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Electrophoretic characterization of inbred maize lines

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ABSTRACT

In this study, 50 inbred lines (S_4) of maize (*Zea mays indentata* Sturt.), which were developed by the Department of Field Crops, Faculty of Agriculture, University of Namık Kemal, were used as a material. In the study, the band patterns of the gliadin protein of inbred lines were determined by the SDS – PAGE method, and regarding the ratio density data of the genotypes, the number of the bands and the spreading of them to the gliadin regions were examined. At the end of the electrophoresis examinations, it was revealed that the band number of the gliadin proteins in the inbred maize lines was between 11 and 20, the relative mobility of the genotypes was between 18 and 90 kDA, and according to the gliadin regions, the bands were mainly in the omega, beta and gamma regions, respectively. It was found out that the relative mobility was minimum in the alpha region. As a result of the study, it was determined that some lines were formed by similar populations with the obtained band patterns, and the majority were different. This indicates that genetic diversity exists in the examined lines, and the obtained data can be used in the breeding studies.

1. Introduction

Maize is an annual summer-growing cereal included in the Poaceae family. Its origin is America, and it was first cultivated in Rio Balsas region of Mexico about 9000 years ago [1]. It can be grown in tropical and subtropical mild temperate zones and can be cultivated almost everywhere in the world. While grain cropping is carried out in about 712 million hectares of the total 1.5 billion hectares of agricultural land in the world, maize is cultivated in 183 million hectares of this area. Maize is the most cultivated grain after wheat and rice [2]. In our country, according to 2016 data, maize was grown in an area of 6.820.000 decares, and its production was 6.300.000 tons [3]. Under optimum conditions, in Adana, Sakarya and Aydın provinces (under main product conditions), around 1400-1600 kg/da yield is obtained per unit area, and our country is generally above the average of the world and EU countries in terms of maize yield [4]. Maize production has indispensable importance in terms of agriculture and economy of our country. The contribution of grain maize production to Turkey's economy is calculated as approximately 4.05 billion TL according to 2014 data of the TUİK (Turkish Statistical Institute) [5].

Maize is rich in fat and protein and it is consumed as human food and animal feed. Furthermore, in terms of industry, it is abundantly used in the production of starch, syrup, beer, alcohol and whiskey [6]. The proteins stored in the endosperm of cereals are classified into two main groups as prolamines and glutelins. Prolamines are the proteins that are soluble in alcohol. Maize includes zein from the prolamine group [7]. In the studies carried out by the SDS-PAGE method, it was determined that the zein protein is composed of two different polypeptides. These polypeptides are hordein that is abundant in barley and gliadin that is abundant in wheat [8]. There are many

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studies related to zein in maize in the literature [9-10-11-12 -13]. However, the studies related to gliadin were mostly carried out in wheat [14-15-16-17]. The basic amino acids are disproportionately found in the structure of zein [11]. For this reason, zeins are classified as α, β, γ and δ according to their molecular mass (18). δ-zein (10-kDa) is very rich in terms of methionine, and if it is not available, the quality of maize is negatively affected. It is thought that maize is rich in terms of methionine before it is cultivated, but its high methionine characteristic is lost after it is cultivated [19]. Therefore, while breeders try to improve the grain quality, they attempt to reduce the zein content and thus to facilitate the accumulation of albumins and globulins, which have a more balanced amino acid ratio, or to produce lines containing high lysine, methionine and phenylalanine by obtaining hybrids through using the ancestors of corn [20].

Maize is the plant on which the breeding studies are most intensely performed in the world. Hybrid maize breeding studies started in our country in the 1950s. Along with the breeding studies carried out until today, valuable populations, a large number of inbred lines and hybrid maize varieties have been developed [4]. The basic step in the hybrid variety breeding is to obtain inbred lines. When the studies carried out on maize in our country are examined, it is observed that inbred lines or hybrid maize varieties have been developed; however, there is no study on zein in the content of these materials or gliadin or hordein in the structure of zein. In this study, it was aimed 1- to examine the gliadin band patterns of the inbred lines by the SDS-PAGE method, and 2- to determine the similarities and differences of the lines according to band patterns.

2. Material and Method

2.1 Material

In the study, 50 inbred *Zea mays indentata* Sturt. lines at S_4 generation belonging to the Department of Field Crops, Faculty of Agriculture, University of Namık Kemal, were used as the material (Table 1).

2.2 Method

2.2.1 Planting and cultivation of maize seeds

The seeds of the 50 inbred maize lines at S_4 generation developed by selection and inbreeding in previous years were planted in the trial field of the Faculty of Agriculture, University of Namık Kemal. In the experiment set up with three repetitive, the parcel length was 5.00 m, inter-row spacing was 0.70 m, and intra-row spacing was 0.25 m. In the experiment, each line was manually planted in a single row (three seeds for each line). The germination of the seeds and the growth of the plants were monitored, and irrigation, hoeing, and fertilization were performed if required. During the harvest-time, the cobs of each line were picked one by one, put into paper bags and stored in the seed chamber of the Department of Field Crops.

2.2.2 SDS-PAGE Method

The SDS-PAGE method was used to reveal the genotypic differences of the lines used in the experiment. Five seeds for each line were examined at this stage. The electrophoresis procedures used in the determination of the protein bands of the genotypes were performed by following the steps below;

- One grain maize was thoroughly crushed and then ground, 0.04 g was taken from the sample and placed into the Eppendorf tube. Then, 500 µl of 70% ethanol was added on this sample, and it was kept waiting for 2 hours. During waiting, the tubes were mixed in a vortex mixer for 1 minute every 10 minutes. At the end of this period, the tubes were centrifuged at 13.000 rpm for 5 minutes. 100 µl was taken from the centrifuged sample and transferred to a separate tube.
- 100 µl of SDS solution, 25 µl of Mercaptoethanol, 190 µl of 60% Glycerin, and 190 µl of 0.005% Bromphenol blue solution were added to the tubes, and they were kept waiting in hot water bath (90 ^{O}C) for 2.5 minutes. 10 µl of sample was taken from the tubes and loaded into the gel.

Two gels (loading gel and running gel) were prepared for electrophoresis. The following stocks were made ready before these gels were prepared.

Water: 4 ml of deionized water was used to prepare 10 ml gel.

Acrylamide-Bisacrylamide mixture: 9 g of acrylamide and 0.24 g of bisacrylamide were weighed and completed to 30 ml with deionized water. After the prepared 30% acrylamide was mixed in the mechanical shaker for an hour, it became ready for use. After the usage, the remaining part can be stored in a dark environment.

SDS (10%): 1g of SDS was taken and completed to 10 ml with deionized water, and it was stirred and allowed to dissolve. The prepared solution was stored at room temperature until use.

1.5 M Tris (pH 8.8): 9.075 g of Tris was weighed and completed to 50 ml with deionized water, and the pH value of the solution was measured. The pH value of the solution was adjusted to 8.8 by using concentrated HCl.

1 M Tris (pH 6.8): 6.055 g of Tris was weighed and completed to 50 ml with deionized water, and the pH value of the solution was measured. Then, the pH value was adjusted to 6.8 by using concentrated HCl.

10% APS: After 1 g of APS (ammonium persulphate) was weighed, it was completed to 10 ml with deionized water to prepare the solution. During the use of this solution, attention was paid to prepare it as fresh.

TEMED: The ready-to-use TEMED solution was used.

Table 1. Inbred Zea mays indentata Sturt. Lines numbers, relative mobility value of bands, band number of gliadin regions (Ω, γ, β,α) and total band numbers used as a material in the experiment

BN	GR				Relative Mobility (Rm)																				LN
	α	β	γ	Ω					87	82	76	70	60	53	49	46	41	39	37	31	25	24	21	19	SM 158
17	1	2	2	12				88	82	77	68	61	57	55	48	47	42	39	37	33	31	26	23	21	SM 21
14	-	3	1	10							83	81	76	69	59	53	49	45	40	37	30	26	24	19	SM 191
14	1	2	2	9							87	83	76	73	62	59	57	54	42	38	33	27	24	21	SM 140
15	-	2	4	9						81	76	74	70	61	60	53	50	47	42	40	37	31	25	24	SM 252
17	1	2	3	11				88	83	76	73	68	61	57	51	48	47	41	37	35	32	26	23	21	SM 143
12	1	3	2	6									86	83	79	75	71	60	55	45	40	37	32	24	SM 34
11	1	1	3	6										87	83	72	66	61	52	46	41	38	30	26	SM 247
14	1	3	2	8							87	84	81	75	69	60	51	47	42	38	36	32	29	24	SM 206
16	-	3	3	10					84	80	77	73	68	60	57	53	49	48	46	41	38	36	33	26	SM 17
16	-	4	2	10					85	82	76	75	72	62	56	52	48	43	41	38	35	32	27	23	SM 60
13	-	4	2	7								85	81	77	76	70	62	58	51	47	42	38	32	27	SM 107
15	-	3	3	9						85	82	76	74	69	61	58	53	46	41	38	33	31	27	23	SM 243
14	-	3	2	9							85	82	76	70	61	58	51	48	43	39	37	33	26	22	SM 31
13	-	4	1	8								85	82	77	75	62	58	50	47	44	41	37	26	24	SM 61
14	-	3	2	9							83	81	76	71	62	59	54	50	47	43	39	35	32	25	SM 12
15	-	3	3	9						84	81	76	72	68	61	55	52	48	42	39	35	30	25	23	SM 8
14	-	3	2	9							83	80	76	72	61	53	49	47	41	38	35	33	27	24	SM 59
13	-	3	1	9								83	80	75	60	52	49	47	41	37	31	27	24	22	SM 48
14	-	3	2	9							83	80	75	72	64	59	53	49	45	41	37	33	26	23	SM 59
15	-	4	2	9						85	80	79	78	67	60	55	53	48	40	38	35	31	27	25	SM 68
14	1	3	2	8							86	84	79	75	67	63	54	48	44	39	35	30	27	25	SM 30
15	1	4	2	8						88	84	81	79	75	67	60	47	44	39	37	34	29	28	26	SM 4
14	1	3	2	8							89	85	78	75	67	63	56	46	40	35	30	29	26	25	SM 209
15	1	3	2	9						88	84	79	78	66	60	55	47	42	36	34	31	28	26	24	SM 201
12	-	2	2	8									85	78	65	60	55	49	38	35	34	29	26	25	SM 242
13	1	2	1	9								90	84	78	65	59	50	45	38	35	29	27	26	24	SM 214
13	-	3	1	9								84	79	75	65	58	49	46	42	35	29	27	25	23	SM 63
14	1	3	1	9							88	85	78	77	65	58	45	36	35	31	29	26	25	23	SM 134
15	1	3	2	9						88	84	80	78	73	67	59	51	46	41	37	34	30	26	24	SM 193
16	2	2	4	8					88	86	81	78	71	68	66	60	52	44	38	34	30	25	23	18	SM 579
15	1	3	2	9						88	85	81	79	71	66	58	54	50	43	39	34	27	22	17	SM 164
14	1	2	1	10							87	83	81	70	58	53	50	44	41	39	34	28	21	19	SM 245
13	2	1	3	7								89	87	81	74	70	66	56	50	44	37	33	27	18	SM 200
13	-	2	2	9								81	77	70	67	59	56	50	42	37	32	26	20	18	SM 184
14	2	2	2	8							88	87	81	76	69	65	57	49	38	32	29	25	21	19	SM 108
11	-	3	1	7										85	81	78	70	59	51	44	36	29	21	18	SM 223
13	2	2	2	7								88	87	81	76	70	61	53	44	38	34	27	21	19	SM 228
14	1	3	2	8							89	84	81	77	71	68	59	52	44	39	33	27	22	19	SM 93
14	-	3	1	10							85	81	76	70	59	52	46	42	38	34	30	25	22	19	SM 144
17	2	1	1	13				89	88	85	70	59	53	45	41	36	34	30	28	26	25	22	21	18	SM 104
17	1	2	3	11				86	85	82	74	69	63	57	52	46	41	34	27	26	23	22	21	18	SM 45
20	2	3	2	13	88	87	85	81	75	69	63	57	53	50	45	40	35	32	27	26	24	22	20	18	SM 220
19	2	3	2	12		88	86	85	78	75	71	62	53	50	45	42	37	33	29	28	26	23	22	20	SM 242
18	2	2	2	12			89	88	85	82	71	69	58	53	49	45	41	33	30	28	26	25	22	18	SM 79
17	1	3	1	12				89	85	83	78	70	57	53	44	41	36	33	29	27	26	24	22	20	SM 15
17	2	2	2	11				89	86	83	79	72	67	58	52	47	40	34	33	29	27	26	23	20	SM 132
17	2	1	2	12				88	86	83	73	68	57	53	47	43	41	36	28	26	25	23	21	20	SM 246
16	1	2	2	11					89	85	79	73	67	58	51	46	43	40	32	27	26	24	22	20	SM 55
16	1	2	2	11					89	85	80	72	61	49	46	40	35	31	28	27	25	24	22	20	SM 118

LN: Line number GR: Gliadin regions BN: Band numbers Ω: Omega γ: Gamma β: Beta α: Alpha

For the preparation of 10 ml of 10% running gel, 4 ml of water, 3.3 ml of acrylamide, 2.5 ml of Tris (pH 8.8), 0.100 ml of SDS and 0.100 ml of APS were taken from the above stocks, and they were thoroughly mixed in a suitable container. The prepared gel was mixed with an addition of 10 µl of TEMED immediately before being poured, and the gel (sub-gel= running gel) was quickly poured between the glass plates of the electrophoresis apparatus so that the gel would not be solidified (Figure 1A).

For the preparation of 5 ml of loading gel, 3.4 ml of water, 0.830 ml of acrylamide mixture, 0.625 ml of 1 M Tris (pH 6.8), 0.050 ml of SDS and 0.050 ml of APS were taken from the above stocks, and they were thoroughly mixed in a suitable container. Before the obtained gel was poured on the running gel, 5 µl of TEMED (0.005 ml of TEMED) was added and quickly mixed. After the gels were poured, the combs were placed between the glass plates (Figure 1B, 1C).

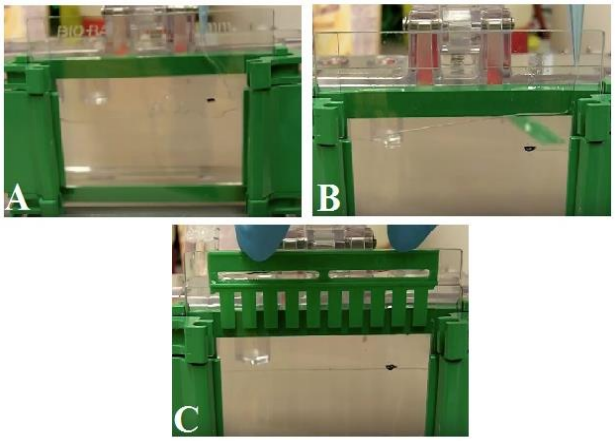

Figure 1. A: Running gel was poured between the glass plates of the electrophoresis apparatus B: Loading gel was poured between the glass plates C: The combs were placed between the glass plates

After waiting for 1 to 1.5 hours for the freezing of gels, the gels were fixed to their place in the electrophoresis apparatus and added solution (Figure 2A and 2B). By using micro-injector, 0.005 ml of sample liquid was injected from previously prepared samples into the comb housing (Figure 2C and 2D). The electrophoresis apparatus was capped and run at 10 amperes for 15 minutes and then it was run at 16 amperes until the end of the process. When the process was completed, the gels were taken out and put into the dye solution. 2% Coomassie-blue R was used for the dyeing of gels. 24 ml of glacial acetic acid and 16 ml of ethyl alcohol were added to 400 mg % Coomassie-blue R to prepare 200 ml of the dye solution. The solution was completed to 200 ml with deionized water and thoroughly mixed.

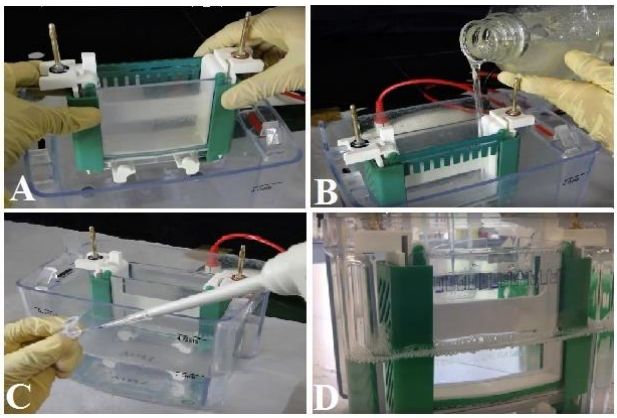

Figure 2. A and B: The gels were fixed to their place in the electrophoresis apparatus and added solution, C and D: Sample liquid was injected into the comb housing by using micro-injector

The gel taken from the electrophoresis apparatus was left in the dye solution, which was poured into a container, it was waited overnight, and through this way, the gel was ensured to be dyed. During the dyeing, the container was shaken in a magnetic stirrer. The dye removal solution was prepared to remove dye from the dyed gels. While preparing the solution, 24 ml of glacial acetic acid, 16 ml of ethyl alcohol and 160 ml of deionized water were used. The gels taken from dyeing process were placed into the dye removal solution and washed. This process was repeated 3 times with 15-minute intervals. The gels taken from there were fixed in 5% glycerol.

2.2.3 Calculation of the relative mobility values of the genotypes of the inbred maize lines

The electrophoregrams were evaluated on 9 x 13 cm size photographs [21]. While calculating the relative mobility values of the protein bands, the Low Molecular Weight Standard M 5630 was used as the standard. The protein types and molecular weights in the structure of the standard are presented in Table 2.

Using the molecular weight values of this standard type, the relative mobility values of the genotypes of the inbred maize lines were calculated with the UvıPhotoMW software.

Table 2. Protein types and molecular weights in the standard used.

Protein	Molecular weight
Aprotinin	6500
Alfa-Lactalbumin	14200
Trypsinogen Inhibitor	20000
Trypsinogen	24000
Carbonic Anhydrase	29000
Glyceraldehyde- 3- phosphate Dehidrogenase	36000
Ovarbumin	45000
Albumin	66000

2.2.4 Evaluation of gliadin band patterns:

In the evaluation of gliadin band patterns, the genotype formulas were obtained by tabulating the relative mobility (Rm) values calculated for each inbred line genotype. By benefiting the relative mobility values of the bands in the genotype formula and according to the French system also used by Bushuk and Zilman (1978), it was accepted that the Rm values between 0-59 were Ω(Omega) gliadin region, between 59-74 were γ (gamma) gliadin region, between 74-85 were β (Beta) gliadin region, and between 85-100 were α (alpha) gliadin region. The distribution patterns of the gliadin bands of the relevant sample were determined by using this information [23- 24].

3. Results and Discussion

In this study, the gliadin protein bands of the 50 inbred maize lines at S_4 generation that were included in the experiment were examined by the electrophoresis method, and their genotypes were investigated. The gliadin band patterns obtained for these lines are presented in Figure 3. The end band belongs to the standard.

When the obtained values for the gliadin protein bands of the maize lines were examined (Table 1), it was determined that the band number of the gliadin proteins in the inbred maize lines showed variances between 11 and 20. In addition, the relative mobility values of the genotypes varied between 18 and 90 kDa, and according to the gliadin regions, the bands were mainly in the omega region, and it was followed by the beta and gamma regions. It was also found out that the relative mobility values were minimum in the alpha region.

When the genotypes were compared in terms of the gliadin protein band structure, it was observed that the lines SM1, SM3, SM6, SM9, SM13, SM14, SM16, SM17, SM18, SM19, SM20, SM22, SM24, SM25, SM28, SM30, SM32, SM39, SM40, SM42, SM49 and SM50 had similar bands.

When the relative mobility values of the bands of the lines were examined, it was determined that the lines SM1, SM49 and SM50 had 20, 24, 25, and 40 kDa bands, the lines SM3 and SM40 had 19, 30, 59, 76 and 81 kDa bands, the lines SM6 and SM42 had 21, 23, 26, 41, 57 and 88 kDa bands, the lines SM9, SM39, SM22 and SM24 had 39, 67, and 75 kDa bands, the lines SM13 and SM17 had 23, 61, and 76 kDa bands, the lines SM14, SM16, SM18 and SM20 had 26, 33, 38, 50, 76 and 83 kDa bands, the lines SM19 and SM28 had 27, 49, and 75 kDa bands, and the lines SM25, SM30 and SM32 had 26, 34, 66, 79 and 80 kDa bands. It is also determined that the other band values were generally close to each other. The code required for the synthesis of proteins is given from DNA and each living being has its own unique protein structure. The fact that the lines have similar gliadin band patterns indicates that they are also similar to each other in terms of genotypes.

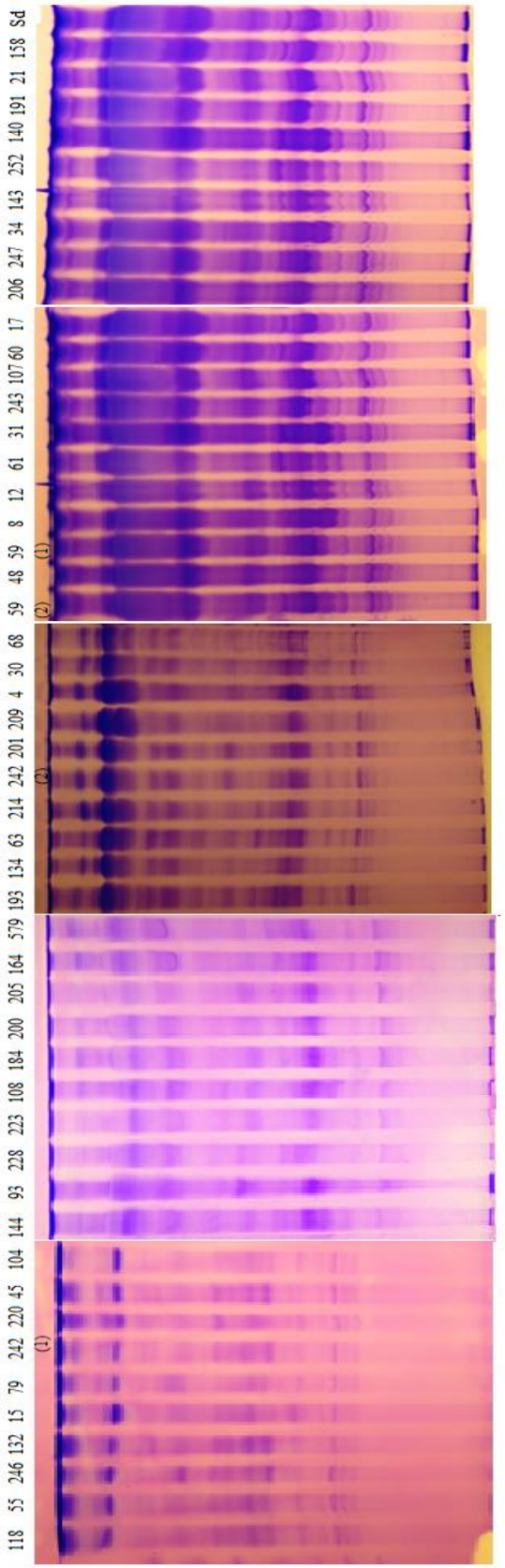

Figure 3. Gliadin protein band patterns of the inbred *Zea mays* indentata Sturt. lines at S_4 generation. Sd: Standard

As a result of the examination, it was determined that the inbred maize lines of SM 247, SM 252, SM 242, SM 61, SM 579, SM 245, SM 200, SM 184 and SM 15 showed significant differences in terms of the gliadin protein band structure compared to other lines. During the study, some morphological characteristics of the maize lines were also examined, and it was attempted to identify the relationship between band patterns and some morphological characteristics. When the morphological characteristics of similar genotypes in terms of the gliadin protein band structure were compared, it was observed that there were significant differences in all characters. Whereas some of the lines taking values far below or above the average in terms of characteristics such as stalk diameter, grain yield, cob length, thousand kernel weight, corncob diameter, diameter weight and leaf area are early-season, some others are late-season, and they have the same band pattern. It was determined that the lines that were found to differ significantly from other lines in terms of the gliadin protein band structure generally took place in the late-season groups in terms of the tasseling period and the ear silking period, and that their the leaf areas, stalk diameters, plant appearances, corncob diameter, and weights were usually above the average. The performance of the lines in terms of cob length, number of grains in the cob, and thousand kernel weight showed significant changes comparing to the general average, and they were usually below the average. In terms of grain yield, it was determined that the lines usually had the values close to the average. The obtained results show that these lines are significantly different from other lines in terms of protein band distribution and various morphological characteristics.

Depending on in which areas (silage, food for people, oil production, grain, etc.) the maize will be used, the desired morphological characteristics vary. Therefore, the lines to be proposed in the breeding studies to be performed will also vary. For example, when the morphological characters of maize lines were examined together with protein band structures, it was determined that SM 30 and SM 68 lines, which had the earliest silking time, carried 25, 27, 35, 48, 67 and 79 kDa bands. For high quality and efficient silage, it is important that the lines are early [25-26]. Corn lines with a lower stove diameter, stove weight and stem diameter are more valuable as silage. It was determined that SM 8, SM 246 and SM 134 lines, which had the lowest stove diameter and stove weight, were carrying 23, 25, 31 and 35 kDa bands. It was also identified that SM 45, SM 245, SM 21 and SM 63 lines with the lowest stem diameter generally carried 21, 23, 27, 34, 41 or 42, 46 or 47, 57 or 58, 82 kDa bands .

In the selection of silage corn lines, the characteristics such as leaf area, plant grain yield, ear length, grain number on the ear and thousand kernel weight should be above the average [25-26]. It was determined that SM 79 and SM 579 lines, which have the highest leaf area, carried 18, 25, 30, 71, and 88 kDa bands; SM 214 and SM 193 lines, which have the highest grain number on the ear and the highest plant grain yield, carried 24, 26, 59, 78 and 84 kDa bands; SM 61 and SM 184 lines having a maximum of thousand kernel weight carried 26, 37, 50 and 77 kDa bands; and SM 63 and SM 243 lines with the maximum length of the ear carried 23, 27, 46 and 58 kDa bands.. According to these obtained data, in terms of the examined characters, the presence of the bands, whose relative mobility values are same, in the similar lines jointly may explain the effect of these bands on the emerging of the characters. Therefore, these lines, whose band distributions and the common bands they carried were given, can be considered suitable for developing silage corn cultivar in the future.

Gliadin is one of the proteins involved in the gluten structure. The alpha, beta, gamma gliadins cause celiac disease in humans having gluten-sensitive enteropathy. Therefore, it is tried to reduce the amount of gliadin in cereals. In this study, it was found that the amount of lysine increased in gliadin-reduced wheat [27]. Lysine is considered the most important essential amino acid, and because it is not synthesized in animals, it must be acquired through diet. Especially in developing countries where the diet is mainly composed by a single cereal, there is a great interest in increasing the content of lysine in cereal crops since it has both an economic and humanitarian importance [28]. Mutant high-Lys lines have been obtained in maize opaque-2 mutants and opaque-2- derived quality protein maize (QPM) lines [29]. Genetic engineering approaches have been also used to increase the lysine content in maize and rice [30]. Previous studies with opaque-2 mutants and QPM maize lines have demonstrated the enhancement of the nutritional properties in animal (rats, pigs, and chickens) and human nutrition, with higher utilizable protein values as a consequence of the lysine increase and a more balanced amino acid composition [27]. If corn is to be produced as food for humans, lines with a small number of alpha, beta and gamma gliadin bands should be preferred. In this study, it was determined that SM48, SM63, SM144, SM 191 and SM 223 lines did not carry an alpha band. The protein band number in the beta and gamma regions was determined as 4. These lines are recommended for the nutrition of people with celiac disease.

4. Conclusion

As a result of this study, it was determined that whereas some of the examined 50 maize lines had similar gliadin band patterns, the majority of them were different.

This result indicates that the available lines at S4 generation have genetic diversity. The obtained data can be evaluated and used by breeders in the development of new varieties with desired characteristics in terms of silage or grain.

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