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Determination of changes on minerals, amino and organic acids on different growing periods of buckwheat and cereal genotypes

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Abstract

The objective of this study was to determine changes of some mineral, amino and organic acid characteristics in developmental of stages of buckwheat, bread wheat, durum wheat and triticale genotypes. Analyses of above-ground parts in buckwheat and cereal genotypes showed that nutritional quality characteristics of buckwheat are similar to bread and durum wheat, barley and triticale genotypes in minerals, amino acid and organic acid compositions. Our results confirm that when compared with Sönmez (bread wheat), Dumlupınar (durum wheat), Ince (barley) and Karma (triticale) genotypes; Aktaş, Güneş and buckwheat genotypes are rich in minerals, amino and organic acid compositions. They are mostly formed by genotype × environment interaction.

Key words: Buckwheat, cereals, durum wheat, barley, triticale, organic acids

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Karabuğday ve tahıl çeşitlerinde farklı gelişme dönemlerinde mineral madde, amino ve organik asit düzeylerindeki değişimin belirlenmesi

Özet

Bu çalışmada ekmeklik buğday, makarnalık buğday, arpa, triticale ve karabuğday çeşitlerinde farklı gelişme devrelerinde yaprak ve saplarda mineral madde, amino asit ve organik asit değişimleri incelenmiştir. Yapılan incelemede karabuğday çeşitlerinin mineral madde, amino asit ve organic asit düzeyleri yönünden zengin ve diğer incelenen yakın özellik gösterdiği belirlenmiştir. Ayrıca mineral madde, amino asit ve organic asit düzeylerinin, genotip x çevre interaksiyonundan oldukça etkilendiği sonucuna varılmıştır.

Anahtar kelimeler: Karabuğday, tahıllar, makarnalık buğday, arpa, tritikale, organik asit

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is cultivated, produced, consumed in many countries of the world and it is important cultivated crop in many countries of the world (Prakash et al., 1987; Ohnishi, 2003; Gupta et al., 2002; Taghipour and Salehd, 2008; Wronkowska et al., 2010; Awasthi and Thakur, 2010). Having low use of moisture, buckwheat could supply many advantages in rotation systems by breaking up disease cycles, surpressing weeds and keeping moisture in soil (Sedej et al., 2011). Buckwheat is better adapted to low-fertile soils than most other crops (Clark et al., 1994; Dabney et al., 2001) and is not tolerant crop in frost, flooding, soil crusting and extreme drought conditions (Clark, 1994; Cruse, 1995; Delgado, 1998). It has wide usability potential in food industry and is commonly used-processed into products such as breakfast foods, flour and noodles etc. Not only as a green crop but as a grain buckwheat could be substituted for other crops in feeding livestock, linseed meal in a ration consisting of tankage, linseed meal, and alfalfa hay. The best use of buckwheat in animal feeding is suggested as mixture of it with other suitable fodder crops (Gupta et al., 2002; Dogra, 2010). This crop seems to be high in protein, amino acid

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composition and vitamins, starch, minerals, dietary fibre (Murray, 1999; Veraverbeke and Delcour, 2002; Sedej et al., 2011); biochemical, nutritional quality and nutraceutical characteristics of above-ground parts in buckwheat seem similar to major cereals (Matsuo and Dexter, 1980; Gupta et al., 2002; Wronkowska et al., 2010). Tomatake et al. (2006) determined content in buckwheat grains as 2.3 per cent and tryptophan content as 2.0 per cent. Tang et al. (2007) stated amino acid profile of tartary buckwheat seeds and reported methionine content as 0.29 per cent and tryptophan content as 0.08 per cent. Dogra (2010) pointed out methionine content in buckwheat grains as 57.9 to 103.4 mg/g N and tryptophan content as 62.2 to 79.2 mg/g N. The objective of this study was to determine changes of some mineral, amino and organic acid characteristics in developmental of stages of buckwheat, bread wheat, durum wheat and triticale genotypes.

2. Materials and methods

This study, a pot experiment, was carried out in greenhouse conditions at Osmangazi University, Agricultural College Eskişehir, Turkey (30°32'E 39°46' N, at an altitude of 792 m) in the 2012–2013 cropping seasons. Seeds were sown in PVC containers (0.75 m width, 1 m length, and 0.75 m height) containing 75 kg of loamy textured soil (33.7% sand, 37.0% silt, and 27.3% clay). Soil also had 0.44% $CaCO_3$, 241.4 mmol/kg P_2O_5, 385.1 mmol/kg K_2O, and 2.09% organic matter, 6.04 pH, and 2.71 dS/m electrical conductivity.

Sönmez: Bread wheat, winter habit, kernel red-hard, 100-110 cm plant height, 32-50 g thousand kernel weight, 80-84 kg/hl test weight, 12-14% protein content, resistant to drought and cold. **Ince:** Barley, two-row spike, kernel white, 95-105 cm plant height, 32-50 g thousand kernel weight, 62-73 kg/hl test weight, 7-13% protein content, resistant to cold.

Dumlupınar: Durum wheat, white kernel, 90-95 cm plant height, 40-45 g thousand kernel weight, 81-84 kg/hl test weight, 12-14% protein content, resistant to cold. **Karma:** Triticale, winter habit, kernel red brown, 110-120 cm plant height, 35-40 g thousand kernel weight, 78-80 kg/hl test weight, 11-13% protein content, resistant to drought and cold. **Aktaş:** Buckwheat, spring habit, kernel brown, 60-115 cm plant height, 25-35 g thousand kernel weight, 52-58 kg/hl test weight, 11-14% protein content. **Güneş:** Buckwheat, spring habit, kernel browned, 70-110 cm plant height, 24-33 g thousand kernel weight, 50-60 kg/hl test weight, 11-13% protein content. **Local variety:** Buckwheat, spring habit, kernel brown, 65-100 cm plant height, 23-35 g thousand kernel weight, 54-62 kg/hl test weight, 11-12% protein content. Cereals including cv. Sönmez (bread wheat) cv. Ince (two-row barley), cv. Dumlupınar (durum wheat) and cv. Karma (triticale) were sown during the first two weeks of September at a seed rate of 500 seed/m^2. Sixty kg N ha^{-1} (½ at sowing stage and ½ at tillering stage) and 60 kg ha^{-1} P_2O_5 (at sowing) were applied. Ammonium sulfate (21% N) and triple superphosphate (46% P_2O_5) were used as fertilizers in the study. Buckwheat cultivars (Aktaş Güneş and local variety) were sown at the third week of April and they were planted in pots with the account of 50 kg/ha seed rate, 150 kg/ha nitrogen and 100 kg/ha phosphorus. Containers in the experiment were protected from bird damage by netting. Experimental design was a randomized complete block design (RCBD) with three replications. Irrigation at sowing, at stem elongation (Feekes 6.0), and at flowering (Feekes 10.51) was applied, and after this stage waterlogging was applied. Water was given in buckwheat as needed. Seasonal changes on mineral, amino acid and organic acid levels of genotypes were determined. Mineral, amino acid and organic acid levels of genotypes were determined in seedling, flowering and maturity periods of genotypes.

Amino Acid Analysis; for the amino acid analysis, 5 mL of 0.1 N HCl was added to 5 mg plant sample. The samples were homogenized and dispersed using an IKA Ultra Turrax D125 Basic homogenizer and incubated at 40C for 12 hours. Then, the homogenized samples were vortexed. After these sample suspensions were centrifuged at 1200 rpm for 50 minutes, the supernatants were filtered using a 0.22 µm Millex Millipore filter. Next, the supernatants were transferred to vials for amino acid analysis using HPLC as described (Henderson et al. 1999). The quantities of amino acids found in the plant samples, including aspartate, glutamate, and asparagine, were determined after 26 minutes of HPLC derivation and are reported as pmol µl-1. **Organic acid analysis;** for the analysis of organic acids, 10 mL of deionized water was added to mg plant sample, which were homogenized using an IKA Ultra Turrax D125 Basic homogenizer. After centrifugation at 1200 rpm for 50 minutes, the supernatants were filtered through a 0.22 µm pore Millex Millipore filter and collected in vials. The supernatants were subjected to HPLC analysis using a Zorbax Eclipse-AAA 4.6 x 250 mm, 5 µm column (Agilent 1200 HPLC), and the absorbance at 220 nm was read using a UV detector. The flow speed was 1 mL min-1, and the column temperature was 250C. The organic acid contents of the bacterial suspensions, including oxalic and propionic acids, were determined using 25 mM potassium phosphate pH 2.5 as the mobile phase.

Hormone analysis; the extraction and purification processes were executed as described (Davies, 1995). For hormone analysis, 5 mL of cold (-400 C) 80% methanol was added to 5 mg plant sample. The plant suspensions were homogenized for 10 minutes using an IKA Ultra Turrax D125 Basic homogenizer, and then the plant suspensions were incubated for 24 hours in the dark. The plant suspensions were filtered using a Whatman No: 1 filter, and the supernatants were filtered again using a 0.45 µm pore filter. The hormones were analyzed by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC), and the absorbance was read at 265 nm using a UV detector. Gibberellic acid, salicylic acid, indole acetic acid (IAA), and abscisic acid (ABA) were determined using 13%

acetonitrile (pH 4.98) as the mobile phase. **Enzyme activities of PGPR;** phosphatase activity was determined using para-nitro-phenyl phosphate (pNPP) as an ortho-phosphate monoester analog substrate (Tabatabai, 1982). We calculated the p-nitrophenol content using a calibration curve obtained with standards containing 0, 10, 20, 30, 40 and 50 ppm of p-nitrophenol. **Antioxidant enzymes analysis of PGPR;** for antioxidant enzyme assays, frozen plant samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 0.1 mM phosphate buffer, pH 7.8, containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulfonylfluoride (PMSF) and 0.5% polyvinylpyrrolidone (PVP). The superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzyme activities in the apoplastic fractions were measured using a spectrophotometer (Sairam and Srivastava, 2002). **Element analysis;** the Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine the total N content (Bremner, 1996) of PGPR strains. The Ca, Mg, Na, K, P, S, Fe, Cu, Mn, Zn, Pb, Ni and Cd contents were determined using an Inductively Coupled Plasma spectrometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA (Mertens, 2005). **Statistical analysis;** Descriptive statistics (maximum, minimum, mean) in characters were computed (Düzgüneş et al., 1987). Biplot analysis was performed to determine similarities/differences, performance and stability of genotypes and characters by using Minitab 17 software pocked programs (Olgun et al., 2015).

3. Results

The nutritional values of cereals are extremely important and this importance of cereals is mainly due to their usage in the different form the basic ingredients of bread and other bakery products (Aubrecht and Biacs, 2001). Buckwheat proteins are high in lysine, but digestibility of protein is so low due to the high fiber and tannins content. The concentration of several amino acids in buckwheat is so higher than that of traditional cereal proteins. Buckwheat products are very nutritious containing high amount of some essential amino acids (Nicholson et al., 1976).

Environmental effects could cause significant damages on plant grain development, leading significant changes in yield components, minerals, amino acids and organic acids in crops (Setter and Waters, 2003; Uddling et al., 2007). Performances of crops including yield, yield components, minerals, amino and organic acids are formed under genotype x environment interaction. So, they are expected to be influenced by severity of waterlogging, so big variations are more likely to occur among genotypes (Sayre et al., 1994; Mengel and Kirkby, 2001). Changes including minimum, maximum and mean values in characters are given in Table 1. Minimum and maximums as pmol/ul in amino acids were 7865,0-12722,0 in asparagine, 7405,0-11977,0 in glutamine, 3008,6-4866,4 in glycine, 878,6-1421,1 in valine, 1851,2-2994,3 methionine, 1661,6-2687,5 in tryptophan, 1625,9-2629,9 in fenilalanine, 4119,0-6663,0 in lysine, 1663,2-2690,2 in hydroxyl proline and 105,76-171,06 in proline.

Table 1. Minimum, maximum and mean values on characters in buckwheat and cereals.

Variables		**Minerals** Variable: **Mean:** **Min.-Max:**	**N (%)** 2,1393±0,2427 1,6210-2,6220	**P (mg/kg)** 2470,7±259,7 2035,0-2886,0
K (mg/kg) 9115,0±2466,0 4010,0-12362,0	**Ca (mg/kg)** 4688,4±471,2 3713,0-5536,0	**Mg** (mg/kg) 1762,0±631,0 598,0-2468,0	**Na (mg/kg)** 377,6±134,5 136,0-515,9	**Fe (mg/kg)** 86,98±36,87 18,90-136,29
Cu (mg/kg) 30,85±13,98 7,12-48,15	**Mn (mg/kg)** 24,48±8,73 7,99-40,28	**Zn (mg/kg)** 28,43±6,58 16,53-41,54	**Amino Acids** Variable **Mean** **Min.-Max:**	**Asparagine (pmol/ul)** 10380,0±1178,0 7865,0-12722,0
Glutamine (pmol/ul) 9772,0±1109,0 7405,0-11977,0	**Glycine (pmol/ul)** 3970,5±450,4 3008,6-4866,4	**Valine (pmol/ul)** 1169,5±131,5 878,6-1421,1	**Methionine (pmol/ul)** 2443,0±277,2 1851,2-2994,3	**Tryptophan (pmol/ul)** 2192,7±248,8 1661,6-2687,5
Fenilalanine 2145,7±243,4 1625,9-2629,9	**Lysine (pmol/ul)** 5436,0±617,0 4119,0-6663,0	**Hydroxy Proline (pmol/ul)** 2194,9±249,0 1663,2-2690,2	**Proline (pmol/ul)** 139,56±15,83 105,76-171,06	**Organic Variable Acids Mean** **Min.- Max:**
Oxalic (ng/ul) 1,2055±0,1585 0,9078-1,4658	**Propionic (ng/ul)** 1,8825±0,2136 1,4265-2,3074	**Butyric (ng/ul)** 4,1715±0,4733 3,1610-5,1129	**Lactic (ng/ul)** 18,205±2,065 13,795-22,313	**Citric (ng/ul)** 2,6955±0,3058 2,0425-3,3037
Malic (ng/ul) 2,6527±0,3009 2,0101-3,2513	**Giberellic (ng/ul)** 87,62±16,33 57,53-123,39	**Salicylic (ng/ul)** 38,91±5,96 28,24-51,56	**Indole Acetic (ng/ul)** 2,4645±0,3126 1,7972-3,0153	**Abscisic (ng/ul)** 0,19855±0,03591 0,14420-0,26430

Moreover, minimum and maximum levels in organic acids as ng/ul were 0,9078-1,4658 in oxalic acid, 1,4265-2,3074 in propionic acid, 3,1610-5,1129 in butyric acid, 13,795-22,313 in lactic acid, 2,0425-3,3037 in citric acid, 2,0101-3,2513 in malic acid, 57,53-123,39 in giberellic acid, 28,24-51,56 in salicylic acid, 1,7972-3,0153 in indole acetic acid and 0,14420-0,26430 in abscisic acid (Table 1). Comparative analysis of buckwheat and cereals were made in two main part; first analyses above-ground parts in seedling, flowering and maturity periods developmental stages, second analyses of kernel characteristics.

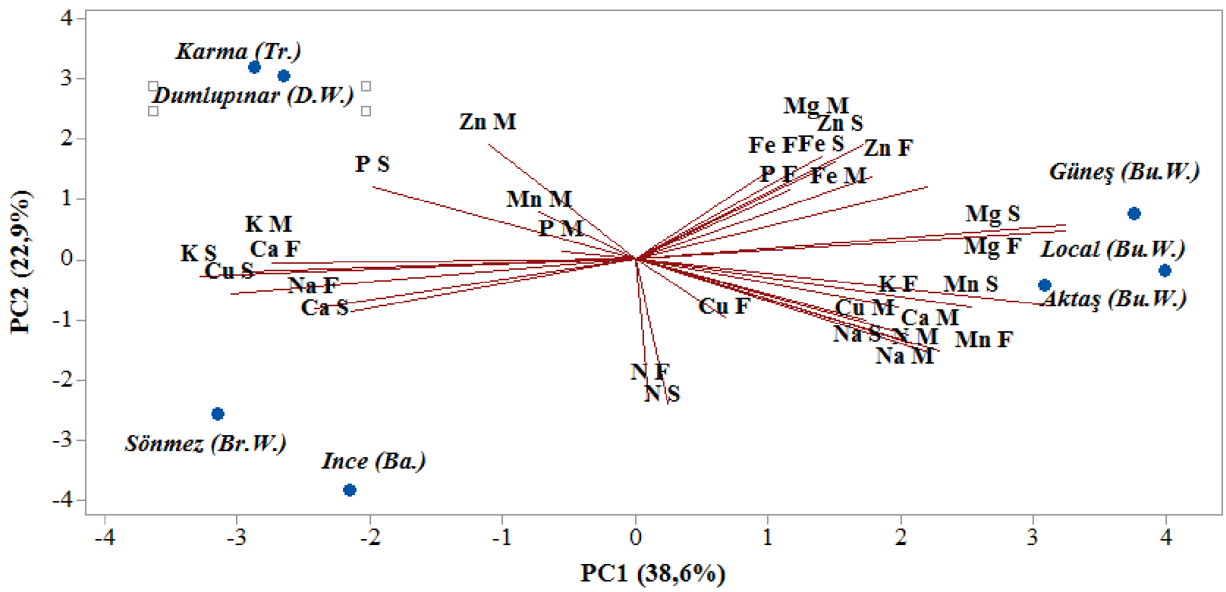
Seasonal Changes in Characteristics of Genotypes: Analyses of above-ground parts were made for minerals, amino acids and organic acids. Distribution of mineral elements in developmental stages of buckwheat and the other cereal genotypes were given in Table 2.

Table 2. Distribution of mineral elements in developmental stages of buckwheat and the other cereals genotypes

	N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
	%	mg/kg								
Seedling Period										
Aktaş (Bu.W.)*	2,11	2136	8652	3895	2215	513	86,53	36,58	24,16	32,44
Güneş (Bu.W.)	2,06	2215	8214	3713	2451	425	92,31	32,51	28,53	30,26
Local (Bu.W.)	2,2	2163	8569	4215	2365	496	84,16	33,48	24,15	35,41
Sönmez (Br.W.)	2,27	2501	10845	4512	1748	431	75,69	46,52	22,37	26,58
Ince (Ba.)	2,30	2035	11478	4158	1653	469	81,06	47,51	24,13	27,46
Dumlupınar (D.W)	1,84	2418	10674	4168	1788	386	82,13	48,15	21,52	35,45
Karma (Tr.)	2,02	2415	11263	4102	1875	412	92,06	40,12	20,16	30,85
Mean$_{Buckwheat}$	**2,12**	**2171,33**	**8478,33**	**3941,00**	**2343,67**	**478,00**	**87,67**	**34,19**	**25,61**	**32,70**
Mean$_{Cereals}$	**2,11**	**2342,25**	**11065,00**	**4235,00**	**1766,00**	**424,50**	**82,74**	**45,58**	**22,05**	**30,09**
Flowering Period										
Aktaş (Bu.W.)	2,36	2715	11524	4865	2236	425	116,35	42,15	29,54	31,26
Güneş (Bu.W.)	2,42	2812	11365	4715	2345	412	119,63	40,32	31,26	30,13
Local (Bu.W.)	2,45	2652	10256	4263	2468	426	102,35	38,75	32,15	34,52
Sönmez (Br.W.)	2,59	2776	10303	5505	1888	474	94,61	40,01	26,40	24,72
Ince (Ba.)	2,62	2259	10904	5073	1785	516	101,33	40,86	28,47	25,54
Dumlupınar (D.W)	2,10	2684	10140	5085	1931	425	102,66	41,41	25,39	32,97
Karma (Tr.)	2,32	2615	10213	5261	1925	510	124,53	36,25	25,41	26,59
Mean$_{Buckwheat}$	**2,42**	**2726,33**	**11048,33**	**4614,33**	**2349,67**	**421,00**	**112,78**	**40,41**	**30,98**	**31,97**
Mean$_{Cereals}$	**2,41**	**2583,50**	**10390,00**	**5231,00**	**1882,25**	**481,25**	**105,78**	**39,63**	**26,42**	**27,46**
Maturity Period										
Aktaş (Bu.W.)	2,24	2235	10568	4865	2169	475	126,39	35,47	32,68	30,15
Güneş (Bu.W.)	2,26	2315	11241	4765	2214	482	123,48	36,24	34,41	34,11
Local (Bu.W.)	2,31	2241	9685	4963	2168	461	135,67	38,12	32,16	33,26
Sönmez (Br.W.)	2,17	2554	11642	4899	2039	436	105,02	35,21	31,94	31,15
Ince (Ba.)	2,20	2078	12322	4515	1928	475	112,47	35,96	34,45	32,18
Dumlupınar (D.W)	1,76	2469	11459	4526	2086	391	113,96	36,44	30,73	41,54
Karma (Tr.)	2,05	2103	12362	4103	2235	426	136,29	30,12	40,28	35,24
Mean$_{Buckwheat}$	**2,27**	**2263,67**	**10498,00**	**4864,33**	**2183,67**	**472,67**	**128,51**	**36,61**	**33,08**	**32,51**
Mean$_{Cereals}$	**2,05**	**2301,00**	**11946,25**	**4510,75**	**2072,00**	**432,00**	**116,94**	**34,43**	**34,35**	**35,03**
Grand Mean$_{Buckwheat}$	**2,27**	**2387,11**	**10008,22**	**4473,22**	**2292,34**	**457,22**	**109,65**	**37,07**	**29,89**	**32,39**
Grand Mean$_{Cereals}$	**2,19**	**2408,92**	**11133,75**	**4658,92**	**1906,75**	**445,92**	**101,82**	**39,88**	**27,61**	**30,86**

***Bu.W:** Buckwheat, **Ba:** Barley, **Br. W:** Bread wheat, **D. W:** Durum wheat, **Tr:** Triticale

Having different and significant metabolic and physiological functions, minerals are assumed as essential for optimum human and animal nutrition and buckwheat was found to contain perceptible levels in minerals and similar with other cereals (Thacker et al., 1983; Gupta et al., 2002 Gopalan et al., 2004). While Ince in N and K, Sönmez in P and Ca, Güneş in Mg, Fe and Mn, Aktaş in Na and Zn, Dumlupınar in Cu had the highest levels; Karma in N and Mn, Aktaş in P and Ca, Güneş in K and Cu, Sönmez in Fe and Zn, Ince in Mg and Dumlupınar in Na the lowest mineral contents in seedling period. Besides, buckwheat genotypes in N, Mg, Na, Fe, Mn and Zn; cereal genotypes in P, K, Ca and Cu seemed to be richer. Mineral levels increased in various amounts toward to flowering period in buckwheat and other cereal genotypes. In flowering period, Ince in N and Na, Gün in P and Fe, Aktaş in K, Cu and Mn, Local genotype in Mg and Zn, and Sönmez in Ca were found as the highest genotypes. Lowest ones were determined as Dumlupınar in N, K and Mn, Ince in P and Zn, Sönmez in Mg and Fe, Local genotype in Ca, and Karma in Cu. Higher levels of N, Mg, Fe, Cu, Mn and Zn in buckwheat genotypes; P, K, Ca and Na in cereal genotypes were found in flowering period. Although, mineral levels decreased in maturity period when compared with previous period, mineral contents in maturity period were still higher than that of seedling period. Local genotype in N, Ca, and Cu, Dumlupınar in P and Zn, Karma in Mg, Fe and Mn, Gün in Na and Sönmez in P showed up the genotypes with the highest mineral contents. Moreover, Dumlupınar in N and Na, Karma in Ca and Cu, Sönmez in Mg, Fe and Mn, Ince in P and Local genotype in K had the lowest mineral content. Higher levels of N, Ca, Mg, Na, Fe and Cu in buckwheat genotypes; P, K, Mn and Zn in cereal genotypes were found in maturity period. With overall assumption, N, P, Mg, Na, Fe, Mn and Zn in buckwheat genotypes; K, Ca and Cu in cereal genotypes were found to be higher (Table 2). Minerals are known to be essential healthy nutrition and nutritional value of buckwheat is reported as similar with cereals (Dietrych-Szostak and Oleszek, 1999; Christa and Soral-Smietana, 2008).



Bu.W: Buckwheat, **Ba:** Barley, **Br. W:** Bread wheat, **D. W:** Durum wheat, **Tr:** Triticale, **S:** Seedling period, **F:** Flowering period, **M:** Maturity period

Figure 1. Biplot analysis of mineral elements in developmental stages of buckwheat and the other cereals

Researchers reported that levels of N, P, K, Ca, Mg, Mn, Zn, Fe, Cu and Na varied between1,60-2,80%, 2050-2500, 4200-1140, 2800-450, 1620-2450, 22,40-36,50, 16,80-27,50, 80,60-105,40, 30,20-43,60 and 420-530 mg/kg, respectively (Wang et al., 1995; Gupta et al., 2002; Ikeda et al., 2005). Biplot analysis of mineral elements in developmental stages of buckwheat and the other cereals genotypes are shown in Figure 1. Second component values (PC_2) assigned that buckwheat genotypes (Güneş, Aktaş and Local cv.) showed better stability performance and Karma cv., Dumlupınar cv. and Sönmez cv., Ince cv. had instabile characteristics in minerals. Stability performances for PC_2 varied in minerals. Cu, Mn, Ca and K minerals were more stabile than the other minerals in PC_2. N F, N S, Cu F, P M, Mn M and Zn M play small role in explaining the variation on PC_1. Mg F, Mg S, Mn F, Mg S, Mn S, Ca M, N F, Na F, Na M, Na F, K F, Zn F, Mg M, K S, Cu S, Na F, Ca S, Ca F, K M, P S had greater impact than the other minerals for PC_1. Including considerable levels of some minerals, the nutritional characteristics, mineral levels on different stages in buckwheat aren't thoroughly elucidated (Pomeranz, 1983). Mineral levels in different stages varies, increase toward flowering stage, and mineral levels during developmental stages are influenced by genetic factors, environmental conditions and processing conditions (Pomeranz and Robbins, 1972; Pomeranz, 1983; Ikeda et al., 1995; James, 1995). It could be said that genotypic differences are important, genotypic differences have more effective on mineral changes than that of seasonal changes, and variations. It could be said that genotypic differences are important, genotypic differences have more effective on mineral changes than that of seasonal changes. Levels of minerals in genotypes and their seasonal trends substantially depend upon genotype × environment interaction.

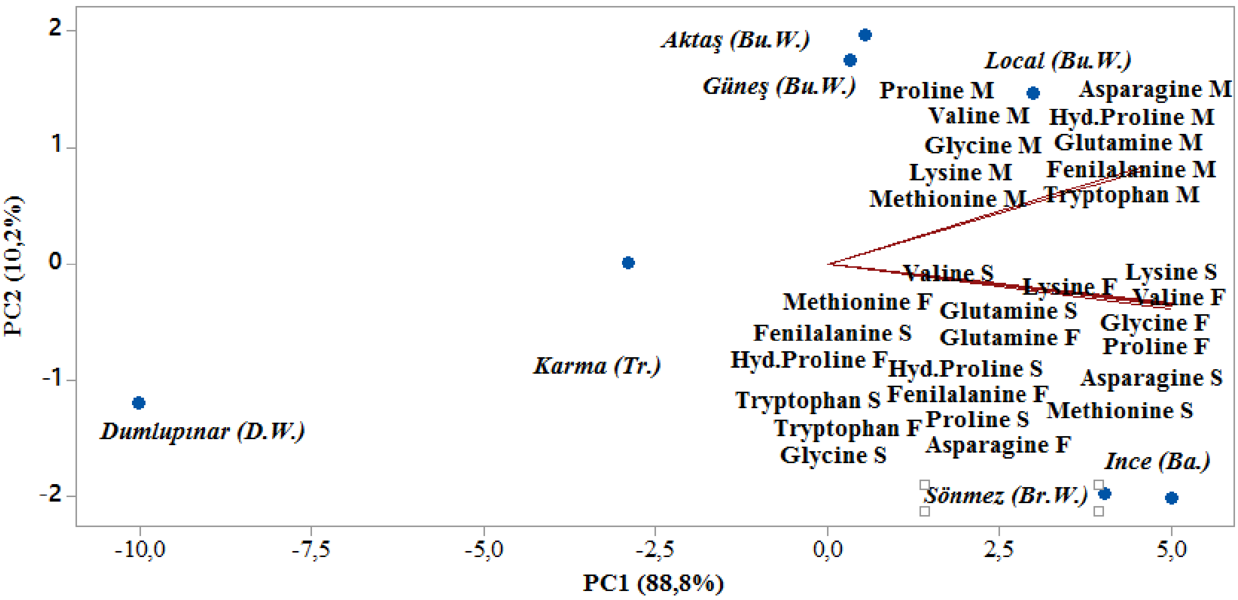
Being basis ingredients in protein synthesis, amino acids influence the metabolic and physiological processes in crops (Dietrych-Szostak and Oleszek, 1999; Olgun et al., 2015). Amino acid contents of proteins in buckwheat seemed to be balanced and high level of biologic value (Kato et al. 2001). Buckwheat is rich in arginine and lysine, and low in methionine and threonine in comparison to cereals (Aubrecht and Biacs, 2001). Comparative amino acid levels in buckwheat and cereal genotypes were given in Table 3. Amino acid composition in genotypes were examined in three periods; seedling, flowering and maturity periods. As a means of seedling, flowering and maturity periods and grand means, levels of asparagine, glutamine, glycine, valine, methionine, tryptophan, phenylalanine, lysine, hydroxy proline and proline in buckwheat were higher than cereals. Amino acid contents in all amino acids ranged as flowering period > maturity period > seedling period. In seedling period, Ince in and Dumlupınar in phenylalanine were found as the highest genotypes. Dumlupınar in asparagine, glutamine, glycine, valine, lysine and proline; Karma in methionine, tryptophane, phenylalanine and hydroxyl proline had the lowest amino acid contents. Besides in flowering period, the lowest values belonged to Dumlupınar in all amino acids. Ince in asparagine, methionine, tryptophane, phenylalanine, lysine, hydroxyl proline and proline; Sönmez in glutamine and valine had the highest amino acid levels. In maturity period, while Dumlupınar had the lowest amino acid levels, the highest values were recorded in Local variety (Table 3). Compared to other cereals, the amino acid contents in buckwheat are well more or less similar or richer in some amino acids. Buckwheat is particularly rich in lysine and arginine, which is generally known as limiting amino acid in wheat and barley (Bhagmal, 1994; Wei et al., 2003).

Table 3. Distribution of amino acids in developmental stages of buckwheat and the other cereal genotypes.

	Asparagine	Glutamine	Glycine	Valine	Methionine	Tryptophan	Fenilalanine	Lysine	Hydroxy Proline	Proline
	pmol/ul									
Seedling Period										
Aktaş (Bu.W.)*	10238	9638	3916	1144	2410	2163	2116	5362	2165	138
Güneş (Bu.W.)	9995	9410	3823	1117	2353	2112	2066	5234	2114	134
Local (Bu.W.)	10674	10050	4083	1192	2512	2255	2207	5590	2257	144
Sönmez (Br.W.)	11014	10369	4213	1230	2592	2327	2277	5768	2329	148
Ince (Ba.)	11160	10506	4269	1247	2627	2358	2307	5844	2360	150
Dumlupınar (D.W)	8928	8405	3415	997	2101	1886	1846	4675	1888	120
Karma (Tr.)	9801	9227	3749	1095	2307	2071	2026	5133	2073	132
Mean$_{Buckwheat}$	**10302,33**	**9699,33**	**3940,67**	**1151,00**	**2425,00**	**2176,67**	**2129,67**	**5395,33**	**2178,67**	**138,67**
Mean$_{Cereals}$	**10225,75**	**9626,75**	**3911,50**	**1142,25**	**2406,75**	**2160,50**	**2114,00**	**5355,00**	**2162,50**	**137,50**
Flowering Period										
Aktaş (Bu.W.)	11451	10780	4380	1279	2695	2419	2367	5997	2421	154
Güneş (Bu.W.)	11742	11055	4492	1312	2764	2481	2427	6149	2483	158
Local (Bu.W.)	11887	11192	4547	1328	2798	2511	2457	6225	2514	160
Sönmez (Br.W.)	12556	11821	4803	1403	2955	2652	2596	6576	2655	169
Ince (Ba.)	12722	11977	4866	1421	2994	2688	2630	6663	2690	171
Dumlupınar (D.W)	10178	9582	3893	1137	2395	2150	2104	5330	2152	137
Karma (Tr.)	11257	10598	4306	1257	2649	2378	2327	5895	2380	151
Mean$_{Buckwheat}$	**11693,33**	**11009,00**	**4473,00**	**1306,33**	**2752,33**	**2470,33**	**2417,00**	**6123,67**	**2472,67**	**157,33**
Mean$_{Cereals}$	**11678,25**	**10994,50**	**4467,00**	**1304,50**	**2748,25**	**2467,00**	**2414,25**	**6116,00**	**2469,25**	**157,00**
Maturity Period										
Aktaş (Bu.W.)	10868	10232	4157	1214	2558	2296	2247	5692	2298	146
Güneş (Bu.W.)	10966	10324	4195	1225	2581	2317	2267	5743	2319	147
Local (Bu.W.)	11208	10552	4287	1252	2638	2368	2317	5870	2370	151
Sönmez (Br.W.)	10547	9930	4034	1178	2482	2228	2180	5524	2230	142
Ince (Ba.)	10686	10061	4088	1194	2515	2258	2209	5597	2260	144
Dumlupınar (D.W)	8549	8049	3270	955	2012	1806	1767	4477	1808	115
Karma (Tr.)	9947	9364	3805	1111	2341	2101	2056	5209	2103	134
Mean$_{Buckwheat}$	**11014,00**	**10369,33**	**4213,00**	**1230,33**	**2592,33**	**2327,00**	**2277,00**	**5768,33**	**2329,00**	**148,00**
Mean$_{Cereals}$	**9932,25**	**9351,00**	**3799,25**	**1109,50**	**2337,50**	**2098,25**	**2053,00**	**5201,75**	**2100,25**	**133,75**
Grand Mean$_{Buckwheat}$	**11003,22**	**10359,22**	**4208,89**	**1229,22**	**2589,89**	**2324,67**	**2274,56**	**5762,44**	**2326,78**	**148,00**
Grand Mean$_{Cereals}$	**10612,08**	**9990,75**	**4059,25**	**1185,42**	**2497,50**	**2241,92**	**2193,75**	**5557,58**	**2244,00**	**142,75**

***Bu.W:** Buckwheat, **Ba:** Barley, **Br. W:** Bread wheat, **D. W:** Durum wheat, **Tr:** Triticale

Level of lysine and glycine are two of the main factors determining the cholesterol lowering properties of proteins having the highest cholesterol lowering properties. These amino acids contribute to regulate the hepatic LDL receptors, and lowering the serum cholesterol, and indirectly helping to prevent formation of arteriosclerosis (Murray, 1999; Wronkowska et al., 2010). Similar to our findings, Bhagmal (1994) reported that glycine, phenylalanine, methionine and lysine as mg/kg ranged from 3100 to 4300, 1850 to 2450, 1950 to 2750, 3900 to 5800, respectively in wheat, barley and maize. Moreover, methionine tryptophane and lysine content as mg/kg ranged 2250-2800, 1800-2500 and 4900-5900, respectively (Bonafaccia et al., 2003; Gopalan et al., 2004). Biplot analysis of amino acids in developmental stages of buckwheat and the other cereals genotypes are shown in Figure 2. According to second component values (PC_2), Karma cv. was stabile genotype, whereas the other genotypes were instabile in levels of amino acids. Meanwhile, amino acids in period had instabile characteristics (PC_2). Buckwheat genotypes occupied same group; Sönmez cv. and Ince cv. joined in one group. In the same way, amino acids in seedling and flowering periods had same group and they in maturity period created another group. Amino acids in different periods have great impact in explaining the variation on PC_1. Periodic differences play more important role on levels of amino acids if compared with genotypic differences. Besides, genotype x environment interaction stamps out behavior and level of amino acids in genotypes. Levels of organic and amino acids are more likely to be correlated with the photosynthetic activities in crops. More photosynthetic activities cause higher levels of amino acids (Wronkowska et al., 2010). This explains higher values on amino acid levels in seedling and flowering period (Table 3 and Figure 2). Being in plant metabolism organic acids play vital role in anabolic and catabolic reactions such as energy production, making up leading indicators for amino acid synthesis and regulating crop to environmental conditions (Fornal et al., 1987; Hoffland et al., 1992). Citric acid and malic acid were the main organic acids in growing tomato (Kamilova et al., 2006; Hoffland et al., 1992). The role of organic acids in the protection of the root apex from Al stress, and in its tissue detoxification and their importance in its xylem transport have been shown in *F. esculentum* and *Hydrangea* (Ma et al., 1997). Contends of organic acid increase during plant growth and citric and malic acids represent the major organic acids in tomato, cucumber, and sweet pepper (Kamilova et al., 2006).



Bu.W: Buckwheat, **Ba:** Barley, **Br. W:** Bread wheat, **D. W:** Durum wheat, **Tr:** Triticale, **S:** Seedling period, **F:** Flowering period, **M:** Maturity period

Figure 2. Biplot analysis of amino acids in developmental stages of buckwheat and the other cereals

Comparisons of organic acid levels in buckwheat and cereal genotypes were given in Table 4. As a mean, buckwheat seemed to be superior in three growing periods for most organic acids. In seedling period, buckwheat in oxalic acid, propionic acid, butyric acid, lactic acid, citric acid, malic acid, salicylic acid and indole acetic acid; cereals in giberellic acid and abscisic acid had higher organic acid levels. In flowering period, higher organic acid levels belonged to buckwheat in oxalic acid, butyric acid, citric acid, malic acid, giberellic acid and salicylic acid; to cereals in propionic acid, lactic acid, abscisic acid and indole acetic acid, respectively. Buckwheats were determined as better in all organic acids except in abscisic acid that belonged to cereals in maturity period and in general evaluation (Table 4). Analyses of above-ground parts in buckwheat and cereal genotypes showed that nutritional quality characteristics of buckwheat are similar to bread and durum wheat, barley and triticale genotypes. Buckwheat could be well alternative as a fodder crop if buckwheat is harvested early in flowering having respectable quality level in quality characters such as minerals, amino and organic acids and protein (Gopalan et al., 2004).

Organic acids draw similar trend like amino acids. In second component values (PC_2), Karma cv. and Dumlupınar cv. were more stabile genotypes than the other genotypes. Organic acids showed instabile performances in different levels. Some organic acids such as butyric acid S, propionic acid S, malic acid S, oxalic acid F, and indole acetic acid S were more stabile organic acids (PC_2). Like Figure 2, Buckwheat genotypes occupied same group; Sönmez cv. and Ince cv. joined in one group. Organic acids in seedling and flowering periods had same group and they in maturity period occupied another group. Variation on PC_1 stemmed from changes on organic acids in different periods. Organic acids in three different periods achieve significant role in explaining the variation on PC_1. Periodic differences, environmentao conditions affecting metabolic processes are therefore more important factor on levels of organic acids if compared with genotypic differences. Besides, genotype x environment interaction plays also effective role in behavior and level of organic acids in genotypes (Table 4 and Figure 3).

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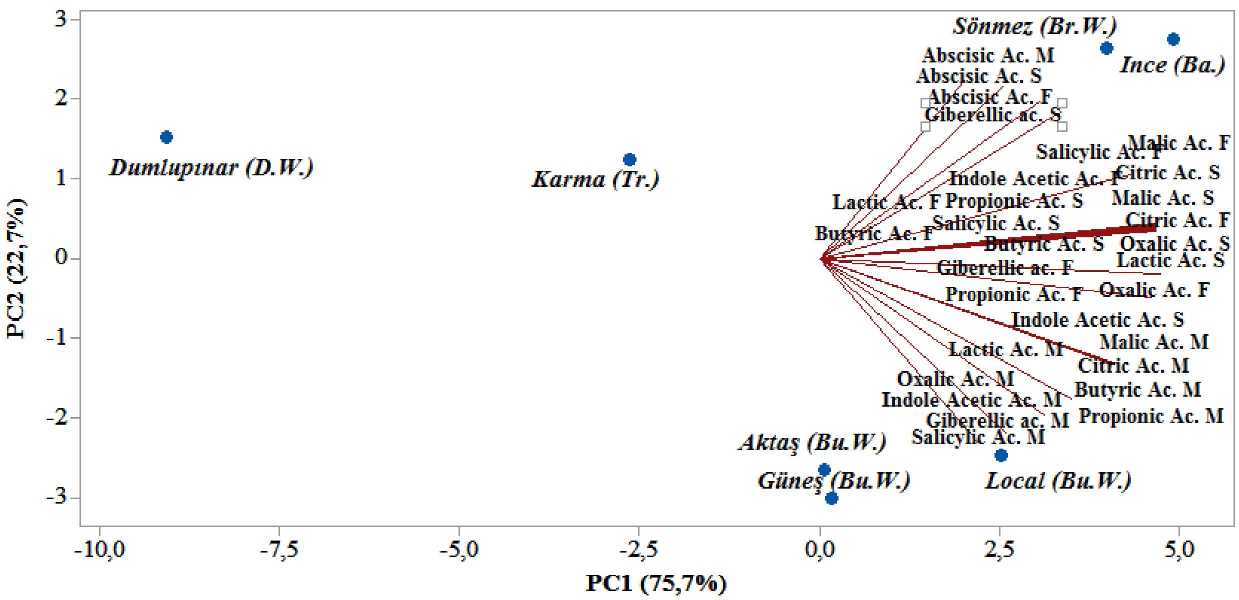
Accumulation of organic acids in the night and utilization come about during the day and the leaves of crop contain about 1.5 times as much total acid as the stem (Schiffner et al., 2001; Morita et al. 2006; Gleixner and Mügler, 2007).

Table 4. Distribution of organic acids in developmental stages of buckwheat and the other cereal genotypes.

	Oxalic Acid	Propionic Acid	Butyric Acid	Lactic Acid	Citric Acid	Malic Acid	Giberel-lic Acid	Salicy-lic Acid	Indole Acetic Acid	Absci-sic Acid
	ng/ul									
Seedling Period										
Aktaş (Bu.W.)*	1,18	1,86	4,11	2,62	17,96	2,66	101,60	36,76	2,72	0,15
Güneş (Bu.W.)	1,15	1,81	4,02	2,55	17,53	2,60	99,19	35,89	2,66	0,14
Local (Bu.W.)	1,23	1,94	4,29	2,73	18,72	2,77	105,93	38,32	2,84	0,15
Sönmez (Br.W.)	1,27	2,00	4,43	2,81	19,32	2,86	121,79	39,54	2,79	0,18
Ince (Ba.)	1,29	2,02	4,49	2,85	19,57	2,90	123,40	40,07	2,83	0,18
Dumlupınar (D.W)	1,03	1,62	3,59	2,28	15,66	2,32	98,72	32,05	2,26	0,15
Karma (Tr.)	1,13	1,78	3,94	2,50	17,19	2,55	108,37	35,19	2,48	0,16
Mean$_{\textbf{Buckwheat}}$	1,19	1,87	4,14	2,63	18,07	2,68	102,24	36,99	2,74	0,15
Mean$_{\textbf{Cereals}}$	1,18	1,86	4,11	2,61	17,94	2,66	113,07	36,71	2,59	0,17
Flowering Period										
Aktaş (Bu.W.)	1,37	2,08	4,60	2,93	20,08	2,97	91,21	41,11	2,62	0,19
Güneş (Bu.W.)	1,40	2,13	4,72	3,00	20,59	3,05	93,53	42,16	2,69	0,19
Local (Bu.W.)	1,42	2,16	4,78	3,04	20,85	3,09	94,69	42,68	2,72	0,20
Sönmez (Br.W.)	1,45	2,28	5,05	3,21	22,02	3,26	100,02	45,08	2,98	0,23
Ince (Ba.)	1,47	2,31	5,11	3,25	22,31	3,30	101,34	45,68	3,02	0,24
Dumlupınar (D.W)	1,17	1,85	4,09	2,60	17,85	2,64	81,07	36,54	2,41	0,19
Karma (Tr.)	1,30	2,04	4,52	2,88	19,74	2,92	89,67	40,41	2,67	0,21
Mean$_{\textbf{Buckwheat}}$	1,40	2,12	4,70	2,99	20,51	3,04	93,14	41,98	2,68	0,19
Mean$_{\textbf{Cereals}}$	1,35	2,12	4,69	2,99	20,48	3,03	93,03	41,93	2,77	0,22
Maturity Period										
Aktaş (Bu.W.)	1,32	1,97	4,37	2,78	19,06	2,82	86,58	50,00	2,51	0,20
Güneş (Bu.W.)	1,33	1,99	4,41	2,80	19,23	2,85	87,35	50,44	2,53	0,20
Local (Bu.W.)	1,36	2,03	4,50	2,86	19,66	2,91	89,28	51,56	2,59	0,21
Sönmez (Br.W.)	1,22	1,91	4,24	2,70	18,50	2,74	70,97	37,87	2,22	0,26
Ince (Ba.)	1,23	1,94	4,29	2,73	18,74	2,78	71,91	38,37	2,25	0,26
Dumlupınar (D.W)	0,99	1,55	3,44	2,18	14,99	2,22	57,53	30,69	1,80	0,21
Karma (Tr.)	1,15	1,80	4,00	2,54	17,45	2,58	66,93	35,71	2,09	0,25
Mean$_{\textbf{Buckwheat}}$	1,34	2,00	4,43	2,81	19,32	2,86	87,74	50,67	2,54	0,20
Mean$_{\textbf{Cereals}}$	1,15	1,80	3,99	2,54	17,42	2,58	66,84	35,66	2,09	0,25
Grand Mean$_{\textbf{Buckwheat}}$	1,31	2,00	4,42	2,81	19,30	2,86	94,37	43,21	2,65	0,18
Grand Mean$_{\textbf{Cereals}}$	1,23	1,93	4,26	2,71	18,61	2,76	90,98	38,10	2,48	0,21

***Bu.W:** Buckwheat, **Ba:** Barley, **Br. W:** Bread wheat, **D. W:** Durum wheat, **Tr:** Triticale



Bu.W: Buckwheat, **Ba:** Barley, **Br. W:** Bread wheat, **D. W:** Durum wheat, **Tr:** Triticale, **S:** Seedling period, **F:** Flowering period, **M:** Maturity period

Figure 3. Biplot analysis of organic acids in developmental stages of buckwheat and the other cereals..

4. Conclusions and discussion

Buckwheat (*Fagopyrum esculentum* Moench) is commonly used as green vegetable at their early seedling stage, or as a grain crop. Its sprouts as a green vegetable could be used as a fresh vegetable, in salads, or seasoned vegetables and it possesses high antioxidant activity, phenolic acids, flavonoids, phytic acid, vitamins (Kreft and Skrabanja, 2002; Schiffner et al., 2001; Morita et al. 2006). Another consumption as a grain buckwheat is commonly used as a baking material for breads, cakes, or pancakes with the other cereals. Besides, whatever usage in buckwheat is made, the important phenomena is that the more powerful vegetative grow occur, the more yield is formed. Our results confirm that when compared with Sönmez (bread wheat), Dumlupınar (durum wheat) Ince (barley) and Karma (triticale) genotypes; Aktaş, Güneş and buckwheat genotypes are rich in minerals, amino and organic acid compositions. They are mostly formed by genotype x environment interaction. Further detailed and systematical studies will help to determine biochemical composition, to make better usability, to breed new genotypes in buckwheat.

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