Composition and molecular weight distribution of albumin and globulin protein isolates from durum wheat genotypes.

Hailegiorgis D[1]*, Lee[2] CA, Yun SJ[3]

[1]Department of Plant Sciences, Wollo University, Dessie, Ethiopia
[2]Department of Crop Science and Biotechnology, Chonbuk National University, Jeonju 561-756, Republic of Korea
[3]Institute of Agricultural and Life Sciences, Chonbuk National University, Jeonju 561-756, Republic of Korea

Abstract

This paper attempts to evaluate the banding patterns of non-gluten protein isolates from the grain of durum wheat varieties. Under reduced condition, polyacrylamide-gel electrophoresis has revealed a number of different sized albumin and globulin protein bands. The electrophoretic pattern of globulin showed more polymorphisms than that of albumin. High polymorphism, both in band intensity and occurrence, was observed between 15 kDa and 35 kDa. Most of the protein bands were scored in the range of 10 kDa and 85 kDa in the two protein fractions. At a cut-off point 2.5, cluster analysis based on the SDS-PAGE of globulin proteins classified the durum wheat varieties into three major family-groups. Generally, the experiment showed the suitability and usefulness of globulin protein fractions *ASA* genetic marker in discriminating durum wheat genotypes.

Keywords: Durum wheat, Albumin fraction, Globulin fraction, SDS-PAGE, Sedimentation volume.

Accepted on 16 September 2019, 2019

Introduction

Seed proteins are most abundant and highly diverse classes of biomolecules. The gliadin and glutenin proteins are commonly used protein markers for the identification of germplasms in genetic diversity analysisand as a breeding tool to select plants with desirable trait [1]. The reason for this is thatthe gluten proteins are highly diverse and they are found abundantly [2]. In contrast, the use of electrophoretic patterns of albumin and globulins for genetic diversity analysis are less common and barely used.

Albumin and globulin proteins, also known as Leukosin and Edestin, respectively, represent 10 to 30% of total flour protein [3,4]. These proteins are found mainly in the embryo and seed aleurone layer [1]. Albumins are believed to have roles as nutrient reserve for the germinating embryo, defense to insects and fungal pathogens, and influence on grain hardness [5,6]. In addition, they also act as enzymes and enzyme inhibitors [7].

Apart from their structural, protective and metabolic functions, some high molecular weight albumins and certain globulin proteins are believed to have storage function too [8,9].

From nutrition point of view, albumin and globulin proteins are considered to be best in terms of their amino acid compositions. These protein fractions have higher lysine and methionine contents as compared to the gliadin and glutenin proteins [10]. The most common albumins and globulin proteins are α-amylase/trypsin, serpins and purothionins [5].

The main objective of this research was to study the composition and electrophoretic pattern of non-gluten protein fractions from durum wheat.

Materials and Methods

Experimental material

Twenty improved durum wheat varieties of Ethiopian origin were included in the present study. Their local names, pedigrees, origin and adaptation areas are given in Table 1.

Protein fractionation

One hundred milligrams of finely ground wheat flour was used to sequentially extract the four major protein fractions by the Osborne procedure [11]. Albumin proteins were extracted by deionized water with intermittent vortexing every 10 min for half an hour. The supernatant obtained following centrifugation at 2,000 rpm for 5 min (albumin extract) was saved for further analysis. The procedure has been repeated twice to remove all the albumin fractions before proceeding to the next step.

The pellet from the final albumin extraction was used to extract globulin fraction. The extraction was conducted using 0.5 N NaCl for 30 min with intermittent vortexing every 10 min. In similar fashion, the supernatant obtained following centrifugation at 2,000 rpm for 5 min (globulin extract) was kept in refrigerator for further analysis. Extraction was repeated twice to avoid carry over (cross-contamination) of globulin fraction to the subsequent steps. The centrifugate was washed with distilled water to reduce the effect of the salt in the extraction of other proteins in the subsequent steps.

SDS-PAGE analysis

Protein fractions were analyzed under reduced conditions (in the presence of beta-mercaptoethanol) using discontinuous SDS-PAGE [12]. The electrophoresis was conducted in 12% separating gel and and 6% stalking gel. Separation of protein bands was conducted at a constant voltage of 200 V until bromophenol blue passes the stacking gel and then raised gradually to 500 V. Following electrophoresis, the gels were stained with 0.2% (w/v) CBB in 45% (w/v) methanol and 10% (w/v) acetic acid under constant agitation. Destaining was conducted in 45% methanol and 10% acetic acid solution.

The acrylamide solution was made from 30 g acrylamide monomer and 0.8 g bisacrylamide in distilled water. The resolving gel buffer was composed of 36.6 g Tris base and 40 ml of 0.1 M HCl and the pH was adjusted to 8.8. Stacking gel buffer composition was 6.06 g Tris base dissolved in 40 ml of distilled water. The pH of the stalking gel adjusted to 6.8 using weak acid.

Molecular weights of the protein bands (polypeptides) were estimated by using Thermo Scientific PageRuler Plus prestained Protein Ladder having a mixture of nine blue, orange, and green dyed proteins (10-250 kDa).

The protein bands on the destained gel were quantitated using AlphaEaseFC 4.0 software (Alpha Innotech Corporation, San Leandro, CA).

Determination of protein content

The extracted samples were mixed with a buffer containing 2 mM DTT, 0.1% Triton X-100 and 63 mM Tris-HCl (pH-6.8). The reagent solution was prepared by mixing the Bradford reagent (100 mg of CBB G-250 in 50 ml of 95% ethanol) with 100 ml of 85% ortho-phosphoric on a magnetic stirrer. The resulting solution was filtered through filter paper (Whatman) and stored in a dark bottle at 4°C. Standard curve was plotted by using seven concentrations [0 (blank), 0.2, 0.4, 0.6, 0.8, 1, 1.2 mg/ml] of bovine serum albumin (BSA) against a blank (deionized water). Absorbance was measured from 1 ml of the reaction solution at 595 nm after 3 min of incubation at room temperature. The absorbance of blank was subtracted from standards concentrations to obtain the actual concentration of the sample. Quantification of proteins was performed in triplicate. The spectrophotometric absorbance was read on Biotek Synergy 2 Micro-plate reader instrument using a Gen5 computer software program.

The absorbance reading was converted to protein concentration using a standard curve established with BSA dissolved in lysis buffer. The protein content was calculated using the following equation:

$$\text{Protein (\%, w/w)} = CVD/M \times 100$$

Where C is protein concentration (mg/ml) obtained from standard curve

V is volume (ml) of the lysis buffer used to resuspend the biomass

D is the dilution factor

M is the amount of biomass (mg)

Table 1. *List of durum wheat varieties, source of seed, pedigree, year of release and adaptation.*

Yerer	CIMMYT	CHEN/TEZ/GVILI/C11	2002	1800-2700
Hitosa	CIMMYT/Ethiopia	CHEN/ALTAR84...CDS-97-B00265. IQX ... 6DZR	2009	1800-2650
Denbi	CIMMYT/Ethiopia	AJAIAIBAUSHEN...CSS98IY00025-0MXI-3QK-4DZR	2009	1800-2650
Mangudo	CGIAR germplasm	MRF1/STJ2/3/1718/BT24//KARIM	2012	1900 - 2700
Kilinto	CIMMYT/Ethiopia	ILLUMILO/INRAT9/BHA/3/HORA/4/CIT 71/JORI	1994	1600-2700
Mukiye		STJ3 //BCR /LKS4/3/TER-3	2012	1900 - 2700
Candateutuba	CIMMYT/Ethiopia	Omruf1/Stojocri2/3/1718/BeadWheat24//Kari m	2015	1800-2750
Cocorit-71	CIMMYT/Ethiopia	RAE/4*TC60//STW63/3/AA”s”DZ27617-18-64-OM	1976	2200-2500
Tob-66 (Arsi Robe)	CIMMYT/Ethiopia	REICHENBACHII/LD357//DUCK/YEL	1996	2000-2500
Assesa	CIMMYT/Ethiopia	CHO/TARUS//YAV/3/FG/CRA/5/FG/DOM/6/HUI or CHORLITO/YAVAROS//FREE-GALLIPOLI/3/FREE-GALLIPOLI/CANADIAN-RED/4/FREE-GALLIPOLI/DON-PEDRO/5/HUITLE	1997	1680-2400
Bichena	CIMMYT/Ethiopia	IM/CIT 71	1995	900-2600
Boohai	CIMMYT/Ethiopia	COO"S"/CII or COOT(SIB)/CANDEAL-II or COCORIT71/CANDEAL-II	1982	1800-2500
Foka	CIMMYT/Ethiopia	COCORIT71/CANDEAL-II	1993	1800-2700
Gerardo (Jorro)	CIMMYT	VZ466/61-130xLdsxGII's'CM9605	1976	1800-2500
Ginchi	CIMMYT/Ethiopia	BOOHAI/ULNV - DZ1050	2000	2000-2300
Quamy	CIMMYT/Ethiopia	ADS//PGO/CANDEALII/7/JO”S”/CR”S”//GS”S”/SBA81/3/FG”S”/4/FG”S”/CR”S”/5/F”S”DOM”S”/6/ HUI”S”CD75533-A	1996	1600-2200
Robe	CIMMYT/Ethiopia	Hora/cit's'//Jo's'/GS's'/3//4/Hora Respinnegro//CM9908/3/Rahum or ACONCHI-89/3/MAGHREBI-72/ RUFFOUS//ALGERIAN-86/RUFINA/4/LABUD-27	1999	2000-2500
Ude	CIMMYT/ CGIAR germplasm	CHEN/ALTAR84//J069	2002	1800-2700
LD-357	CIMMYT/Ethiopia	LD308/NUGGET	1979	2200-2500
Werer	CIMMYT/Ethiopia	No information	2009	450-1200

Source: Jemanesh K [22], Crop variety register Issue 1-12 and http://wheatatlas.org/website
CGIAR: Consultative Group for International Agricultural Research; CIMMYT: International Maize and Wheat Improvement Centre

Micro-Kjeldahl method

The total protein content of the durum wheat varieties was estimated by micro-Kjeldahl method using nitrogen analyzer by taking 5.7 as a conversion factor. Three replicate measurements were taken to estimate error variance.

SDS-sedimentation test

SDS-sedimentation volume was measured following Axford method [13].

Statistical analysis

All analyses were replicated three times and means were compared by Fisher´s least significant differences (LSD) at at P<0.05 and P<0.01 using SAS statistical package [13]. Dendrogram was constructed from the electrophoretic data using Xlstat software.

Results and Discussion

The water soluble albumin and dilute salt soluble globulin proteins were separated and quantified. It is found that variety *Robe* had the highest albumin content (19.35%) while *Boohai* (15.34%) lowest. Albumin concentration in wheat grain ranging from 18% to 21% were also reported elsewhere [14].

In globulins, the highest amount was recorded for varieties *Cocorit-71*(10.70%), while for *Gerardo* (6.07%) the lowest. The albumin and globulin content of most common wheat proteins is reported to be in the range of 20-25% [2]. Our findings also confirmed similar results with an average albumin and globulin content of 27%.

The data presented in Table 2 showed that, the content of total protein is statistically significant (P < 0.01) suggesting the presence of considerable variation in protein content among durum wheat varieties. In the present study, protein content in grain of durum wheat ranged from 8.08% (*Candate-Utuba*) to 14.28% (*Boohai* and *Tob-66*). Other previous research findings reported grain protein content in the range of 7 to 12.5 among 15 Ethiopian durum wheat varieties [15]. The protein content of grain is affected mainly by genetic factor. However, environment and many other factors may also play a great role in determining the protein content of the crop [16,17].

The experimental results based on the absorbance reading revealed gliadin proteins (also known as large monomeric gluten proteins), to be predominant protein fractions (51.47%). Albumins and globulins accounted only for 27% of the total protein. The ratio of gliadin to glutenin proteins was close to 4:1.

The correlation between total protein content and individual protein fractions was evaluated statistically (results not shown here). There was no significant correlation among the individual protein fractions and the total protein content. Contrary to the present finding,there are reports of strong relationship between albumin-globulin fractions and total protein content [18]. Sedimentation volume test had some positive correlation with protein content and this shows that the volume of sediment is a good indicator of protein content.

SDS-PAGE pattern

The albumin-globulin protein bands were identified by means of electrophoresis. The electrophorogram showing the patterns and molecular weight distribution of albumin and globulin proteins is presented in Figures 1-4.

The globulin fractions showed polypeptides of a wide range of molecular weights ranged 10 kDa to 70 kDa in size. Seven globulin dominant genes were revealed. Out of which there were five major globulin polypeptides with a size of 15, 20, 35, 55, and 70 kDa. Trace amounts of other polypeptides are

Table 2. *Distribution individual protein fractions according to their solubility in durum wheat varieties.*

Varieties	Total Protein	Soluble (non-gluten) proteins		Large monomeric proteins (Gliadin) (w/w)	Soluble and insoluble Glutenin (w/w)	Ratio of gliadin to glutenin proteins % ratio	SDS Sedimentation (ml)
		Albumin (w/w)	Globulin (w/w)				
Werer	12.45	19.09	7.40	54.44	15.29	3.56	5.5
Yerer	11.87	17.53	6.26	45.68	18.23	2.51	4.3
Asasa	10.37	17.12	7.10	54.76	19.47	2.81	4.6
Hitosa	10.25	18.20	10.11	54.96	14.01	3.92	4.9
Candate-utuba	8.08	17.12	10.07	51.63	17.51	2.95	3.4
Ude	11.21	17.64	7.82	49.80	16.92	2.94	5.1
Foka	13.73	17.79	9.73	53.75	11.73	4.58	5.7
Denbi	12.35	17.60	9.94	49.05	14.99	3.27	4.3
Mangudo	8.13	16.01	10.08	45.39	14.21	3.19	3.7
Bichena	13.62	17.68	10.24	50.45	10.06	5.01	5.8
Tob-66	14.28	17.79	10.14	44.44	14.54	3.06	5.8
LD 357	11.91	18.05	10.14	51.30	19.67	2.61	4.5
Ginchi/ DZ-1050	12.53	18.01	9.84	54.41	11.73	4.64	6.0
Kilinto	8.58	18.23	9.73	55.61	8.16	6.81	4.2
Cocorit 71	12.69	18.57	10.70	55.94	13.33	4.20	6.6
Quamy	13.67	18.42	9.87	46.30	15.58	2.97	6.2
Boohai	14.28	15.34	9.94	54.24	16.01	3.39	6.1
Mukiye	11.75	18.49	10.35	45.48	6.14	7.41	3.6
Robe	12.56	19.35	7.76	54.44	15.16	3.59	5.5
Gerardo	12.33	19.09	6.07	57.22	17.35	3.30	5.4
Mean	**11.69**	**17.85**	**9.16**	**51.46**	**14.50**	**3.83**	**5.1**

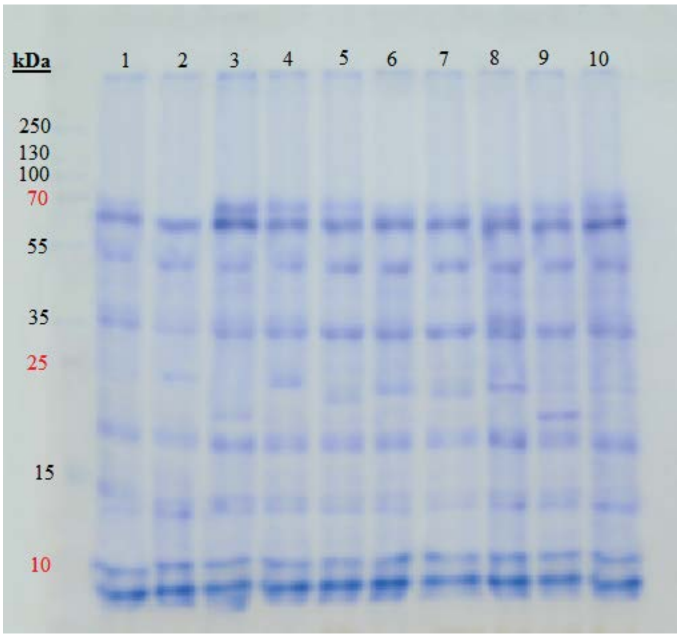

Figure 1. *Electrophorogram showing banding patterns of globulin protein fractions from selected varieties of Ethiopian durum wheat.*

1: Werer; 2: Yerer; 3: Asasa; 4: Hitosa; 5: Candate-utuba; 6: Ude 7: Foka; 8: Denbi; 9: Mangudo; 10: Bichena

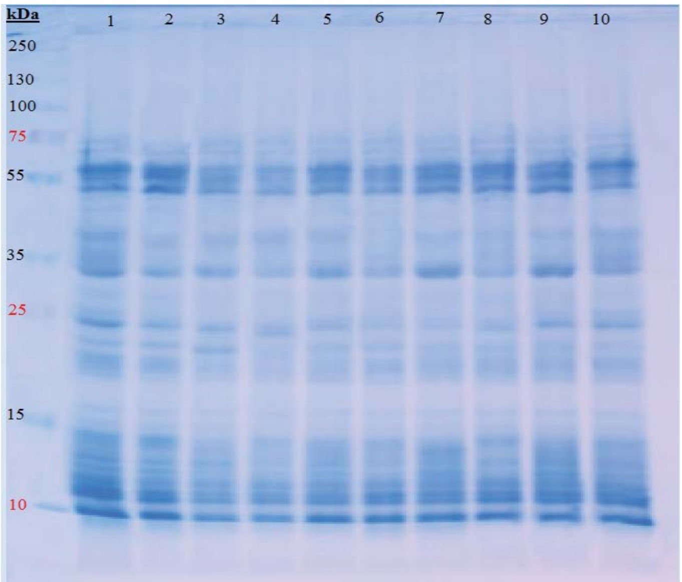

Figure 2. *Electrophorogram showing banding patterns of albumin protein fractions from selected varieties of Ethiopian durum wheat.*

Lane 1: Werer; lane 2: Yerer; lane 3: Asasa; lane 4: Hitosa; lane 5: Candate-utuba; lane 6: Ude lane 7: Foka; lane 8: Denbi; lane 9: Mangudo; lane 10: Bichena

also present (Figure 1). Results of present study are almost consistent with previous research findings [3,19] who reported the molecular weight ranging from 12.4 kDa to76.4 kDa.

In the globulin proteins, high polymorphism both in band intensity and occurrence was observed between 15 and 35 kDa. The presence of polymorphism in this region suggests that globulins could also be used as suitable and useful genetic markers to discriminate genotypes.

Polymorphism usually arises as a result of gene silencing in some varieties encoding a specific protein [20]. The gel

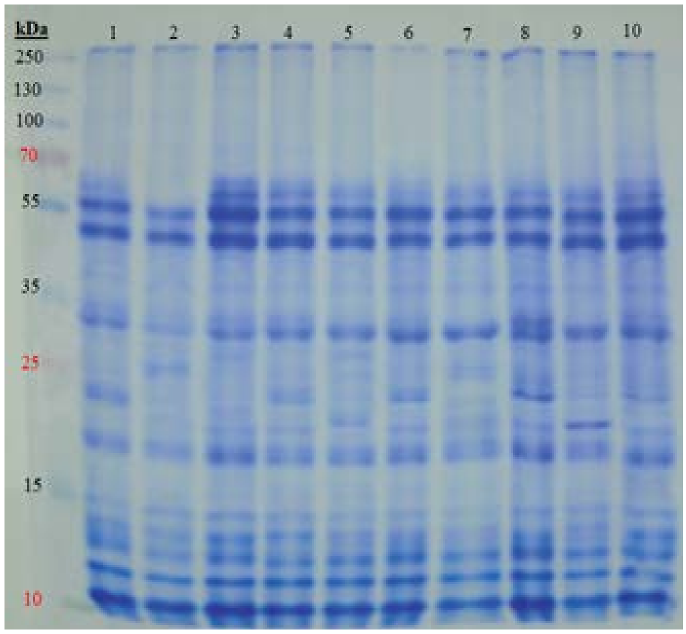

Figure 3. *Electrophorogram showing banding patterns of albumin-globulin protein mixtures from selected varieties of Ethiopian durum wheat.*

Lane 1: Werer; lane 2: Yerer; lane 3: Asasa; lane 4: Hitosa; lane 5: Candate-utuba; lane 6: Ude lane 7: Foka; lane 8: Denbi; lane 9: Mangudo; lane 10: Bichena

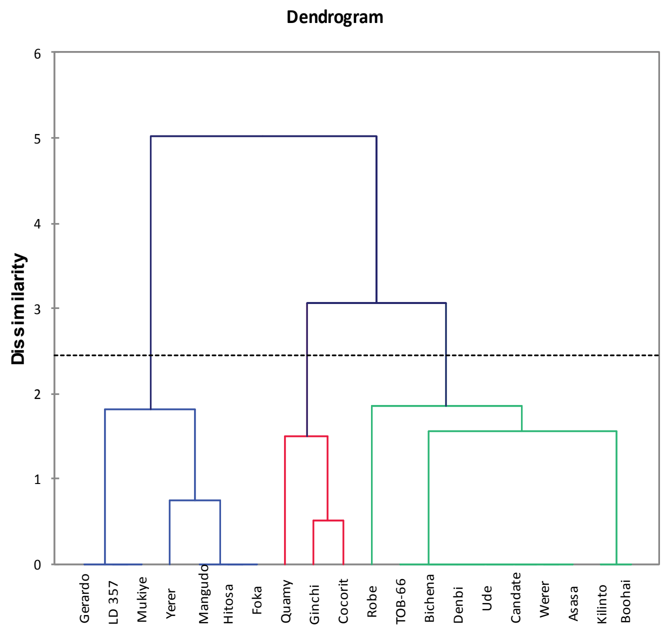

Figure 4. *Dendrogram constructed based on the electrophoretic data of globulin protein fractions of the 20 durum wheat varieties.*

electrophoresis result of albumin proteins did not show any significant variation among the genotypes. Almost all bands were monomorphic across all wheat genotypes. The banding pattern ranged from 10 kDa to 65 kDa and the number and position of bands were similar for all varieties. Unlike globulins, there were only three to four prominent bands in the case of albumins. Generally, around 20 different polypeptides were revealed in the albumin fraction. Likewise, the number of bands in globulin proteins was from 10 to 16. This is in agreement with other previous research findings [3].

The heterogeneity of albumin and globulin was demonstrated in the earlier research works [21]. This pioneering research on albumins has reported the presence of three protein components in the water soluble proteins. Following this finding, there were some contradicting reports on the presence of noted molecular weight heterogeneity in both in the water-soluble and salt-soluble proteins.

Genetic relationship among varieties

The dendrogram constructed based on the electrophoretic data of globulin protein fractions of the 20 durum wheat varieties is shown in Figure 4. There was fairly clear separation of the varieties. The cluster analysis result of globulin proteins classified the durum wheat varieties into three major lineage groups at a cut-off value 2.5. The varieties in cluster 3 were composed of widely adapted types, whereas the varieties in cluster 1 and cluster 2 were primarily from highland areas.

Conclusion

The present finding has revealed fairly significant polymorphism in the globulin protein fractions. Variability was observed in the bands corresponding to molecular weights from 15 kDa to 35 kDa. Contrary to this, albumin proteins did not show any significant variation among genotypes. Generally, apart from the gliadins and glutenins, the globulin has also a potential to serve as biochemical markers for evaluation of polymorphism and genetic diversity in durum wheat varieties. Use of globulin polymorphism in diversity studies could facilitate efforts to improve the quantity and quality of durum wheat varieties and could influence the selection of better raw materials for improved agronomic traits.

References

1. Belderok, B, Mesdag J, Donner DA. Bread-making quality of wheat: A century of breeding in Europe, 2000.
2. Harsch S, Günter T, Kling CH, et al. Characterization of spelt (*Triticum spelta* L.) forms by gel-electrophoretic analyses of seed storage proteins-I, The gliadins. Theor Appl Genet. 1997; 94(2):52-60.
3. Merlino M, Leroy P, Chambon C, et al. Mapping and proteomic analysis of albumin and globulin proteins in wheat kernels. Theoretical Appl Gene. 2009;18:1321-1337.
4. Zilic S, Barac M, Pesic M, et al. Genetic variability of albumin-globulin content, and lipoxygenase, peroxidase activities among bread and durum wheat genotypes. Genetika. 2011;43:503-516.
5. Dupont FM, Altenbach SB. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. J Cereal Sci 2011;38:133-146.
6. Morris CF. Puroindolines: The molecular genetic basis of wheat grain hardness. Plant Molecul Biol. 2002;48, 633-647.
7. Shin O, Toshiaki K, Yoshio S. Properties of wheat albumin. Ass J JapaSoc Medic Func Foods. 2006;6:467-470.
8. Dong K, Ge P, Ma C, et al. Albumin and Globulin Dynamics during Grain Development of Elite Chinese Wheat Cultivar Xiaoyan 6. J Cereal Sci. 2012;56:615-622.
9. Yadav D, Singh NK. Wheat triticin: A potential target for nutrition quality improvement. Asian J Biotechnol. 2011;3:1-21.
10. Lasztity R. The chemistry of cereal proteins, 1984.
11. Osborne TB. The proteins of the wheat kernel, 1907.
12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1970;227:680- 685.
13. Axford DW, McDermott EE, Redman DG. Note on the sodium dodecyl sulphate test of breadmaking quality: Comparison with Pelshenke and Zeleny tests. Cereal Chem. 1979;56:582.
14. Gafurova DA, Tursunkhodzhaev PM, Kasymova TD, et al. Fractional and amino-acid composition of wheat grain cultivated in Uzbekistan. Chemistry of Natural Compounds. 2002;38462-465.
15. Tadesse D, Labuschagne MT, Deventer CS. Quality of Ethiopian durum wheat lines in two diverse environments. J Agron Crop Sci. 192;2006:147-150.
16. Dodig D, Zoric M, Knezevic D, et al. Surlan Momirovic, assessing wheat performance using environmental information. Genetika. 2007;39:413-425.
17. Woyema A, Bultosa G, Taa A. Effect of different nitrogen fertilizer rates on yield and yield related traits for seven Durum Wheat (*Triticum turgidum* L. var Durum) cultivars grown at Sinana, South Eastern Ethiopia. African Journal of Food, Agriculture, Nutrition & Development. 2010;12: 36-79.
18. Stehno Z, Konvalina P, Dotlacil L. Emmer wheat growing. Praha. VÚRV, 22. 2008.
19. Hassan HM, Afify AS, Basyiony AE, et al. Nutritional and functional properties of defatted wheat protein isolates. Australian Journal of Basic and Applied Sciences. 2009;4(2):348-358.
20. Lawrence GJ, Shepherd KW. Variation in glutenin protein sub-units of wheat. Aust J Biol Sci. 1980;33:221-233.
21. Laws WD, France WG. A comparative study of some protein fractions of wheat flour. Cereal Chem. 1948;25:231-243.
22. SAS. Statistical Analysis System Institute – SAS/STAT Procedure guide for personal computers. 1981.

***Correspondence to:**

Daniel Hailegiorgis
Department of Plant Sciences
Wollo University
Dessie, Ethiopia
Tel: +251922551742
E-mail: sjyun@jbnu.ac.kr