamin group fractions were observed to be intense. γ-zein fractions were not found in lines with high oil content.

Conclusion

As a result of the study, twelve doubled haploid lines from local maize populations were developed. It was noted that the inducer line used in terms of HIR value had a relatively lower value in greenhouse conditions than its previously reported HIR value. It was determined that there was a remarkable variation between the lines in terms of the oil and zein content. However, in SDS-PAGE analysis, a similar profile was observed in most of the lines in terms of subfractions of zein proteins. Although the agronomic performances of the developed lines are considered sufficient under greenhouse conditions, they should also be tested under field experiments. Among the developed homozygous materials, two lines were high-oil (>6%) maize (numbered 2 and 6). In addition, it was determined that three lines (16, 17, and 19) had higher zein content than other lines. However, there is a need to test these genotypes both under field conditions and to evaluate their combination abilities with other lines. Registration of parent lines and hybrids may be possible if promising hybrid combinations are available.

Acknowledgements

Author Contributions

FK: Designed, Performed, Analyzed, Writing - review and editing; AK: Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing - original draft; AMG: Investigation, Methodology, Writing -review and editing; and NNY: Data Curation, Formal Analysis, Investigation, Methodology.

References

Anderson, T.J., & Lamsal, B.P. (2011). Zein extraction from maize, maize products, and coproducts and modifications for various applications: A Review. *Cereal Chemistry,* (88), 159-173. https://doi.org/10.1094/CCHEM-06-10-0091

Anonymous, (2019). Zein protein market forecast, trend analysis & competition tracking - global Review 2018 to 2028. https://www.factmr.com/report/654/zein-protein market

Baldin, M., Ying, Y., Fan, Y., Roth, G., Casper, D. P., & Harvatine, K. J. (2018). Characterization of linoleic acid (C18:2) concentration in commercial corn silage and grain hybrids. *Journal of Dairy Science, 101*(1),222-232. https://doi.org/10.3168/jds.2017-12972

Bayhan, M., Özkan, R., Albayrak, Ö., Yıldırım, M., & Akıncı, P. D. C. (2021). İn vivo double haploid tekniği ile yerel çeşitlerden elde edilen haploid bitkilerin saf hat olarak kullanilabilirliğinin belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 24(5), 1029-1036. https://doi.org/10.18016/ksutarimdoga.vi.825121

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemestry, (*722), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Cengiz, R., & Esmeray, M. (2021). Development of late temperate *in vivo* haploid inducers, *Genetika*, Vol. (53), No1, 51-64. https://doi.org/10.2298/GENSR2101051C

Cerit, İ., Cömertpay, G., Oyucu, R., Çakır, B., Hatıpoğlu, R. & Özkan, H. (2016). Melez Mısır Islahında In-vivo Katlanmış Haploid Tekniğinde Kullanılan Farklı Inducer Genotiplerin Haploid İndirgeme Oranların Belirlenmesi "Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi" 52-57. https://doi.org/10.21566/tarbitderg.280162

Chaikam, V., Nair, S. K., Babu, R., Martinez, L., Tejomurtula, J., & Boddupalli, P. M. (2015). Analysis of effectiveness of R1-nj anthocyanin marker for *in vivo* haploid identification in maize and molecular markers for predicting the inhibition of R1-nj expression. *Theorotical Applied Genetics 128*(1),159–171. https://doi.org/10.1007/s00122-014-2419-3

Chalyk, S. T. (1994). Properties of maternal haploid maize plants and potential application to maize breeding. *Euphytica 79,* 13–18. https://doi.org/10.1007/BF00023571

Chidzanga, C., Muzawazi, F., Midzi, J. & Hove, T. (2017). Production and use of haploids and doubled haploid in maize breeding: A review. *African Journal of Plant Breeding* 4: 201-213.

Eder, J., & Chalyk, S. (2002). In vivo haploid induction in maize. *Theor. Appl. Genet, 104(*4), 703-708. https://doi:10.1007/s00122-001-0773-4

Erdal, S., Cengiz, R., & Ozturk, A. (2019). Breeding doubled haploid maize inbred lines for methionine and lysine amino acid composition. Maydica, *64*(2), 1-10.

Erenstein, O., Chamberlin, J., & Sonder, K. (2021). Estimating the global number and distribution of maize and wheat farms. *Global Food Security*, *30*, 100558. https://doi.org/10.1016/j.gfs.2021.100558

Feng, L., Zhu, J., Wang, G., Tang, Y., Chen, H., Jin, W., Wang, F., Mei, B., Xu, Z., & Song, R. (2009). Expressional profiling study revealed unique expressional patterns and dramatic expressional divergence of maize alpha- zein super gene family. *Plant Molecular Biology*, (69), 649–659. https://doi.org/10.1007/s11103-008-9444-z

Foley, T. (2009). Plants and seeds of high oil corn variety HOOO1, *US Patents,* US007495155B2.

Holding, D. R. (2014). Recent advances in the study of prolamin storage protein organization and function. *Frontiers in Plant Science*, (*5*), 276. https://doi.org/10.3389/fpls.2014.00276

Kahrıman F. (2016). Mısırda polen etkisi ve bu etkinin kontrolünde uygulanan yöntemler, Lambert Academic Publishing, Saarbrükhen.

Kahrıman, F., Songur, U., Dişbudak, A., Kızık, S. & Vural, B. (2022). Effects of donor x inducer interaction on the success of haploid induction and comparison of haploid seed identification methods in the *in vivo* maternal haploid technique in maize. *Journal of Agricultural Sciences, 28*(3), 385-395, 22-22. https://doi.org/10.15832/ankutbd.824114

Kahrıman, F., (2021). Polen toplama ve filtreleme sistemi, Ulusal Faydalı Model, Başvuru Numarası: 2021/018211.

Kahrıman, F., Sütal, A., Topçakıl, M. & Gezer, Ö. (2021) Prototype near-infrared (NIR) reflectance spectrometer for the analysis of maize flour, *Instrumentation Science & Technology*, *49*(5), 521-531. https://doi.org/ 10.1080/10739149.2021.1890611

Kahriman, F., Egesel, C. Ö., Orhun, G. E., Alaca, B., & Avci, F. (2016). Comparison of graphical analyses for maize genetic experiments: Application of biplots and polar plot to line x tester design. *Chilean Journal of Agricultural Research*, *76*(3), 285-293. https://doi.org/10.4067/S0718-58392016000300004

La Grange, A., Le Roux, S., & Gardner-Lubbe, S. (2009). BiplotGUI: Interactive biplots in R. *Journal of Statistical Software 30*(1), 1-37. https://doi.org/10.18637/jss.v030,i12

Lambert, R., (2001). High-oil corn hybrids. In: Zallauer A (ed) specialty maizes, 2nd edn. *CRC Press, Boca Raton, pp* 131–154.

Mazur, M., Vila, S., Brkić, I., Jambrović, A. & Šimić, D. (2019). The development of homozygous maize lines using an in vivo haploid induction in the Croatian germplasm. *Poljoprivreda, 25*(1), 19-25. https://doi.org/10.18047/poljo.25.1.3

Mousavi, S. M. N., & Nagy, J. (2021). Evaluation of plant characteristics related to grain yield of FAO410 and FAO340 hybrids using regression models. *Cereal Research Communications,* (*49*)**,** 161–169. https://doi.org/10.1007/s42976-020-00076-3

Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., van der Putten, W.H., Kleyer, M., Schurr, U., & Postma, J., (2016). Pampered inside, pestered outside, differences and similarities between plants growing in controlled conditions and in the field. *New Phytologist*, (*212*), 838-855. https://doi.org/10.1111/nph.14243

Prasanna, B. M., Chaikam, V., & Mahuku, G. (2012*).* Doubled haploid technology in maize breeding: *Theory and practice*. Mexico, D. F., CIMMYT.

R Core Team. (2019) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ray, K., Banerjee, H., Dutta, S., Hazra, A. K., & Majumdar, K., (2019). Macronutrients influence yield and oil quality of hybrid maize (*Zea mays* L.*). Plos One, 14(5), e0216939.* https://doi.org/10.1371/journal.pone.0216939

Ren, J., Wu, P., Trampe, B., Tian, X., Lübberstedt, T., & Chen, S. (2017). Novel technologies in doubled haploid line development. *Plant biotechnology journal*, *15*(11), 1361–1370. https://doi.org/10.1111/pbi.12805

Rotarenco, V., Dicu, G., & Fuia, S. (2010). New inducers of maternal haploids in maize. *Maize Genetics Cooperation Newsletter*, 84, 21-22.

Sadek, S. E., Ahmed, M. A., & Abd El-Ghaney, H. M. (2006). Correlation and path coefficient analysis in five parents inbred lines and their six white maize (Zea mays L.) single crosses developed and grown in Egypt. *Journal of Applied Sciences Research*, *2*(3), 159-167.

Schmidt W. (2003) Hybrid maize breeding at KWS SAAT AG. In: Bericht über die Arbeitstagung der Vereinigung der Pflanzenz üchter und Saatgutkaufleute Österreichs, Gumpenstein, Österreich, 25–27 November, pp. 1–6.

Seitz G. (2005) The use of doubled haploids in corn breeding. In: Proc. of the 41th Annual Illinois Corn Breeders' School 2005. Urbana-Champaign, Il, USA, p. 1–7.

Singh, N., Vasudev, S., Yadava, D., Chaudhary, D., & Prabhu, K. (2014). Oil Improvement in Maize: Potential and Prospects. In Maize*: Nutrition Dynamics and Novel Uses*. Springer, Indian, 77-82. https://doi.org/10.1007/978-81-322-1623-0_6

TTSM, (2018). Mısır Teknik Talimatı. https://www.tarimorman.gov.tr/BUGEM/TTSM

TUIK, (2020). Türkiye İstatistik Kurumu Verileri. www.tuik.gov.tr

Yau, J. C., Bockholt, A. J., Smith, J. D., Rooney, L. W. &Waniska, R. D. (1999). Maize endosperm proteins that contribute to endosperm lysine content. *Ceral Chemistry*, (76), 668-672. https://doi.org/10.1094/CCHEM.1999.76.5.668

Zabirova, E. R, Chumak, M. V., Shatskaia, O. A. & Scherbak, V. S. (1996). Technology of the mass accelerated production of homozygous lines (in Russian). Kukuruza Sorgo 4: 17-19

Zararsız, D., Yanıkoğlu, S., Öztürk, L., Turgut, İ., Kızık, S., & Bilgin, B. (2019). Production of double haploid plants using *in vivo* haploid techniques in maize. *Journal of Agricultural Sciences*, *25*(1), 62-69. https://doi.org/0.15832/ankutbd.539000