

Screening of genetic variability in Turkish maize landraces for protein and starch related traits

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Abbreviations:

PV: Phenotypic variance, GV: genotypic variance, EV: environmental variance, GCV: Genetic coefficient of variability, PCV: Phenotypic coefficient of variability, ECV: Environmental coefficient of variability, hBS: Broad sense heritability, GA: Genetic advance, GAM: Genetic advance over to mean, NIR: Near infrared reflectance.

Abstract

Local populations of maize are valuable resources to maintain the genetic variability within the species. Breeding programs in many different countries try to characterize and exploit such germplasm. The objective of this study was to evaluate 192 Turkish maize landraces for their variation in terms of protein ratio, the amino acids that affect the protein quality, starch ratio as well as its fractions. Field experiment was carried out at the Crop Research and Application Unit of Çanakkale Onsekiz Mart University Agricultural Faculty Farm, and used an augmented design with 6 blocks. Each block contained 32 landraces and 7 standard hybrids. Data were collected on protein, lysine, tryptophan, starch, amylose, and amylopectin content. Analysis of variance was run, and genetic calculations were utilized to determine the heritability values. The results suggested that Turkish maize landraces possess a considerable variation for protein and starch traits. The ranges determined for protein, starch, amylose, amylopectin, lysine, and tryptophan were 6.56-16.50%, 56.38-79.63%, 2.09-35.25%, 64.75-97.91%, 0.12-0.93%, and 0.03-0.09%, respectively. The broad sense heritability values for the investigated traits were between 16-53%. Several landraces were detected to be superior to the standard varieties for some traits and considered to be valuable genetic material for the breeding studies to come.

Introduction

Today, hybrid varieties are widely used in maize production. However, genetic variation in maize has been significantly reduced by hybrid breeding (Troyer, 2001). In recent years there has been a trend towards the development of specialty maize varieties for food and industrial use. In the breeding programs initiated for this purpose, local maize populations became important breeding sources for the development of maize genotypes with enhanced grain quality characteristics (Newton et al., 2010). In this context, efforts have been made to screen local maize populations in terms of major or minor grain quality components and to integrate them into modern breeding purposes.

The major biochemical components in maize are starch, proteins, and lipids. The proportional quantity of these components and the composition of the sub-components have a decisive role in the use and quality of maize products. In normal maize genotypes, the protein

content varies between 8 and 11%. Protein quality is of no less value than protein content and it is an important aspect that is desired to be developed in maize used especially for food purposes. Zein proteins, which are the predominant type of storage proteins in maize kernel, are deficient in lysine and tryptophan amino acids (Vasal, 2000). The decrease in this protein group allows increasing the concentration of essential amino acids lysine and tryptophan (Prasanna et al., 2001), which are the target amino acids to increase protein quality in maize grain. Another major biochemical component in maize kernel is starch. The starch content varies between 61-78% in a normal maize grain (Watson, 2003), and the sub-components of starch are amylose and amylopectin. These two sub-components, which differ in their chain structure, have a decisive feature in the use of maize grain for different purposes (Ai and Jane, 2016).

Studies have been conducted in different countries in

order to screen local maize genotypes in terms of both agronomic characteristics and grain quality characteristics. With these studies, the existing potentials of maize genetic resources have been tried to be revealed. Berardo et al. (2009) conducted a study to determine the available variation for protein, oil, and carotenoid concentrations in 1245 different local maize accessions, and stated that they had a significant variability in the characteristics examined. From these materials, a hybrid with high carotenoid content was developed and introduced into the Italian industry. VazPatto et al. (2009) evaluated 46 different landraces cultivated in Portugal for their protein, oil, amylose content, fiber content and viscosity properties and emphasized that these populations were important sources in food research and should be protected. In comparison with other countries, there are studies where Turkish maize landraces has been screened for major grain quality characteristics such as protein and oil (Öner 2011; Cömertpay et al., 2016), but the studies on screening minor components of protein and starch quality are rather limited (Ünlü et al., 2018). Therefore, a material screening study was needed in order to determine the utilization potential of Turkish maize landraces.

This study aims to determine the variation of protein and starch content and sub-components of these components in Turkish local maize populations and to evaluate their heritability in quality breeding programs.

Materials and Methods

Plant Material and Experimental Details

In this study, a total of 192 local maize landraces collected from different regions of Turkey were used as plant material (Table 1). In addition to local maize, seven maize varieties (Hido, Synove, 75MAY75, 72MAY80, Calicio, Caraella and Reserve) were included in the experiment as standard hybrids. The field experiment to evaluate the families was carried out in summer growing season of 2018, at Çanakkale Onsekiz Mart University (ÇOMÜ) Agricultural Farm, Plant Production Research and Application Unit, in Turkey (long: 26.4°N and lat. 40.1°E). The experiment used an augmented design which consisted of six blocks. Each genotype was planted in 4 m single-rows plots with 0.7 x 0.2 m spacing. Each block consisted of 39 rows and standard varieties were planted in random order in each block. The plots were drip-irrigated as needed and fertilized with a total of 180 kg ha⁻¹ nitrogen and 80 kg ha⁻¹ phosphorus in two occasions (before planting and before flowering). To preserve the genetic structure in the local populations, 3-5 plants were hand pollinated in each plot as described by Kahrıman (2016).

Measurement of Kernel Quality Traits

Protein and Starch Content

Protein and starch ratios were determined by NIR (Near Infrared Reflectance) spectroscopy. For NIR measurements, kernels taken from 3-5 hand-pollinated ear samples from each plot were grinded in a laboratory mill (Fritsch pulverisette14, Germany) through a 0.5 mm sieve, and approximately 50 g of sample was placed in the rotary container of NIR device (Spectrastar 2400D, USA). Then, the spectra taken at each nanometer between 1200-2400 nm were applied to local calibration models previously developed in our laboratory (Egesel and Kahrıman, 2012) to estimate the protein and starch ratios of the samples.

Tryptophan Content

Tryptophan contents of the samples were determined according to the method proposed by Galicia et al. (2009). For tryptophan analysis, 30 mg oil extracted samples were taken into Eppendorf tubes. 1.125 mL of papain solution was added onto the samples. Samples were maintained at 64 °C for 16 hours and vortexed 1 hour after being placed in the oven and 1 hour before removal. After cooling to room temperature, the samples were centrifuged at 3600 rpm for 5 min and the supernatant was taken to a clean tube for analysis. 50 µL were taken from the supernatant, placed in a 96-well microplate and 150 µL of colorimetric solution was added. After vortexed, the samples were incubated at 64 °C for 30 min and then allowed to cool down to room temperature. After the samples cooled, absorbance values at 560 nm were recorded in the microplate reader. The tryptophan contents of the samples were determined using a standard curve prepared with tryptophan standard.

Lysine Content

The lysine contents of the samples were determined according to the method proposed by Galicia et al. (2009). For lysine analysis, 100 mg of oil extracted samples were taken into a 15 mL falcon tube. 5 mL of papain solution was added to the samples. The samples were kept at 64 °C for 16 hours and vortexed 1 hour after placing in the oven and 1 hour before removal. Having cooled to room temperature, the samples were centrifuged at 2500 rpm for 5 minutes and the supernatant was taken to a clean tube for analysis. 1 mL of the supernatant was taken, and 0.5 mL of carbonate buffer solution and 0.5 mL of copper phosphate solution were added. The samples were shaken for 5 minutes and then centrifuged at 2000 rpm for 5 min. 1 mL of supernatant was transferred to a new tube and 0.1 mL of

Table 1 - The maize landraces used in this study

Code	Region	Code	Region	Code	Region	Code	Region
TR36986	Karadeniz	TR38101	Akdeniz	TR48461	Karadeniz	TR50798	Ege
TR37006	Ege	TR38104	Karadeniz	TR48477	Marmara	TR50816	Karadeniz
TR37105	Marmara	TR38128	Ege	TR48891	Ege	TR51719	Marmara
TR37115	Karadeniz	TR38141	Akdeniz	TR48893	D. Anadolu	TR51727	Karadeniz
TR37543	Karadeniz	TR38147	Karadeniz	TR49168	G. Anadolu	TR52003	Karadeniz
TR37573	G. Anadolu	TR38172	Ege	TR49171	Ege	TR53247	Marmara
TR37583	Karadeniz	TR38208	Karadeniz	TR49197	Karadeniz	TR53254	Marmara
TR37596	Marmara	TR38240	Karadeniz	TR49225	Karadeniz	TR54192	Karadeniz
TR37597	Marmara	TR38243	Ege	TR49234	Karadeniz	TR54193	Karadeniz
TR37600	Karadeniz	TR38256	Karadeniz	TR49245	İç Anadolu	TR54196	Karadeniz
TR37601	Marmara	TR38289	Marmara	TR49260	Karadeniz	TR54197	Ege
TR37603	Ege	TR38292	Marmara	TR49271	Karadeniz	TR54199	Marmara
TR37605	Karadeniz	TR38323	Karadeniz	TR49277	Marmara	TR54216	Marmara
TR37611	Karadeniz	TR38329	Karadeniz	TR49303	Marmara	TR54217	Karadeniz
TR37618	Karadeniz	TR38337	Marmara	TR49313	Karadeniz	TR54712	Karadeniz
TR37630	Karadeniz	TR38339	Marmara	TR49318	Marmara	TR55452	Karadeniz
TR37653	Marmara	TR38341	Akdeniz	TR49323	Karadeniz	TR55461	Karadeniz
TR37719	Karadeniz	TR38343	Marmara	TR49578	Marmara	TR55463	Karadeniz
TR37720	Ege	TR38350	Marmara	TR49579	Ege	TR55464	Karadeniz
TR37735	Marmara	TR38375	Marmara	TR50125	Marmara	TR55471	Karadeniz
TR37746	Karadeniz	TR38389	Karadeniz	TR50126	Marmara	TR55476	Ege
TR37754	Marmara	TR38401	Karadeniz	TR50130	Ege	TR55479	Ege
TR37810	Karadeniz	TR38422	Karadeniz	TR50131	Akdeniz	TR55480	Ege
TR37861	Karadeniz	TR38435	Marmara	TR50216	Ege	TR55481	Marmara
TR37876	Marmara	TR38439	Ege	TR50220	Karadeniz	TR55484	Karadeniz
TR37882	Karadeniz	TR38451	Ege	TR50250	Marmara	TR55485	Ege
TR37912	Karadeniz	TR38457	Karadeniz	TR50505	Marmara	TR55486	Ege
TR37918	G. Anadolu	TR40604	Marmara	TR50511	Marmara	TR55488	Karadeniz
TR37924	Ege	TR42576	Karadeniz	TR50513	Karadeniz	TR55491	Karadeniz
TR37932	Marmara	TR42591	Marmara	TR50515	Karadeniz	TR55492	D. Anadolu
TR37940	Karadeniz	TR42641	Ege	TR50516	Karadeniz	TR55502	Karadeniz
TR37941	Karadeniz	TR42689	Marmara	TR50524	Marmara	TR55506	Ege
TR37953	Karadeniz	TR42703	Karadeniz	TR50547	D. Anadolu	TR55507	Karadeniz
TR37955	Ege	TR42712	Marmara	TR50549	Karadeniz	TR55508	Karadeniz
TR37958	Karadeniz	TR42725	G. Anadolu	TR50550	Karadeniz	TR55510	G. Anadolu
TR37969	Karadeniz	TR42750	Karadeniz	TR50551	Marmara	TR55513	Karadeniz
TR37970	Marmara	TR42856	Karadeniz	TR50555	Karadeniz	TR55518	Marmara
TR37974	Karadeniz	TR42868	Karadeniz	TR50558	Karadeniz	TR55521	Karadeniz
TR37984	Ege	TR42877	Ege	TR50559	Ege	TR55522	Karadeniz
TR37986	Ege	TR42948	Akdeniz	TR50564	Karadeniz	TR55527	Karadeniz
TR37995	G. Anadolu	TR42949	Karadeniz	TR50566	Marmara	TR55528	Marmara
TR37998	Karadeniz	TR42985	Karadeniz	TR50585	Marmara	TR55533	Karadeniz
TR38008	Karadeniz	TR44385	Karadeniz	TR50587	Marmara	TR55534	Marmara
TR38024	Marmara	TR44410	Marmara	TR50588	Marmara	TR55540	Marmara
TR38026	Karadeniz	TR44501	Marmara	TR50641	Marmara	TR55542	Marmara
TR38040	Karadeniz	TR45102	Ege	TR50642	Ege	TR55545	Ege
TR38064	İç Anadolu	TR48449	Marmara	TR50670	Karadeniz	TR57654	Karadeniz
TR38100	Marmara	TR48454	Ege	TR50683	Marmara	TR57658	Marmara

2-chloro-3,5-dinitro-pyridine was added and vortexed. The samples were awaited at room temperature for 2 hours while shaken in every 30 min. 5 mL of 1.2 N HCl was added to the samples and vortexed. Then 5 mL ethyl-acetate was added, and the tubes were turned upside down 10 times. The supernatant was removed by polyethylene syringe and the absorbance value of the sample at 390 nm was determined by spectrophotometer. To determine the lysine content, a standard curve prepared with lysine standard was used.

Amylose and Amylopectin Content

The amylose and amylopectin contents of the samples were determined by the method of Kaufman et al. (2015). Samples of 5 mg were taken into a 2 mL centrifuge tube, then 1 mL of 90% dimethyl sulfoxide (DMSO) in water was added on and vortexed every 10 min and heated to 95 °C for 60 min. Following starch dispersion, the tubes were cooled down for 5 min and 100 µL sample from each tube was taken into a well on a 96-well plate. Then, 100 µL 90% DMSO 3.04 g/L iodine was added to each well and plate was shaken for 2 min. Two wells were filled with the control blank, 100 µL 90% DMSO plus 100 µL 90% DMSO 3.04 g/L iodine. From each well 20 µL sample was pipetted into an empty plate, then 180 µL of deionized water was added to each well and plate was shaken for 2 min. Then, the plate was analyzed for absorbance at 620 nm and 510 nm. A regression equation was determined by using both the absorbance value at 620 nm and Diff ABS (ABS620 - ABS510) for the standard curve in each plate analyzed. The amylose content of the samples was calculated using following equation: Amylose content (%) = (Diff ABS – y-intercept of regression/slope of regression).

Statistical Analysis

Data collected from the study were analyzed based on augmented experimental design. Analysis of variance was performed in the R program (R Core Team, 2018) using the augmented-RCBD package (Aravind et al., 2019). Descriptive statistics such as mean, standard deviation, standard error, skewness and kurtosis values were calculated to show variability within the tested materials. Additionally, boxplot graphs were

used to compare standard varieties and populations for the investigated traits. Each boxplot displayed the 3 landraces with the highest and 3 landraces with the lowest values as well the standard varieties for the respective traits. For each trait, phenotypic variance (PV), genotypic variance (GV), environmental variance (EV), genetic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), environmental coefficient of variability (ECV), broad sense heritability (hBS), genetic advance (GA) and genetic advance over to mean (GAM) values were calculated by Aravind et al. (2019). The correlations among the protein and starch traits were investigated by Pearson Moment correlation.

Results

Analysis of Variance

Descriptive statistics for the investigated traits are presented in Table 2. It was found that there was a significant variation in the genotypes for all the variables. When the mean values were taken into consideration, the materials used here were similar to the values obtained from normal maize genotypes within the limits of scientific literature. However, minimum and maximum values obtained suggested that some genotypes could be classified as specialty maize within these materials. For example, the maximum value for tryptophan content was above the limit value specified in the literature (Table 2). On the other hand, skewness and kurtosis values for the investigated traits indicated that the distribution of data was close to normal except for protein content, which showed a skewed distribution to the left (Table 2).

Analysis of variance results are shown in Table 3. The results of the analysis of variance showed that there were statistically significant differences in lysine and protein content for the complete set of genotypes used when the block effect was ignored. The in-group variation was significant for the standard varieties for protein, amylose and amylopectin content. In local populations, lysine content has been found to have a significant variation (Table 3).

Protein content, amylose content and lysine content were higher in the local maize populations than in the

Table 2 - Descriptive statistics of observed traits for all genotypes

Trait	n	Mean	Std.Err.	Std. Dev.	Min.	Max.	Skewness	Kurtosis
Protein	199	10.41	0.13	1.83	6.29	15.65	0.62 **	3.27 ns
Lysine	199	0.47	0.01	0.18	0.12	0.93	0.22 ns	2.53 ns
Tryptophan	199	0.06	0.001	0.01	0.02	0.09	0.07 ns	2.69 ns
Starch	199	68.17	0.22	3.15	58.28	78.05	-0.04 ns	3.53 ns
Amylose	199	20	0.47	6.58	2.09	35.25	-0.17 ns	2.54 ns
Amylopectin	199	80	0.47	6.58	64.75	97.91	0.17 ns	2.54 ns

Table 3 - The results of variance analysis for protein and starch related traits

Source of Variation	df	Amylopectin	Amylose	Starch	Lysine	Protein	Tryptophan
Block (ignoring Treatments)	5	1075.7**	1075.7**	2.1 ^{ns}	0.09 **	1.31 ^{ns}	0.00038 ^{ns}
Block (eliminating Treatments)	5	130.2 ^{ns}	130.2 ^{ns}	10.9 ^{ns}	0.05 *	1.83 ^{ns}	0.00048*
Genotypes (eliminating Blocks)	198	46.59 ^{ns}	46.59 ^{ns}	8.39 ^{ns}	0.03 **	3.37*	0.00014 ^{ns}
Genotypes (ignoring Blocks)	198	70.46 ^{ns}	70.46 ^{ns}	8.17 ^{ns}	0.03 **	3.36 *	0.00014 ^{ns}
Check	6	197.4**	197.4**	5.69 ^{ns}	0.03 ^{ns}	7.42**	0.00026 ^{ns}
Landrace and Landrace vs. Check	192	41.87 ^{ns}	41.87 ^{ns}	8.48 ^{ns}	0.03 **	3.24 ^{ns}	0.00014 ^{ns}
Landrace vs. Check	1	700.36 **	700.3**	5.15 ^{ns}	0.02 ^{ns}	29.41**	0.00000034 ^{ns}
Landrace	191	63.18 ^{ns}	63.18 ^{ns}	8.26 ^{ns}	0.03 **	3.09 ^{ns}	0.00014 ^{ns}
Residuals	30	52.56	52.56	5.11	0.01	1.95	0.00019

* Statistically significant at 0.05 level. ** Statistically significant at 0.01 level. ns: Statistically not significant

standard varieties. The average of starch and tryptophan content was similar between standard varieties and local populations, whereas amylopectin content was significantly higher in standard varieties (Figure a, b, c, d, e). Populations coded TR55507, TR50549, TR54193 were in the top three among populations for protein content among the landraces with higher protein than the standard varieties. The grain protein contents of Calicio, Caraella and Hido varieties were higher than other standards. Among the populations, TR37916, TR37882 and TR37719 were prominent for lysine content, while Synove, Hido and 75MAY75 were the hybrids to have high levels. Standards Caraella, Reserve and Synove had higher tryptophan content than the other varieties, while TR50551, TR37719 and TR53247 coded landraces from the local populations ranked first for this trait. The variation in starch content in the standard varieties was limited, and Reserve, Synove, Hido had relatively higher levels of starch. Among the landraces, TR37754, TR55485 and TR53254 were the first three landraces with high starch content. The first three varieties with high and low values in terms of amylose and amylopectin content are the exact opposite. Of the standards, Calicio, Caraella and Reserve had higher amylose values and Hido, Synove and 75MAY75 had higher values than others with high amylopectin. TR55528, TR54712 and TR55545 from local corn populations had high amylose content, while TR37882, TR37810, TR44501 showed significant differences in amylopectin content from other landraces.

Assessment of Genetic Variability and Cross Relationships for Observed Traits

The components of the variance (PV, GV, EV) and variability coefficients (PCV, GCV, ECV) calculated from the data obtained from this study as well as the heritability values (hBS) of traits and theoretical genetic advance values (GA and GAM) are summarized in Table 4. For all other traits except lysine content, the share of environmental variance (EV) in phenotypic variance (PV) was higher than genotypic variance (GV). This difference has reached five folds, especially for amylose and amylopectin content. GCV values were grouped as high for lysine content, moderate for protein and amylose content, and low for starch content (Table 4). Broad sense heritability values (hBS) were moderate (between 30%-60%) for protein, lysine and tryptophan contents, and low for amylopectin (16.81%). Although the maximum values of the theoretically possible genetic advance (GA) were calculated for amylose and amylopectin content, it was the lysine content (GAM = 40.44%) that this progress was the highest in comparison to the mean value (Table 4). According to these calculated values, it is possible to say that there is a remarkable genetic variation in the material used for lysine content. Correlation analysis results showed that starch content had negative correlation with protein and tryptophan contents. A significant positive correlation was found between protein content and tryptophan content. The other correlations were statistically insignificant (Table 5).

Table 4 - Genotypic and phenotypic variability parameters for the investigated traits.

Trait	Mean	PV	GV	EV	GCV	PCV	ECV	hBS	GA	GAM
Protein	10.41	3.09	1.14	1.95	10.25 ^M	16.89 ^M	13.42	36.86 ^M	1.34	12.84 ^M
Lysine	0.47	0.03	0.02	0.01	26.76 ^H	36.52 ^H	24.85	53.69 ^M	0.19	40.44 ^H
Tryptophan	0.06	0.00014	0.0001	0.00019	0.02	21.23 ^H	24.63	52.6 ^M	0.0001	0.18 ^M
Starch	68.17	8.26	3.15	5.11	2.61 ^L	4.22 ^L	3.32	38.17 ^M	2.26	3.32 ^L
Amylose	20	63.18	10.62	52.56	16.29 ^M	39.73 ^H	36.24	16.81 ^L	2.76	13.78 ^M
Amylopectin	80	63.18	10.62	52.56	4.07 ^L	9.94 ^L	9.06	16.81 ^L	2.76	3.45 ^L

M: Moderate, H: High, L: Low.

Table 5 - The correlation coefficient among protein and starch related traits.

	Starch	Amylose	Amylopectin	Protein	Lysine
Amylose	0.06				
Amylopectin	-0.06	-1.00**			
Protein	-0.54**	0.01	-0.01		
Lysine	0.07	0.06	-0.06	0.04	
Tryptophan	-0.25**	0.11	-0.11	0.33**	0.06

** Statistically significant at 0.01 level.

Discussion

Genetic Variation for Protein and Starch Related Traits

During the last century, due to the increasing use of maize in human and animal nutrition, research on its grain quality has increased (Ai and Jane, 2016). A normal maize grain consists of three main parts (endosperm, embryo, seed coat) and the highest share of these parts belongs to the endosperm. Improving grain quality in maize depends on the amount of macro biomolecules found in these parts and the composition of the subcomponents of these molecules.

The highest share in maize grains belongs to structural and non-structural carbohydrates, while proteins come in the second place (Watson, 2003). Structural carbohydrates form starch, while simple sugars and other sub-components are non-structural carbohydrates. Starch is composed of amylose and amylopectin and, unlike linear and short chain amylose amylopectin is a long chain and multi-branched structure (Hizukuri, 1986). In the genotypes having a waxy endosperm, the amylose content is negligible and the branching structure of the amylopectin molecules varies. In contrast to this type of maize, high amylose genotypes contain 50-85% amylose. Both types of endosperms are specifically used manufacturing many different food and industrial products (Ai and Jane, 2016). On the other hand, digestibility is low in maize grains with high amylose and long amylopectin chain branch structure. Based on all these considerations, it is possible to say that the value of maize grain as food/feed and in other fields greatly depends on the content of starch components, amylose, and amylopectin. The genetic material used in this study was found to have a significant variation in starch content (min = 58.3%, max = 78.5%). Cömertpay et al. (2016) reported a range of 73.3-80% for starch content within 79 Turkish maize populations. When the starch sub-components were taken into consideration, it can be stated that the amylose content of the material used in our study is low (3-35%) and the amylopectin content is high (65-98%). In the study conducted by Ünlü et al. (2018), amylose content range was found to be 5.9% -37.6% in 35 different local corn populations,

while amylopectin values were between 62.4% and 94.1%. Our results coincide with the findings of this research. A remarkable point is that the top three of the tested populations are all different for starch, amylose and amylopectin. However, Synove and Hido standard varieties are the genotypes with the highest ratios of both starch and amylopectin. No doubt that the number of standard varieties and local populations is also important here, but it can be said that there is a high variability in the genotypes for starch properties in local populations.

The second important biomolecules found in maize are proteins. Protein content of the genotypes used in this study was determined between 6.25% and 15.65%. In previous studies conducted with Turkish local maize populations, protein content was found between 6.6% and 11.6% (Cömertpay et al., 2016) and similar results were obtained in a different study (Ünlü et al., 2018). According to these results, it can be said that the detected variation for protein content in the study was wider than those reported in the previous studies. Despite occurring at proportionally lower amounts than starch, proteins in maize grain are highly important for nutrition and their quality is more of a concern than the quantity. To enhance the protein quality, the two deficient amino acids are targeted in the breeding programs (Shewry, 2007). TR37719 was listed within the top three landraces for both lysine and tryptophan. For protein, on the other hand, the top three landraces were different from not only these 2 amino acids, but also all the other investigated traits. Maize with high lysine and tryptophan content is considered as quality protein maize (QPM). Genotypes with tryptophan higher than 0.075% in the whole grain are accepted as QPM (Vasal, 2000). Based on this limit value, it can be stated that the top landraces (TR50551, TR37719, TR53247) for tryptophan content can be considered as having high protein quality (Figure 1).

Inheritance of Protein and Starch Related Traits and Their Relationships

Genetic and environmental variance calculations provide valuable information about the traits studied in the studies on genetic variability. It is possible to determi-

ne whether the variation in a trait is mainly controlled by environmental or genetic factors. When the genotypic variation coefficient is over 20% it is considered to have a significant effect on the trait (Sivasubramanian and Madhavamenon, 1973). Values for heritability, an important parameter in breeding studies, is classified as low ($h < 30$), moderate ($30 < h < 60$) and high ($h > 60$) (Robinson, 1966). Calculations of genetic gain against the mean of the investigated traits also provide valuable information about the material in breeding studies. Genetic advance over the mean (GAM) values of $< 10\%$, $10\text{--}20\%$, and $> 20\%$ are considered to be indication of low, moderate, and high genetic progress, respectively (Johnson et al., 1955). In the light of these evaluations, it has been seen that phenotypic effects play a more important role than genetic effects for all the traits. Genotypic variance (GV) for lysine content was high, for amylose and protein content were moderate and for other traits are low. Broad sense heritability was low for amylose and amylopectin content ($hBS = 16.81\%$), while

for the other traits it was moderate ($hBS > 30\%$). According to these results, a significant improvement (GAM = 40.4%) can be achieved with lysine content in breeding practices. The relationships between grain quality traits are influenced by the environment, and the correlations change as a result of linkage or epistatic interactions between the genes controlling these traits (Cömertpay et al., 2016). In our study, the full negative correlation between amylose and amylopectin ($r = -1.00$, $p < 0.001$) arises from the fact that these are the alternative components of starch. Similarly, there is a general negative relationship between protein and starch, as indicated by our data as well as some earlier studies (Cömertpay et al., 2016) that investigated Turkish maize landraces. In our study, variability was observed in the correlations between the amount of protein and amino acids. The positive correlation detected between protein and tryptophan contents ($r = 0.33$, $p < 0.001$) could be seen as a reason to explain the negative correlation between starch and tryptophan contents ($r = -0.25$, $p < 0.001$).

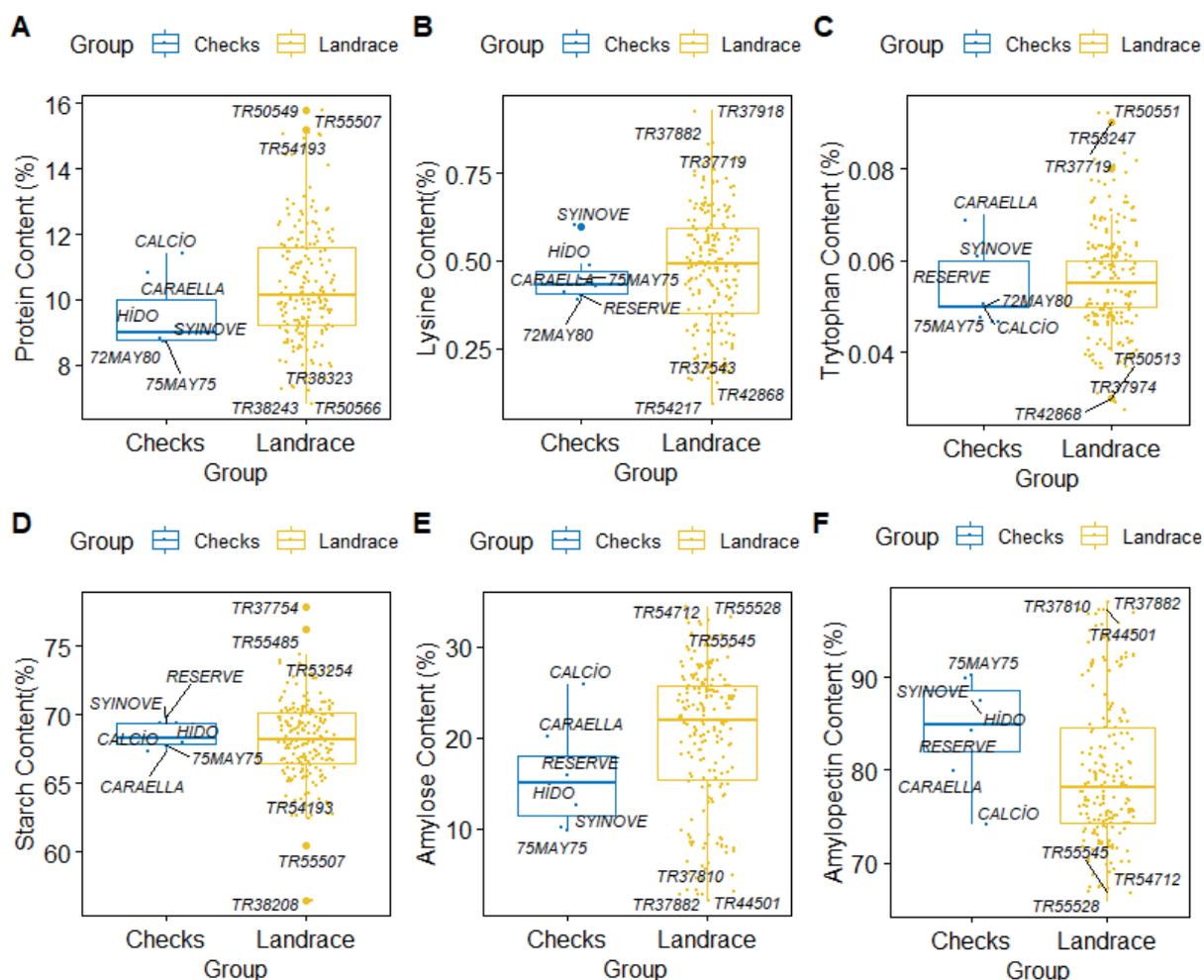


Fig. 1 - Variation in protein and starch related traits in landraces and check varieties.

The significant relationship between tryptophan and protein contents deserves consideration since the level of tryptophan alone is regarded as an indication of the protein quality of maize kernel (Hernandez ve Bates, 1969).

Consequently, it has been found that there is a remarkable variation in protein and starch properties in Turkish local maize populations. In terms of lysine content, it was understood that these materials could be the sources to use in studies aimed at developing maize genotypes with high protein quality. Results indicate that there are populations which have the desired characteristics in terms of protein & starch properties as well as possessing significant advantages over standard varieties in terms of other traits. More detailed and better designed studies (i.e., experimental design, statistical analysis method, more environments etc.) could help exploiting the genetic merit in these promising materials.

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