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Original Article

Determining seed storage proteins of some almond (*Prunus dulcis* (Mill.) D.A. Webb.) genotypes distributed in East and Southeast of Turkey

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Summary. Almonds (*Prunus dulcis* (Mill.) D.A. Webb.) genotypes that has a large number of varieties, are grown in almost every region of Turkey except in the Eastern Black Sea region. In this study, the amount of sub-fractions (albumins, globulin A, globulin B, prolamins, and glutelins) of seed storage proteins of 32 almond genotypes were studied. Almond genotypes collected from different locations in Eastern and Southeastern Anatolian regions of Turkey. In analysis, using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique. As a result the characteristic almond protein sub-fractions band profiles and the other second degree important and variable bands were revealed. These band profiles were evaluated together according to degree of similarity and variations of genotypes.

Key words: *Prunus dulcis* (Mill.) D.A. Webb, seed storage proteins, SDS-PAGE, variation.

«Determinazione delle proteine di riserva dei semi di alcuni genotipi di mandorla (*Prunus dulcis* (Mill.) D.A. Webb.) distribuiti in Oriente e nel Sudest della Turchia »

Riassunto. I genotipi di mandorla (*Prunus dulcis* (Mill.) DA Webb.), che ha un gran numero di varietà, sono coltivati in quasi tutte le regioni della Turchia ad eccezione della regione del Mar Nero orientale. In questo studio sono state esaminate le quantità delle sotto-frazioni (albumine, globuline A, globuline B, prolamine e gluteline) delle proteine di riserva dei semi di 32 genotipi di mandorla. I genotipi di mandorla sono stati raccolti da diverse località delle regioni anatoliche orientali e sudorientali della Turchia. Per l'analisi è stata utilizzata la tecnica dell'elettroforesi su gel di poliacrilamide con il 12% di sodio dodecil solfato (SDS-PAGE). Come risultato si sono evidenziati i caratteristici profili delle bande delle sotto-frazioni proteiche della mandorla e le altre bande variabili importanti di secondo grado. Questi profili delle bande sono stati valutati insieme in accordo con il grado di somiglianza e di variazione dei genotipi.

Parole chiave: *Prunus dulcis* (Mill.) D.A. Webb, proteine di riserva dei semi, SDS-PAGE, varietà

Introduction

Turkey, in terms of plant genetic diversity and its number of endemic species in the world has more than 3,000 of the most genes sources. Economic values of almond taxa come from its fruit which is widely used for food, medicine and pharmacology, and so on (1-3).

Almond (*Prunus dulcis* (Mill.) DA Webb, syn. *P. amygdalus* Batsch, and *P. communis* (L.)) (4) depends on the Prunus species of the family Rosaceae (5-6). The almonds species of Central Asian origin are distributed in cold and arid environments, mountainous areas across regions in western China, Turkestan, and countries including Afghanistan, Iran (7), Syria and

Turkey (6, 8). However, almond trees are widely cultured for fruit in the United States and the climate-condition of the Mediterranean region (2, 4, 6, 9-11). Almonds (*Prunus amygdalus* var. *dulcis*) are grown in almost every region of Turkey except for the Eastern Black Sea region. Davis (12) encountered 12 species mainly *Amygdalus communis* L. (Syn: *A. dulcis* Miller, *Prunus amygdalus* Batsch, *P. communis* L.) in Turkey. However, the numbers of species and varieties has been adversely affected in terms of genetic diversity due to many years of *ad-hoc* irregular usage.

In Turkey, with regards to the different almond fruit yields and quality, wide variations are found in the morphological and genetic structures of the different varieties. For this reason, the identification of the relationships and variations of these genotypes are primarily needed. Thus, along with the growing number of emerging technology which is the basis of many studies one method of protein analysis for the differentiation of genotypes was used in this study. Seed storage proteins are known as storage proteins (13). In the modern classification system, the discrimination is in progress for seed storage proteins, storage proteins, structural and metabolic proteins and protective proteins according to the connection to these classes of multiple major proteins. With regard to dissolution rates, most seed storage proteins are divided into 2S, 7S, 9S, 11S, 13S or 15S (14). Today, the major seed storage protein groups, based on the information on their molecular structure, are classified as prolamin, 2S albumin, globulins 7-8S and 11-12S globulins (15). Because seed storage proteins are physiologically stable and easily obtainable, their traceability has been located substantially in the literature (16-17). The protein profiles of different parts of the plant will be discovered with the SDS-PAGE technique, and in this way, relationships and inter-specific and intra-specific variations of any kind of genus or species can be determined (18- 21). In this study, the SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) technique was used in the diagnosis of the almond protein profiles of the genotypes. Vaz method (22) was modified. With this technique the sub-fractions of almond seed storage proteins and their sub-band profiles were separated and evaluated together with almond genotypes collected from different locations.

Materials and methods

Almond seed resources

The mature seeds of almonds used in the study were collected from 32 different genotypes from Eastern Anatolia Region of Malatya, Elazig, Tunceli, Bitlis, Van and Hakkari provinces and from Southeastern Anatolia Region of Diyarbakır, Mardin, Adıyaman, Siirt, Batman and anlıurfa provinces (Table 1). Among these genotypes, the numbered 37, 42 and 53 are bitter, sample numbered 43 has a bitterish tastes and all other samples consist of sweet almonds.

The chemicals used in protein electrophoresis and preparing solutions

Acrylamide, N, N'-Methylenebisacrylamide, Coomassie Brilliant Blue R-250, Dodecyl Sulfate Sodium Salt, Sodium hydroxide, Trizma base, Ethanol, Glycine, N, N, N', N'-Tetramethyletylenediamine (Temed), 2-Mercaptoethanol, Methanol, Glycerin (Glycerol), Acetic acid, Bio-Rad DC Protein Assay Kit (Protein Standard-II: Reagent A, Reagent B and Reagent S), Acetonitrile, Ammonium peroxodisulphate, EDTA. Albumins and. Fermentas (116, 66.2, 45.0, 35.0, 25.0, 18.4 and 14.4 kDa) were used. 0.1 M Tris-HCl (pH: 7.5), 0.5 M Tris (pH 6.8), 1.5 M Tris (pH 8.8), 10% SDS, 5% glycerol, 1x and 5x running buffer, protein destaining and staining solution, sample buffer (18).

Equipment

UV spectrophotometer (for determining density of proteins), pure water apparatus, vortex, precision scales, automatic pipettes, centrifuge, freezer, for protein purification and density protein filtration membranes (10-100 kDa) used. Protein subfractions to be run in protein electrophoresis system "BIO-RAD Mini-Protean® 3 Cell", and protein bands calculated through gel imaging system (UV transformer).

Obtaining the sub-fractions of almond seed storage protein

To obtain the almond seed storage protein subfractions Vaz et al. (22) method (*Lupinus albus*, lupine)

Table 1. The localities of collected almond (*Prunus dulcis* (Mill.) D.A. Webb genotype

Sample no:	Region	Province	Location	Altitude (m)
2	EA*	Hakkari/ Center	Durankaya town, Tarlabaşı-İskite	1576
4	SEA**	şanlıurfa/ Center	Kızlar village-Kepır	763
6	SEA	şanlıurfa/Bozova	Yaylak, Between şanlıurfa-Bozova	681
8	SEA	Mardin/ Center	Zınnar village, Konak (Kasr) location	1092
11	SEA	Mardin/ Ömerli	Mardin/Ömerli-Midyat, 10. km.	955
12	SEA	Mardin/ Center	Mardin - Ömerli, 2. Km	901
13	SEA	Mardin/ Center	Mardin-Diyarbakır 8. Km (Akresta Gate)	1094
14	SEA	Batman/Hasankeyf	Hasankeyf-Mardin road. 3 km to Gürüş village	675
16	SEA	Batman City border	Yeniköy-Batman border. Bahniri origin.	553
17	SEA	Siirt/ Aydınlar	Between Siirt-Aydınlar roadside	1163
18	SEA	Siirt/ Bağtepe	Between Bağtepe-Akyamaç 1.5. km	1190
19	SEA	Siirt/ Eruh	Yediyaprak village	1010
21	SEA	Diyarbakır/ Dicle	Merkez-Pınar village	900
22	SEA	Diyarbakır/ Hani	Between Hani-Seren village (Lice roadside)	872
24	SEA	Diyarbakır/ Ergani	Zülküf Dağı roadside	1063
27	SEA	Adıyaman/ Center	Adıyaman-Gölbaşı (Aşağı Çöplü village)	812
28	SEA	Adıyaman/ Center	Savaklı-Belören, 2 km from Belören	1111
30	EA	Malatya/ Center	Çulaklı	946
33	EA	Malatya/Polat	Polat-Doğanşehir 4. km.	1401
35	EA	Tunceli/Pertek	Pertek town border – edge of field	976
37	EA	Tunceli/Pertek	Pertek willage border-edge of field	989
39	EA	Van/Gevaş	Akdamar Island	1628
40	EA	Van/Gevaş	Akdamar Island	1663
42	EA	Van/Gevaş	Akdamar Island	1632
43	EA	Bitlis/Tatvan	Keklikdüzü (Hazo)-Kuşlu village	1624
44	EA	Elazığ/Hankendi	Gözeli-Hankendi (Günaçtı village)	1367
46	EA	Elazığ/Keban	Sağdıçlar village-Musallataşı location	1123
47	EA	Elazığ/ Center	Nortwestern of Çöteli village	1085
49	EA	Elazığ/Yarımca	Karagedik village-Hasantepe location	966
52	EA	Elazığ/Keban	Örenyaka village - Karşıtarla location	1348
53	EA	Elazığ/Keban	Kuçulu village- Köyaltı location	1278
54	EA	Elazığ/Keban	Çevrekaya village-Taktak location	1270

**: East Anatolia*
***: South East Anatolia*

modified and applied to the seeds of almonds. In this study, protein filtration membrane was used for protein purification differ from this method. Besides, different centrifugation speed used and different amount of solutions added to samples.

In this study, first 1 gram seed endosperm was crushed in a mortar for each of genotypes were crushed in a mortar. Afterwards the samples extracted with 8.5 ml distilled water for 2 hours and centrifugation was performed with 8600 g for 15 min. Supernatant filtrated with 3.65 ml distilled water overnight at +4 ^{0}C and then it was concentrated by cantrifugation protein filtration membrane at 4200 g for 15 min. Shortly after this concentrated proteins was centrifugation at 12000 g for 20 min and supernatant (albumins) and pellet (globulins A) obtained. In the other hand pellet (include globulins

B, prolamins and glutelins) dried overnight at +4 ^{0}C and then treated with 4 ml 5% (w/v) NaCl for 2 hours and centrifugated 9200 g for 15 min and globulins B (supernatant) was obtained. Pellet was dried overnight and resuspended with 2 ml 75% (v/v) ethanol for 2 hours, then centrifugation was performed at 9200 g for 15 min. At the end of this process prolamins (supernatant) obtained. After that, remaining pellet dried overnight at +4^{0}C and reacted with 1.6 ml 0.25% (w/v) NaOH solutions for 2 hours and later centrifugation processing applied and glutelins (supernatant) was obtained eventually. End of these stages, albumin, globulin A, globulin B, prolamin, and glutelins were obtained respectively. Getting the seed storage protein sub-fractions of each subfraction, the amount of protein was determined with the "Bio-Rad DC Protein Assay Kit" spectrophotometrically at a wavelength of 750 nm. The absorbance value readings are translated into µg using the Excel program. Thus, the density per unit volume of the proteins of each sample was determined.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) application

For SDS-PAGE, the gels were prepared according to (18). 12% separation gel was used. Before loading, an equal volume of the proteins was stirred with sample buffer (SAB: Sample Amplification Buffer for SDS-PAGE) in the eppendorf tubes. And this mixture were kept in a boiling water bath for 5 minutes (23). Laemmli (18) buffer and Fermentas marker (5 µl) were used for electrophoresis. Despite adequate amount of albumin (75 µg) and globulin B (75 µg) load, globulin A (35 µg) and glutelin (50 µg) were lower loading due to the low density of some genotypes. Proteins were conducted at 20 mA constant current for about 4 hours. After gels obtain, they were taken for a 30 min in staining solution (18). Following this stage, the gels were kept in cleaning solution (5% methanol, 7.5% acetic acid with distilled water) and then protein bands can be visible.

Statistical analysis of seed storage proteins

Sub-fractions of seed storage protein gel images were transferred to a computer through a UV transformer. With help of Quantum ST4 program, genotypes' protein bands and their molecular weights were determined. Subsequently, these data were evaluated with Excel and SPSS 17.0 (Statistical Package for Social Sciences) programs. Hierarchical clustering analysis was performed to measure the levels of relationship between genotypes.

Results

Almond seed storage proteins sub-fraction ratios

Spectrophotometer readings were taken according to the preparation of protein standards and the amount of protein sub-fractions concentrations were calculated as mg /g according to the required conversion coefficients.

Almond samples showed variations according to the cumulative amount of protein sub-fractions (Figure 1). Among the samples of almond seed protein sub-fractions, the total rates ranged from 17.64% (sample no: 53) to 28.59% (sample no: 19).

The storage proteins in the almond seeds were evaluated as a percentage with standard deviation, the albumin was found to be 8.82 ± 0.89%, globulin A 4.00 ± 1.2%, globulin B 6.65 ± 0.14%, prolamin 0.22% ± 0.008, and glutelin was found to be 2.11% ± 0.34. The rates of seed storage proteins within the almond seeds consisted of albumin 40.48%, globulin A 18.33%, globulin B 30.52%, prolamin 0.99% and glutelin was 9.68%. The sum of total globulins (globulin A and globulin B) were 48.85% and the sum of albumin and globulins were 89.33%.

The SDS-PAGE results of almond seed storage proteins subfractions

On the gel images the protein sub-fractions bands of almond samples, the molecular weight of bands which is characteristic of almond samples, and the relationship of genotypes that have bands variations were introduced.

Albumin levels of almond samples

Significant variations were found in the almond albumin protein band profiles (Figure 2). In terms of albumin, 24 different bands were found between 11

Average amount of cumulative subfractions of seed storage proteins (mg/g) of almond genotips

Genotip	mg/g Albumin	mg/g Globulin A	mg/g Globulin B	mg/g Prolamin	mg/g Glutelin
2	90,72	45,78	66,30	2,47	21,16
4	77,56	37,56	45,57	1,80	17,98
6	83,06	33,52	68,82	1,46	19,23
8	77,69	29,34	60,23	1,54	17,50
11	86,76	63,71	53,36	1,43	18,37
12	74,37	54,00	62,06	1,95	18,08
13	80,50	24,70	57,48	1,60	20,05
14	95,19	40,84	79,70	2,12	24,38
16	99,02	24,10	81,65	2,17	21,69
17	95,32	38,90	66,07	1,92	23,47
18	90,98	32,47	80,96	2,39	27,27
19	112,46	74,32	69,84	3,12	26,16
21	90,31	42,46	68,16	2,02	21,94
22	99,19	48,20	74,26	3,02	25,55
24	88,76	49,93	70,32	2,17	21,47
27	86,59	52,02	73,31	2,76	28,25
28	82,21	39,83	63,44	2,56	17,09
30	91,60	59,38	69,54	2,28	20,54
33	78,44	16,57	78,75	2,58	29,39
35	89,64	34,03	71,21	2,43	21,39
37	112,21	37,32	55,97	2,53	17,76
39	85,82	41,02	61,95	2,98	18,60
40	85,77	38,69	58,30	2,59	18,29
42	84,32	41,32	66,49	2,45	19,56
43	88,71	37,68	66,91	2,32	19,98
44	90,05	37,79	68,16	2,06	22,58
46	91,03	40,66	63,56	1,77	18,05
47	87,63	41,20	65,89	2,00	14,97
49	84,53	39,77	70,97	2,09	23,51
52	86,59	27,75	62,01	1,76	19,96
53	73,89	18,78	61,05	1,78	20,88
54	82,72	35,22	66,49	1,24	20,35

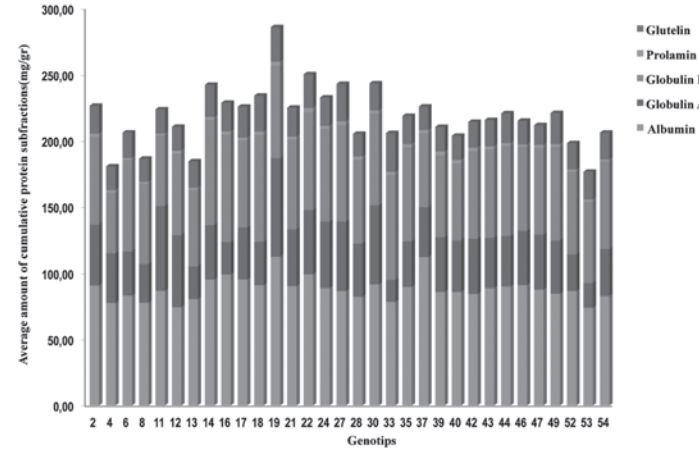

Fig. 1. Composition of the samples and the cumulative amount of seed storage proteins

Figure 1. Composition of the samples and the cumulative amount of seed storage proteins

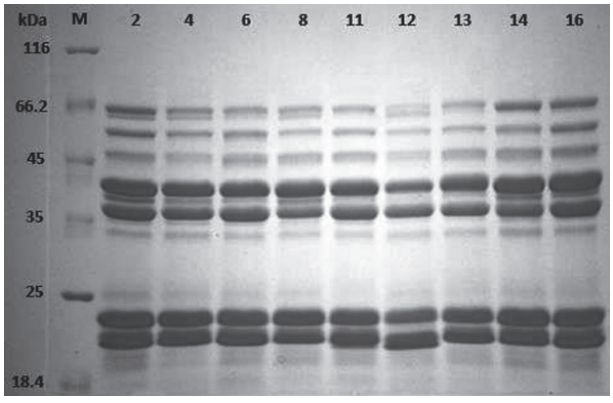

Figure 2. Some of almond samples albumin protein bands on the gel images

kDa - 86.6 kDa. However, large numbers of weak bands were found between 11 kDa - 20 kDa, 25-31.5 kDa and some more weak bands were between 86 kDa - 127 kDa. Prominent characteristic intense bands observed as 35.5 kDa and 40.3 kDa dual bands, and 20.04 kDa and 22.04 kDa dual bands in all almond genotypes. At the Mid-level prominent characteristic bands were observed as 47.4 kDa, 55.4 kDa and 66.0 kDa in all genotypes. 8 bands (4 bands were intense, 3 bands were moderate, and 1 band was lower level) were detected in albumin proteins in all samples significantly. There are variations and differences at medium level among the genotypes protein bands at 31.05 kDa, 37.8 kDa and 60.7 kDa bands, as well as a weak band in terms of 86.7 kDa. 60.7 kDa bands indicated at a few samples from the others bands showing variations.

Globulin levels of almond samples

Some examples of globulin A and B band profiles are shown in Figure 3. In terms of globulin A, over the 25 bands were found as distinctive. The bands ranged from 17 to 78 kDa generally. But, rarely bands were found between 78 to 122 kDa. Globulin A bands were detected in larger amounts than albumin bands according to quantity and varieties. In terms of globulin A, characteristic bands in all samples were detected as two pairs of double bands (21.7 kDa and 23.4 kDa; 37.5 kDa and 41.6 kDa) and two single bands (59.1 kDa and 49.2 kDa) and one triple bands (68.3 kDa-70.4 kDa) were detected as moderate intense. From these, 21.0 kDa, 35.3 kDa, 47.8 kDa, 57.6 kDa, 67.1 kDa, and 68.3 kDa bands showed a significant variation between genotypes.

In terms of globulin B (one example was shown in Figure 4), 20 different bands were found between 14 kDa-97 kDa. Major intense and characteristic bands were found in the range of 20 kDa-22.5 kDa

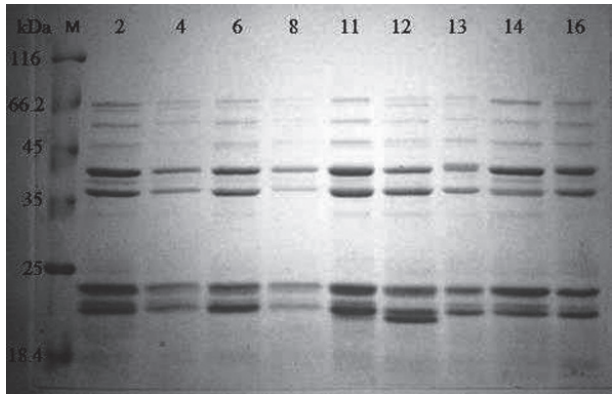

Figure 3. Globulin A protein bands on the gel images of some of the almond samplesgel images

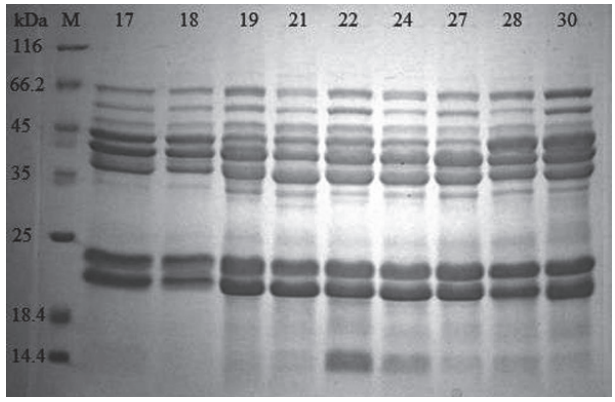

Figure 4. Globulin B protein bands on the gel images of some almonds samples

and 35.7-42.2 kDa. Furthermore, moderately prominent bands were found in the range of 46.7 kDa and 65.5 kDa and distinctive lower level bands were found in the range of 30.02 kDa-34.2 kDa. In all samples, in terms of globulin B, the major intense and characteristic one double bands (20.09 kDa and 22.5 kDa) and one triple bands (35.7 kDa, 39.3 kDa and 42.2 kDa) were detected. Apart from these, one triple band sets (middle level distinctive at 46.7 kDa, 55.2 kDa and 65.5 kDa) are common for all genotypes. These characteristic and common 8 bands showed no variation. However, five globulins B bands (63.4 kDa, 37.1 kDa, 34.2 kDa, 21.9 kDa and 20.1 kDa) showed variations with middle appearance levels. In addition, many variations were identified at the less distinctive level between 14.05 kDa-20 kDa and 22.5 kDa-34.2 kDa. Furtermore, a small number of bands were encountered between 65.5 kDa 96.7 kDa at the very low level.

Prolamin levels of almond samples

Due to the presence of very low amounts of prolamins (identified lower than 1% and band profile conditions occurred with 12% SDS-PAGE electrophoresis), distinctive bands could not be obtained and protein profiles haven't been evaluated at 25 µg loading level of prolamin proteins.

Glutelin levels of almond samples

According to the obtained gel images for glutelin (some examples showed in Figure 5), over 20 different proteins bands were found between 18 kDa-75.2 kDa at different levels. Overall, at intervals of 21.3-23.2 kDa and 35 kDa - 40.2 kDa intense bands and 47.0 kDa-65.8 kDa medium intense bands were identified. The main characteristic bands can be observed in all samples in terms of glutelin 23.02 kDa, 36.0 kDa and 40.2 kDa intense and distinctive bands, and moderate-intense bands at a level of 54.8 kDa. In addition, the level of 65.8 kDa band was found in all samples except for only one sample. It can be observed variations as major bands at the level of 21.03 kDa and 21.7 kDa, with pairs of distinctive and moderately dense bands with 35.0 kDa, 38.5 kDa, 64.5 kDa, 53.9 kDa, 47.0 kDa level and at the 75.2, 43.3 kDa and 22.0 kDa level bands.

Combination of protein fractions and relationship level of samples

The major bands of sub-fractions proteins in the gels of all almond genotypes, total of 57 prominent bands (12 albumin, 17 globulin A, 13 globulin B and 15 glutelin) were evaluated. According to this 57 bands, a dendrogram (Figure 6) was created on the level of relationship between almond genotypes. From these, more significant variations were observed of 4 albumin, 8 globulin A, 5 globulin B, and 7 glutelin bands in particular. However, 8 albumin, 5 globulin A, 8 globulin B and 5 glutelin bands were determined that were characteristic in all samples.

In the dendogram, albumin band structures showed less variation and branching than the other protein sub-fractions. However, globulin A and glute-

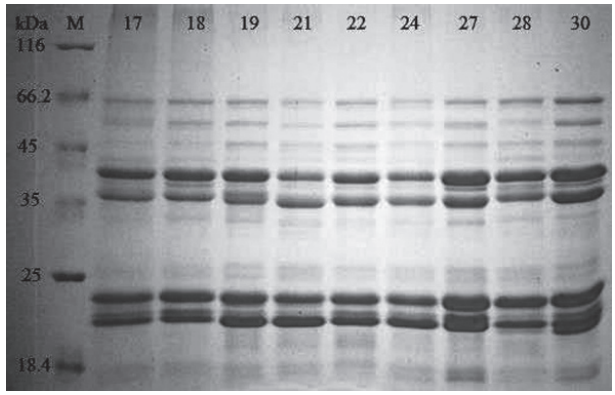

Figure 5. Some examples of almonds glutelin protein bands on the gel images

lin proteins had less distinction in comparison with albumin and globulin B protein according to bands. The variation in the number of broadband growth, making it easier to say that there were distinctions between the genotypes. Therefore, the dendrogram generated from the evaluation of all protein sub-fractions becomes much more diversified with more subdivided numbers of genotypes. This also gives us more information to explain the relations between the genotypes. In general the evaluation of the protein sub-fractions of samples collected close to the geographic areas than in samples

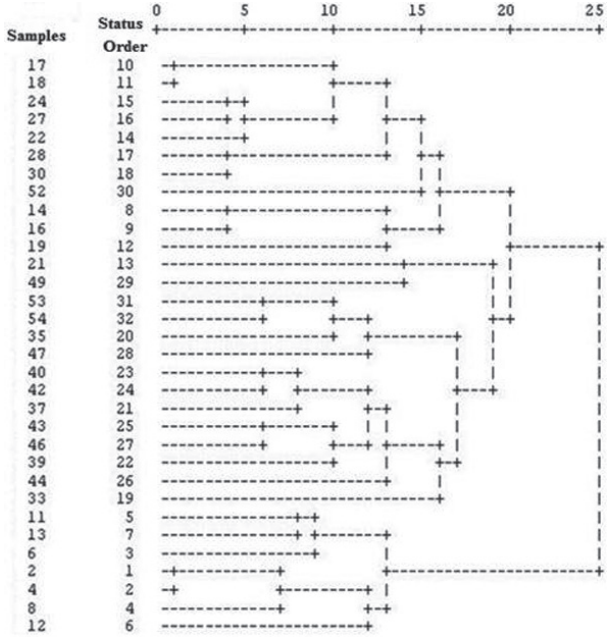

Figure 6. Dendrogram generated by the protein sub-fractions according to the similarity of almond samples

collected from other regions in the dendrogram, clearly see that they have close family relationships. This case depends on genetic structure and gene exchange of genotypes rather than environmental impact.

In the hierarchical classification that albumin, globulin A, globulin B and glutelin sub-fractions of genotypes used two main groups were observed. One of these groups is consists of 7 genotypes (2, 4, 6, 8, 11, 12 and 13 numbered sample) in total. In this group, the samples numbered 2 (Hakkari district), 4 and 6 (anlıurfa district), 8, 11, 12 and 13 (Mardin district) are a combination of all instances of locations. The second group showed a much wider variation. This group is divided into several subgroup. An interesting example is that bitter almond seed samples 37 and 42 were found to be related very closely. In addition to these, another one bitter genotype numbered 53 and a bitterish genotype (numbered 43) which included different subgroups were close to this group. It can be say that this bitter and bitterich genotypes were related to each other.

Discussion

In this study some changes were applied of Vaz et al. (22) procedure and the modified. This modified method was easily used to obtain sub-fractions of seed storage proteins of almond genotypes. When this process used to obtain almond subfraction, protein phases didn't full separate from each other. Also, low density protein subfractions were obtained using this method. To resolve seperation problem, centrifuge speed was increased each of phases of procedure. Besides, to obtain more dense protein, the amount of solvent that were added to each centrifugation step was reduced and protein filtration membrane (10-100 kDa) were used in place of Vaz et al. (22) dialysis membrane. Thus, more dense protein obtained and amount of protein subfractions seperation easier were performed with increased centrifuge speed.

For almond seed protein electrophoresis, the election of clarity and detectability in the direction of the average of the protein bands sub-fractions between 50-75 mg and average 60-70 mg gives us a better idea. In our study, an average of 21.8% almond seed protein

was found. Calixto et al. (24) found the total protein proportions of 18-24 g/100 g that depend on the almond variety. In present study protein sub-fractions with their own individual rates and the percentage of globulins (the sum of globulin A and globulin B) were found as 48.85%. Also, the total amount of albumins and globulins were found to be 89.33%. Calixto et al. (24) studied the sub-fractions of 5 varieties of Mediterranean sweet almonds and its results generally close to the rate of present study. Their implemented method obtained albumins and globulins together as 87.94 - 91.06%. In this modified method, albumin, globulin A and globulin B can be obtained as protein sub-fractions in more detail differ from it.

The almond protein sub-fractions studies using SDS-PAGE method are limited. Likewise, instead of defining the four sub-fractions, many studies have been conducted as 2S albumins, 7S globulins, 11S globulin and 12S globulins. In this study, globulin A (21.7 kDa and 23.4 kDa) and globulin B (20.9 and 22.5 kDa) intense and characteristic main bands were found as first time. These bands correspond to Sathe et al. (10) point out the protein band at levels of 11S globulins. In addition, globulin B protein bands were found to be moderate at the level of 55.2 kDa. that is mentioned by Sathe et al. (10) as 7S globulin proteins. In low conditions the water-soluble proteins from polyacrylamide gel electrophoresis have been divided into two major polypeptide groups. One of these is 42-44 kDa and the other one of them is 25-27 kDa (25). In present study, close values of these polypeptide band groups were obtained. In another article, between 40-42 kDa and 20-22 kDa protein bands were obtained and these bands have allergic reaction (26).

In a study of protein electrophoresis *on Castani sativa* and *C. crenata*, using 2% β-mercaptoethanol, showed a significant increase in the amount of globulin and glutelin. In our study, 2.5% β-mercaptoethanol was used and an average of 21.8% total almond seed protein content was found (27). Also, he identified a seed prolamin rate of 1-2%, and it did not get on band images. Similarly, in our study, prolamin content was obtained from values close to 1% and prolamin band images weren't obtained by 12% SDS-PAGE conditions.

Sathe (28) noted that in conducting almonds electrophoresis studies, the proteins with predominantly all genotype limits of 23.2 kDa and 66.9 kDa protein and only 5 bands have significant variations. Differ from Sathe (28) in this study, a large range of almond protein sub-fractions bands revealed between 20.04 kDa - 70.4 kDa. . It is observed that very distinctive and dominant bands were showed very little variation in all samples. But, the second degree visibly dominant and less dense bands were showed more variations. However, these variations did not occur at the high level. Another study by Sathe et al. (29) emphasized that there were variations between 30-43 kDa bands. Similar to this, in present study, the protein bands were observed as 34.2 kDa and 37.1 kDa level in the globulin band, and 35.0 kDa, 38.5 kDa and 43.3 kDa in the glutelin band. Also, medium level distinctive band was counted as 60.7 kDa and 37.8 kDa and the less distinctive band was counted as 31.05 kDa for albumins. In general, the variations of almond protein bands were observed between 31.5-43.3 kDa. These results are similar to Sathe et al. (29) findings.

In some studies, the 37 kDa level of almond polypeptides expressed the real almond allergen (30). In present study, some bands (37.5 kDa in globulin A, 37.8 kDa encountered in all samples in albumin, and 37.1 kDa globulin B) were identified as close to this allergen protein. The presence of a band close to 37 kDa is mentioned here; the detection of close relations with allergic reaction may be a different study subject. Fling and Gregerson (31) emphasized that the almond protein bands detected by Sathe (28) might have some differences because of differences in the conditions of SDS-PAGE. In our study, it can be said that there were similar differences. Sathe et al. (29) and Sathe (32) subjected to SDS-PAGE analysis of the genotypes almond denatured aqueous extracts obtained regardless of the AMP (Almond Major Protein)'s, the main molecular weight of two polypeptide (38-41 kDa and 20-22 kDa). Also in this study, intensive AMP bands (between the values of 20.4 - 23.4 kDa and 35.5 - 42.2 kDa) were found parallel to Sathe's (32) results. In another study (33), genetic diversity and relatedness between members of 11 species and cultivars of different angiosperm families were determined using SDS-PAGE seed protein banding. In addition, a lot of studies have been conducted on intra-species and inter-species relationships using a large

number of seed samples in protein electrophoresis techniques. Zeinalabedini et al. (8) identified genetic diversity in four Iranian wild almond species (*Prunus eleagnifolia, P. hauskenchtii, P. scoparia* and *P. lycioides*) by electrophoresis, morphology, DNA markers and proteins. The results obtained by electrophoresis support the separation of products and the types of deciduous fruit species and subspecies and uses for comparative purposes (34). Drzewiecki (35) indicated that species and their cultivars can be seperated from each other easily using electrophoresis analysis seed storage proteins of 7 Amaranthus (redroot pigweed) species. Jha and Ohri (21) and Panigrahi et al. (36) demonstrated the genetic polymorphism advantage of the total seed storage proteins in *Cajanus cajan*. In addition, Sing and Eggum (37) determined globulin, albumin, glutelin and prolamin contents in seeds of *Cajanus cajan* L. Our study followed a similar path as the studies mentioned above. However, we traced the method in order to obtain a better band image and to get almond proteins sub-fractions in the pure form necessary for us to do the extraction.

In this study, almond protein sub-fractions were revealed in detail by new modified method. This method was modified of Vaz et al. (22) that used for differentiate of the *Lupinus album* cultivars whith SDS-PAGE method, and now it can be used for separation of the almond genotypes. Thus, this method may be helpful to differentiation and relationship of almond genotypes and to determine some importance particular bands likewise allergen bands for human health or environment.

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