Pak. J. Bot., 54(5): 1741-1748, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-5(17)

ALTERATION IN AMINO ACID CONTENTS IN DIFFERENT GENOTYPES OF BREAD WHEAT AT DIFFERENT DEVELOPMENTAL STAGES

MURAT ARDIÇ

Department of Biology, Faculty of Science and Letters, Eskişehir Osmangazi University, TR-26040, Eskişehir, Turkey

**Corresponding email: mardic@ogu.edu.tr*

Abstract

The aim of this study was to determine changes of amino acid levels in thirteen bread wheat genotypes during growth stages of wheat (tillering, flowering, maturity stages and seeds) in growing periods of 2018-2019. Results suggest that important variations and differences occurred among bread wheat genotypes and their growing stages. The amino acid content was found as higher in tillering and flowering, lower in maturity stage. Once changes in amino acid levels increase/ decrease depending upon biochemical activities and crop growth stages, genotypic capacity and features significantly control of amino acid levels in bread wheat. Amino acid levels are higher until the flowering period than during the maturation period indicates that amino acids are of vital importance in plant development. Especially amino acids responsible for growth and development are very important in bread wheat. In addition, differences between genotypes and plant growth stages indicate that amino acids are highly affected by the genotype x environment interaction.

Key words: Bread wheat genotypes, Amino acid levels, Growth stages, Double dendrogram.

Introduction

Having genetic adaptability to different environments and well resistance to biotic and abiotic stresses, wheat (*Triticum* spp.) is commonly grown and is a major crop in the world. Besides, it performs very important function in human nutrition as food suppliers as protein, minerals etc. (Shewry, 2007). Although, Wheat yields haven't continuously risen and wheat production in the world is still sufficient, wheat production cannot meet this demand due to demands for food to be increased in the near future. Bread wheat covers main position and vital role in crop production and consumption among the food crops in Türkiye. Wheat seed is processed for different purposes (Bouis, 2003). In Türkiye, covering almost 60% of cereal production, bread wheat plays important activity in demands of carbohydrates, proteins and minerals etc. Among cereals, biochemical and nutritional quality of bread wheat are high (Wronkowska *et al*., 2008).

Amino acids are present in plant and form protein. Plants synthesize amino acids from the carbon and oxygen that is obtained from air and hydrogen from water in the soil. Amino acids have various prominent functions in plants. Besides their usage during protein biosynthesis, they also represent building blocks for several other biosynthesis pathways and play pivotal roles during signaling processes as well as in plant stress response (Hildebrandt *et al*., 2015). Amino acids play important role to increase yield and overall quality of crops. The quality and quantity of wheat depends upon the nutritive value of its protein. From nutritional point of view a balance between essential amino acids in the protein complex is important. The protein and amino acid composition of wheat varies with the crop varieties, application of fertilizers, irrigation practices, the soil and climatic conditions of the area (Khan *et al*., 2014).

Determining amino acid levels in different period of crops helps to understand behavior of amino acids and promising new genotypes. The aim of this study was to determine changes of amino acid levels during growth periods of bread wheat genotypes.

Materials and Methods

Current study was carried out on greenhouse conditions at experimental station of Osmangazi University, Agricultural College Eskişehir. Thirteen genotypes of bread wheat (**BW_1:** Es-26, **BW_2:** Bezostaja-1, **BW_3:** Müfitbey, **BW_4:** Altay-2000, **BW_5:** Sönmez-01, **BW_6:** Soyer-02, **BW_7:** Çetinel-2000, **BW_8:** Harmankaya-99, **BW_9:** Sultan-95, **BW_{10}:** Alpu-01, **BW_{11}:** Atay-85, **BW_{12}:** Özdemir and **BW_{13}:** Gerek-79) were used in the current study. The bread wheat genotypes used in the study were obtained from Eskişehir Transitional Zone Agricultural Research Institute (GKTAEM), Turkey. This study was carried out in completed randomized block design with three replications in 2018-2019. Seeds were sterilized by bleach (NaOCl 10%) and put into pots (0.75 m width, 1 m length, and 0.75 m height) having 80 kg of humus soil. Soil also had 28.5 % $CaCO_3$, 297.3 mmol/kg P_2O_5, 385.5 mmol/kg K_2O, and 2.36% organic matter, 6,11 pH, and 2,63 dS/m electrical conductivity. Plants were grown under greenhouse conditions in the relative humidity of 60% and daily (16 h) and nightly (8 h) temperatures of 25 °C. Seed planting was made in 15th of September. By dividing in two (½ at sowing period and ½ at tillering period), 60 kg N ha^{-1} and once (at sowing) 60 kg ha^{-1} P_2O_5 were applied. Normal water (EC=0.8–2.1 dS m^{-1}) was used in the study. Plants grew until tillering period (Zadoks 21) in greenhouse conditions until November then pots were put outside for overwinter, and they kept growing under ambient conditions. Pots were protected from bird damage by netting. Three times irrigation at sowing, at stem elongation (Zadoks 24), and at flowering (Zadoks 65) were Irrigations were made. Precipitations were 366, 9 mm in 2018-2019 and 374, 2 mm in long-term years (Table 1).

Table 1. Average, minimum and maximum temperatures, precipitations in long term years in Eskişehir, Turkey

Climate Param.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	To./Av.
Average temperature (°C)	-0.0	1.6	5.3	10.6	15.4	19.2	21.8	21.8	17.7	12.3	7.0	2.2	11.2
Maximum temperature (°C)	19.2	22.3	29.1	31.2	34.3	36.6	39.2	38.7	36.4	32.8	25.6	21.4	39.2
Minimum temperature (°C)	-23.6	-23.8	-16.5	-7.2	-1.0	0.5	5.0	2.2	-3.7	-7.1	-16.7	-26.3	-26.3
Average of total rainfall (mm)	41.4	35.8	36.9	37.2	45.4	36.0	14.6	7.9	15.3	25.2	30.4	48.1	374.2

*Meteorology Office, Eskisehir, **Long Years 1928-2019

Samples, taken at tillering period, flowering period, maturity period and seed for determining level of amino acids (aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, thionine, arginine, alanine, tyrosine, cysteine, valine, methionine, tryptophan, phenylalanine, isoleucine, lysine, sarcosine and proline) were gathered. Analyzes were made from fresh leaf samples. For determination of amino acid levels; 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 40^{o}C 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*., 2001). 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. Double dendogram was made in NCSS statistic software program.

Results and Discussion

Amino acids are required for vital activities such as protein formation, hormone synthesis and biochemical reactions in humans, animals and plants. Human body is lack of ability to produce 8 essencial amino acids and they must be supplemented. Serving as protein components in plants, amino acids are involved in anabolic and catabolic reactions in the cell and therefore affect a number of physiological processes such as plant growth and development, intracellular pH control, production of metabolic energy or redox power, and resistance to both (Moe, 2013; Zeier, 2013; Fagard *et al.*, 2014; Galili *et al.*, 2014; Pratelli & Pilot, 2014). Besides, amino acids are monitored by genotype x environment interaction. Having distinctive ingredients such as minerals and amino acids, bread wheat plays important role in nutritional scenario of people and it is important for the nutrition of the wheat and the feeding of the community. In addition to this, structural differences in metabolic events, different intensity and speed of them during different developmental periods in wheat cause different levels of amino acids in crops (Wronkowska *et al*., 2008). Distribution of amino acids in tillering stage of bread wheat genotypes were given in Table 2. While $\mathbf{BW_5}$ (Sönmez-01) had the highest levels, $\mathbf{BW_7}$ (Çetinel-2000) included the lowest values in all amino acids. Plant amino acids that respond to different prevailing environmental conditions vary between genotypes. Besides, due to structural differences and different intensity grades of the metabolic events during different developmental periods, the levels of amino acids seem to be different in bread wheat. In this context, amino acid activities start with germination and increase with increases in plant development. Especially since tillering stage, this increase is manifest itself. In this period, different developmental abilities between genotypes due to features such as photosynthetic activity, dry matter production and mineral matter intake, also cause differences in amino acid levels (Lebeau *et al*., 2020). Aspartate is important amino acid, triggering metabolic events such as the cycle of tricarboxylic acid causing energy-consuming metabolic events helping defense systems, leading to the production of signal amino acids (Basavarajappa, 2019). Asparagine and proline are important amino acids promoting nitrogen accumulation and help to adjust cellular sugar balance (Haroun *et al*., 2010).

Another stress amino acid, serine helps to regulate plant tissue against drought, and decrease with increase in proline in drought conditions. In a lot of conditions, proline tends to accumulate tissue and it works for osmoregulation, membrane activity, growth processes and signal of stress conditions (Hare *et al*., 2003). It was pointed out that the serine plays a regulatory role, especially in plant tissues, and plays a regulatory role between stress and normal conditions (Ros *et al*., 2014). Histidine is a stress responsive amino acid that reacts with the plant against abiotic stresses. Therefore, this amino acid acts as a positive regulator against stress with abscisic acid, and the amount of histidine increases with increasing stress in the plant. Distribution of amino acids in flowering stage of bread wheat genotypes were given in Table 3.

Amino acid levels in all the genotypes with crop growth to flowering stage reached the highest level. While $\mathbf{BW_5}$ (Sönmez-01) and $\mathbf{BW_7}$ (Çetinel-2000) had the highest and the lowest levels, respectively in all amino acids. Table 3 showed that substantial differences occurred in amino acid levels among genotypes in flowering stage. Glutamine is an amino acid that acts on metabolic development and acts as a marker to regulate N metabolism and plastid metabolism in the cell (Miflin & Habash, 2002). Another amino acid is glycine that it is synthesized and accumulated in plant that coincided stress conditions. Besides, glycine figures to lower the rate of photosynthesis, to protect plasma membranes and the photosynthetic system, to reduce the level and effect of oxygen-dependent free radicals when availability of water are limited (Chen & Murata, 2008). Valine inhibits the degradation of growth-dependent enzymes by reducing the damage of oxygen-dependent free radicals under stress conditions and act vital position in reducing free oxygen-based radicals in the cell.

Table 2. Distribution of amino acids (pmol µl-1) in tillering stage of bread wheat genotypes.

	Aspartate	**Glutamate**	**Asparagine**	**Serine**	**Glutamine**	**Histidine**	**Glycine**	**Thionine**	**Arginine**	**Alanine**
BW1	4715	2069	10189	8719	9593	4706	3898	6857	18184	14963
BW2	5029	2206	10868	9300	10232	5020	4157	7314	19396	15960
BW3	4557	2000	9850	8429	9273	4549	3768	6628	17578	14464
BW4	4827	2118	10432	8927	9821	4818	3990	7020	18617	15319
BW5	**5096**	**2236**	**11014**	**9425**	**10369**	**5087**	**4213**	**7412**	**19656**	**16174**
BW6	4759	2088	10286	8802	9684	4751	3935	6922	18357	15105
BW7	**4445**	**1950**	**9607**	**8221**	**9045**	**4437**	**3675**	**6465**	**17145**	**14108**
BW8	4737	2078	10238	8761	9638	4729	3916	6889	18270	15034
BW9	4557	2000	9850	8429	9273	4549	3768	6628	17578	14464
BW10	4961	2177	10723	9176	10095	4953	4102	7216	19136	15746
BW11	4849	2128	10480	8968	9867	4841	4009	7052	18703	15390
BW12	4782	2098	10335	8844	9730	4773	3953	6954	18444	15176
BW13	5074	2226	10966	9384	10324	5065	4195	7379	19569	16103
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
BW1	1285	2423	1138	2398	2153	2106	3200	5336	10112	137
BW2	1371	2585	1214	2558	2296	2247	3414	5692	10786	146
BW3	1242	2343	1100	2318	2081	2036	3094	5158	9774	132
BW4	1316	2481	1165	2455	2204	2156	3277	5463	10352	140
BW5	**1389**	**2620**	**1230**	**2592**	**2327**	**2277**	**3459**	**5768**	**10930**	**148**
BW6	1297	2446	1149	2421	2173	2126	3231	5387	10208	138
BW7	**1212**	**2285**	**1073**	**2261**	**2030**	**1986**	**3018**	**5031**	**9534**	**129**
BW8	1291	2435	1144	2410	2163	2116	3216	5362	10160	138
BW9	1242	2343	1100	2318	2081	2036	3094	5158	9774	132
BW10	1353	2550	1198	2524	2265	2217	3368	5616	10641	144
BW11	1322	2493	1171	2467	2214	2166	3292	5489	10400	141
BW12	1304	2458	1154	2432	2183	2136	3246	5412	10256	139
BW13	1383	2608	1225	2581	2317	2267	3444	5743	10882	147
	Aspartate	**Glutamate**	**Asparagine**	**Serine**	**Glutamine**	**Histidine**	**Glycine**	**Thionine**	**Arginine**	**Alanine**
Mean	**4799.12**	**2105.63**	**10372.08**	**8875.70**	**9764.98**	**4790.57**	**3967.56**	**6979.57**	**18510.28**	**15231.06**
S_{ix}	**204.36**	**89.66**	**441.66**	**377.94**	**415.81**	**203.99**	**168.95**	**297.20**	**788.20**	**648.57**
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
Mean	**1308.27**	**2466.90**	**1158.63**	**2441.24**	**2191.13**	**2144.11**	**3257.84**	**5431.88**	**10292.99**	**139.46**
S_{ix}	**55.71**	**105.05**	**49.34**	**103.95**	**93.30**	**91.30**	**138.73**	**231.30**	**438.29**	**5.94**

BW_1: Es-26, **BW_2:** Bezostaja-1, **BW_3:** Müfitbey, **BW_4:** Altay-2000, **BW_5:** Sönmez-01, **BW_6:** Soyer-02, **BW_7:** Çetinel-2000, **BW_8:** Harmankaya-99, **BW_9:** Sultan-95, **BW_{10}:** Alpu-01,**BW_{11}:** Atay-85,**BW_{12}: Özdemir,BW_{13}:** Gerek-79

Moreover, isoleucine is very important for growth and development of biochemical processes in vegetative and generative stages (Mikkelsen & Halkier, 2003). Table 4 shows the distribution of amino acids in maturity stage of bread wheat genotypes. Amino acid levels were determined as lower than tillering and flowering stages. This means that amino acid activity and their levels are significantly related to metabolic activities of crop. Besides being shaped under the influence of the environmental conditions, the more metabolic activities including growth and resistance occur, the more amino acid levels increase. Like two previous stage, **BW_5** (Sönmez-01) and **BW_7** (Çetinel-2000) had the highest and the lowest levels, respectively in all amino acids. Phenylalanine and lysine cause increase in production of organic matter during vegetative and flowering stages. Besides, both amino acids help to increase resistance of crop against stress conditions (Khalifa *et al*., 2020). Sarcosine catalyzes many metabolic events such as the regulation of the osmotic balance, the activation of defense systems (Yancey, 2005). Glutamate is very effective in the carbon and nitrogen mechanism in plants. Glutamate, an important signaling molecule in plants, it is an important element for proline biosynthesis in the regulation of osmotic balance in stress conditions (Forde & Lea, 2007). Alanine monitories crop to adjust against stress conditions. It contributes to regulate many metabolic events, including the reduction of many anabolic and catabolic events during stress, the activation of the plant's water prevention systems (Kalefetoğlu & Ekmekçi, 2010). Tyrosine acts as a trigger in the defense-related metabolic cycle of the plant against stress conditions and plays a triggering and catalytic role in defense-related plant metabolic events and the formation of cycles leading to them (Ghelis, 2011). Methionine and tryptophan play a role in physiological and metabolic events related to plant development. Because they play a role in normal crop development, they decrease in the plant with development regressing under stress conditions. Methionine contribute the completion of DNA sequencing, the formation of proteins, and the completion of many cycles in plants for plant growth, at varying rates in plants (Sairam *et al*., 2002). Tryptophan employs the production of auxins required for yield. It also plays a role in providing osmotic equilibrium, ion transfer, opening of stomata and removal of heavy metals. It therefore decreases with drought (Ravichandran *el al*., 2019). Cysteine is an amino acid that plays an active role in plant growth including anabolic and catabolic events; it acts as a warning in the response mechanisms of the plant against many stress conditions (Kalefetoğlu & Ekmekçi, 2010). Table 5 shows the amino acid levels in seeds of bread wheat genotypes.

Table 3. Distribution of amino acids (pmol µl-1) in flowering stage of bread wheat genotypes.

	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Glycine	Thionine	Arginine	Alanine
BW1	5375	2358	11616	9940	10936	5365	4443	7816	20730	17057
BW2	5733	2515	12390	10603	11665	5723	4739	8338	22112	18194
BW3	5195	2279	11228	9609	10571	5186	4295	7556	20039	16489
BW4	5502	2414	11892	10177	11196	5493	4549	8003	21223	17463
BW5	**5810**	**2549**	**12556**	**10745**	**11821**	**5799**	**4803**	**8449**	**22408**	**18438**
BW6	5426	2381	11726	10035	11040	5416	4486	7891	20927	17220
BW7	**5067**	**2223**	**10952**	**9372**	**10311**	**5058**	**4189**	**7370**	**19545**	**16083**
BW8	5400	2369	11671	9987	10988	5391	4464	7854	20828	17138
BW9	5195	2279	11228	9609	10571	5186	4295	7556	20039	16489
BW10	5656	2482	12224	10461	11509	5646	4676	8226	21815	17951
BW11	5528	2425	11948	10224	11248	5518	4570	8040	21322	17545
BW12	5451	2392	11782	10082	11092	5442	4507	7928	21026	17301
BW13	5784	2538	12501	10697	11769	5774	4782	8412	22309	18357
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
BW1	1465	2763	1298	2734	2454	2401	3648	6083	11527	156
BW2	1563	2947	1384	2916	2617	2561	3892	6489	12296	167
BW3	1416	2671	1254	2643	2372	2321	3527	5880	11143	151
BW4	1500	2828	1328	2799	2512	2458	3735	6228	11802	160
BW5	**1584**	**2986**	**1403**	**2955**	**2652**	**2596**	**3944**	**6576**	**12460**	**169**
BW6	1479	2789	1310	2760	2477	2424	3683	6141	11637	158
BW7	**1381**	**2605**	**1223**	**2578**	**2314**	**2264**	**3440**	**5736**	**10868**	**147**
BW8	1472	2776	1304	2747	2466	2413	3666	6112	11582	157
BW9	1416	2671	1254	2643	2372	2321	3527	5880	11143	151
BW10	1542	2907	1366	2877	2582	2527	3840	6402	12131	164
BW11	1507	2842	1335	2812	2524	2470	3753	6257	11856	161
BW12	1486	2802	1316	2773	2489	2435	3701	6170	11692	158
BW13	1577	2973	1396	2942	2641	2584	3926	6547	12405	168
	Aspartate	**Glutamate**	**Asparagine**	**Serine**	**Glutamine**	**Histidine**	**Glycine**	**Thionine**	**Arginine**	**Alanine**
Mean	**5471.00**	**2400.41**	**11824.17**	**10118.30**	**11132.08**	**5461.25**	**4523.01**	**7956.70**	**21101.72**	**17363.41**
S_{ix}	**232.97**	**102.21**	**503.50**	**430.86**	**474.02**	**232.55**	**192.60**	**338.81**	**898.55**	**739.37**
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
Mean	**1491.43**	**2812.26**	**1320.84**	**2783.02**	**2497.89**	**2444.28**	**3713.94**	**6192.34**	**11734.01**	**158.99**
S_{ix}	**63.51**	**119.75**	**56.24**	**118.51**	**106.36**	**104.08**	**158.15**	**263.68**	**499.66**	**6.77**

BW$_1$: Es-26, **BW$_2$:** Bezostaja-1, **BW$_3$:** Müfitbey, **BW$_4$:** Altay-2000, **BW$_5$:** Sönmez-01, **BW$_6$:** Soyer-02, **BW$_7$:** Çetinel-2000, **BW$_8$:** Harmankaya-99, **BW$_9$:** Sultan-95, **BW$_{10}$:** Alpu-01,**BW$_{11}$:** Atay-85,**BW$_{12}$:** **Özdemir**,**BW$_{13}$:** Gerek-79

Table 4. Distribution of amino acids (pmol µl-1) in maturity stage of bread wheat genotypes.

	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Glycine	Thionine	Arginine	Alanine
BW1	4515	1981	9757	8350	9186	4507	3732	6566	17413	14328
BW2	4816	2113	10408	8906	9798	4807	3981	7004	18574	15283
BW3	4364	1915	9432	8071	8880	4356	3608	6347	16832	13850
BW4	4622	2028	9989	8548	9405	4614	3821	6722	17827	14669
BW5	**4880**	**2141**	**10547**	**9025**	**9930**	**4871**	**4034**	**7097**	**18823**	**15488**
BW6	4558	2000	9850	8429	9274	4549	3768	6628	17579	14465
BW7	**4257**	**1868**	**9200**	**7872**	**8661**	**4249**	**3519**	**6191**	**16418**	**13509**
BW8	4536	1990	9804	8389	9230	4528	3750	6597	17496	14396
BW9	4364	1915	9432	8071	8880	4356	3608	6347	16832	13850
BW10	4751	2085	10268	8787	9667	4743	3928	6910	18325	15079
BW11	4644	2037	10036	8588	9449	4635	3839	6753	17910	14737
BW12	4579	2009	9897	8469	9317	4571	3786	6660	17662	14533
BW13	4859	2132	10501	8986	9886	4850	4017	7066	18740	15420
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
BW1	1231	2321	1090	2297	2061	2017	3065	5110	9683	131
BW2	1313	2475	1163	2450	2199	2151	3269	5451	10328	140
BW3	1190	2243	1054	2220	1993	1950	2963	4940	9360	127
BW4	1260	2376	1116	2351	2110	2065	3138	5232	9913	134
BW5	**1330**	**2509**	**1178**	**2482**	**2228**	**2180**	**3313**	**5524**	**10467**	**142**
BW6	1242	2343	1100	2318	2081	2036	3094	5159	9775	132
BW7	**1160**	**2188**	**1028**	**2165**	**1943**	**1902**	**2890**	**4818**	**9129**	**124**
BW8	1237	2332	1095	2307	2071	2027	3079	5134	9729	132
BW9	1190	2243	1054	2220	1993	1950	2963	4940	9360	127
BW10	1295	2442	1147	2417	2169	2123	3225	5378	10190	138
BW11	1266	2387	1121	2362	2120	2075	3152	5256	9959	135
BW12	1248	2354	1106	2329	2091	2046	3108	5183	9821	133
BW13	1324	2497	1173	2471	2218	2171	3298	5499	10421	141
	Aspartate	**Glutamate**	**Asparagine**	**Serine**	**Glutamine**	**Histidine**	**Glycine**	**Thionine**	**Arginine**	**Alanine**
Mean	**4595.64**	**2016.35**	**9932.31**	**8499.37**	**9350.94**	**4587.45**	**3799.33**	**6683.63**	**17725.44**	**14585.26**
Six	**195.69**	**85.86**	**422.94**	**361.92**	**398.18**	**195.34**	**161.78**	**284.60**	**754.78**	**621.07**
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
Mean	**1252.80**	**2362.30**	**1109.50**	**2337.74**	**2098.23**	**2053.20**	**3119.71**	**5201.56**	**9856.57**	**133.55**
Six	**53.35**	**100.59**	**47.24**	**99.55**	**89.35**	**87.43**	**132.84**	**221.49**	**419.71**	**5.69**

BW_1: Es-26, **BW_2:** Bezostaja-1, **BW_3:** Müfitbey, **BW_4:** Altay-2000, **BW_5:** Sönmez-01, **BW_6:** Soyer-02, **BW_7:** Çetinel-2000, **BW_8:** Harmankaya-99, **BW_9:** Sultan-95, **BW_{10}:** Alpu-01,**BW_{11}:** Atay-85,**BW_{12}: Özdemir,BW_{13}:** Gerek-79

Amino acid levels in seed draw similar trend as seen in previous stages, the highest and the lowest levels of amino acids belonged to **BW_5** (Sönmez-01) and **BW_7** (Çetinel-2000) in all amino acids. Nitrogen is the cornerstone of the amino acid structures necessary for plant growth and development. The nitrogen use of plants in the tillering period is very low compared to other periods. While determining the yield capacity, the parameters of the number of productive heads per m^2, number of seeds and weight per head are very important. Therefore, it is important to have sufficient nitrate levels during the tillering and flowering stages to reach maximum potential yield in common wheat. Proteins stored in seeds are estimated ~40-50%. Naturally, less nitrogen is required during the tillering period compared to the flowering and ripening periods.

Studies have shown that plant growth rate and amount are shaped under the influence of genotype x environment. Especially in the early stage of the plant, the available amount of water and nutrients in the soil significantly affects plant growth. Differences in genotypic capacity, water and nutrient availability and climatic conditions affect plant growth, dry matter production, and therefore amino acid levels. More photosynthesis and assimilate production assign more amino acid levels in crop organs (Khalifa *et al*., 2020). This explains why amino acid levels are higher in the stages where metabolic and biochemical activities are also higher. Another interesting aspect is that the amino acid changes in all genotypes are the same in every period of development, as well as in the seed. The same genotypes had the highest and lowest amino acid levels in each period and seed. In the light of these results, the change in amino acids, which is the basic building block of proteins and play very important role in biochemical events, is shaped by metabolic activities; the genetic capacity of genotypes shaping crop development, determines to the amino acid levels in the wheat genotypes. As a means of growth periods, Figure 1 shows changes of amino acids in all development periods as well as in the seed. All amino acids followed very similar trends throughout the developmental periods examined (Fig. 1). Having certain levels during the tillering period, amino acids was found to be higher than the amount in the ripening period and seed. Although, there is a rapid increase in amino acids in tillering period, the highest increase in amino acids reaches its maximum level during flowering period. In the total amount of amino acids, tillering and flowering periods created the more amino acid levels. Therefore, the highest amount of amino acids in the plant was seen from tillering to flowering stage. This trend has gradually decreased from seed to maturity stage. Therefore, amino acid sequence was determined as flowering, tillering, maturation and seed. The reason for the minimum amount of amino acids in the seed indicates that the amino acids remain in the stem as well as the seed of the plant. In this case, the amount of amino acid, which reaches its maximum level until flowering, shows how effective the plant is in development and growth stages. On the other hand, amino acid levels determined by taking the difference between periods (Fig. 2). In Figure 2, as set out in Figure 1, developments are shown more clearly. While the highest amino acid levels occurred during tillering-flowering period, it decreased to the lowest level during the flowering-maturity period. The amount of amino acids occurring in the maturation-seed formation period was obtained at a higher level than the flower-maturity period. This means that the amount of amino acids obtained at a higher level during the tillering- flowering period was produced at a minimal level in flowering-maturity period. But, huge amount of amino acids accumulation occurred during the seed formation that was higher than flowering- maturity period. The highest contribution to the amino acid accumulation in the seed belonged to tillering- flowering period.

Table 5. Distribution of amino acids (pmol µl-1) in seed of bread wheat genotypes.

	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Glycine	Thionine	Arginine	Alanine
BW1	4153	1822	8977	7682	8451	4146	3434	6041	16020	13182
BW2	4430	1944	9575	8194	9015	4422	3663	6443	17088	14061
BW3	4015	1762	8677	7425	8169	4008	3319	5839	15486	12742
BW4	4252	1866	9190	7864	8652	4245	3516	6184	16401	13496
BW5	**4490**	**1970**	**9703**	**8303**	**9135**	**4482**	**3712**	**6530**	**17317**	**14249**
BW6	4193	1840	9062	7755	8532	4186	3466	6098	16172	13307
BW7	**3916**	**1718**	**8464**	**7243**	**7968**	**3909**	**3238**	**5695**	**15104**	**12429**
BW8	4173	1831	9019	7718	8491	4166	3450	6069	16096	13245
BW9	4015	1762	8677	7425	8169	4008	3319	5839	15486	12742
BW10	4371	1918	9447	8084	8894	4363	3614	6357	16859	13872
BW11	4272	1874	9233	7901	8693	4264	3532	6213	16478	13558
BW12	4213	1848	9105	7791	8572	4205	3483	6127	16249	13370
BW13	4470	1961	9661	8267	9095	4462	3695	6501	17240	14186
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
BW1	1132	2135	1003	2113	1896	1856	2820	4701	8908	121
BW2	1208	2277	1070	2254	2023	1979	3007	5014	9502	129
BW3	1095	2064	969	2042	1833	1794	2726	4544	8611	117
BW4	1159	2186	1027	2163	1941	1900	2887	4813	9120	124
BW5	**1224**	**2308**	**1084**	**2284**	**2050**	**2006**	**3048**	**5082**	**9629**	**130**
BW6	1143	2155	1012	2133	1914	1873	2846	4746	8993	122
BW7	**1068**	**2013**	**945**	**1992**	**1788**	**1750**	**2658**	**4432**	**8399**	**114**
BW8	1138	2145	1008	2123	1905	1864	2833	4723	8951	121
BW9	1095	2064	969	2042	1833	1794	2726	4544	8611	117
BW10	1192	2247	1055	2223	1996	1953	2967	4947	9375	127
BW11	1165	2196	1031	2173	1951	1909	2900	4835	9163	124
BW12	1148	2165	1017	2143	1923	1882	2860	4768	9035	122
BW13	1219	2298	1079	2274	2041	1997	3034	5059	9587	130
	Aspartate	**Glutamate**	**Asparagine**	**Serine**	**Glutamine**	**Histidine**	**Glycine**	**Thionine**	**Arginine**	**Alanine**
Mean	**4227.99**	**1855.04**	**9137.72**	**7819.42**	**8602.87**	**4220.45**	**3495.39**	**6148.94**	**16307.41**	**13418.44**
Six	**180.04**	**78.99**	**389.10**	**332.97**	**366.33**	**179.71**	**148.84**	**261.83**	**694.40**	**571.38**
	Tyrosine	**Cysteine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isoleucine**	**Lysine**	**Sarcosine**	**Proline**
Mean	**1152.57**	**2173.32**	**1020.74**	**2150.72**	**1930.37**	**1888.94**	**2870.13**	**4785.44**	**9068.04**	**122.87**
Six	**49.08**	**92.54**	**43.47**	**91.58**	**82.20**	**80.43**	**122.22**	**203.77**	**386.13**	**5.23**

$\mathbf{BW_1}$:Es-26, $\mathbf{BW_2}$**:** Bezostaja-1, $\mathbf{BW_3}$**:** Müfitbey, $\mathbf{BW_4}$**:** Altay-2000, $\mathbf{BW_5}$**:** Sönmez-01, $\mathbf{BW_6}$**:** Soyer-02, $\mathbf{BW_7}$**:** Çetinel-2000, $\mathbf{BW_8}$**:** Harmankaya-99, $\mathbf{BW_9}$**:** Sultan-95, $\mathbf{BW_{10}}$**:** Alpu-01,$\mathbf{BW_{11}}$**:** Atay-85,$\mathbf{BW_{12}}$**: Özdemir,**$\mathbf{BW_{13}}$**:** Gerek-79

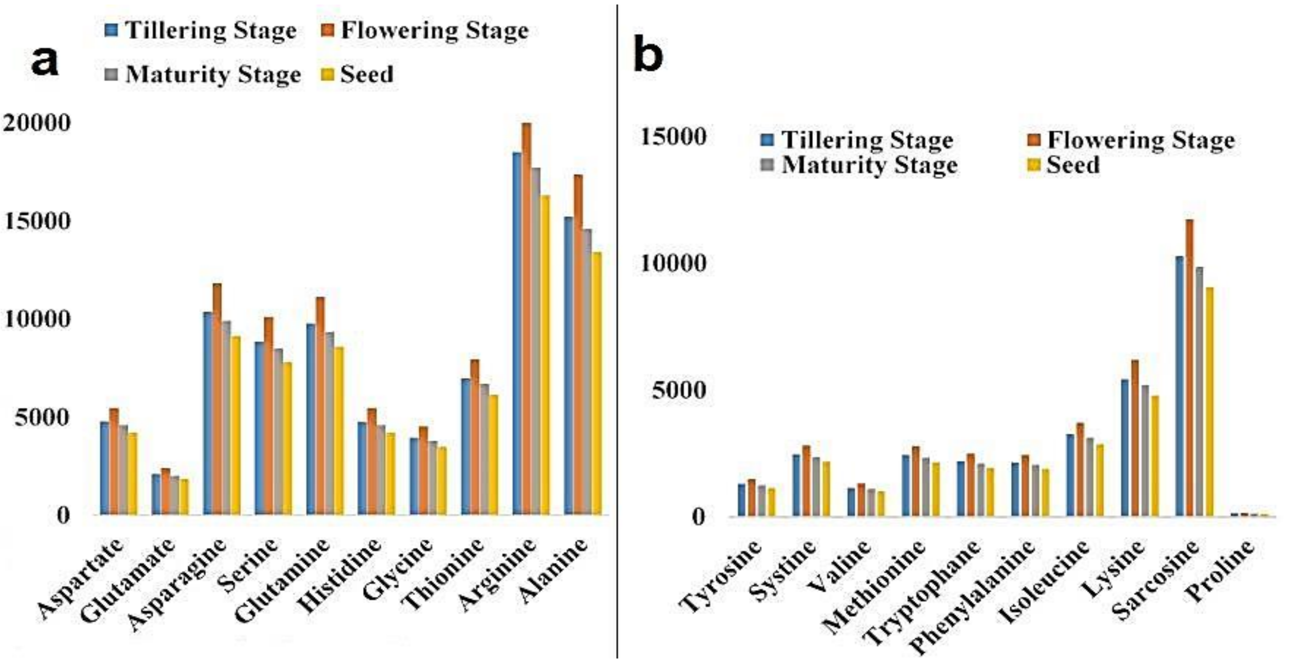

Fig. 1. Levels of amino acids (pmol µl-1) in different development periods and seeds of bread wheat genotypes.

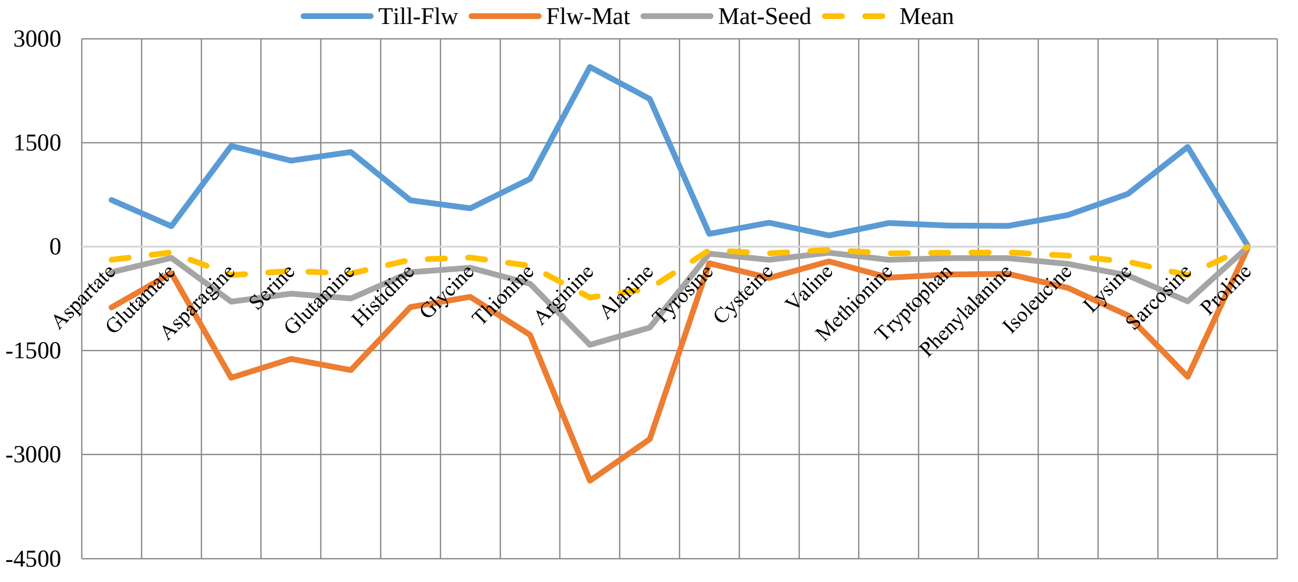

Fig. 2. Amino acid (pmol µl-1) levels occured depending on the difference in development periods in bread wheat genotypes

Conclusion

In this study, the changes in amino acid levels in all stages and seeds showed a similar fluctiations and important differences between both genotypes and development periods. The highest and lowest amino acid levels were determined in BW5 (Sönmez-01) and BW7 (Çetinel-2000) genotypes. While the highest amount of amino acids recorded during tillering and flowering stages, the least amount of amino acids was determined during maturation stage. This means that the more amino acids in tillering and flowering stages had an important effect on speed of amino acid formation and accumulation, thereby the amount of amino acids in the seed. Amino acids, higher in tillering and flowering stages, lower in maturity stage assign that how amino acids are liable for the growth and development of the plants. In this way, the plant grows and matures. Especially in the maturation period, amino acids that promote maturation and senescence are of great importance. Amino acid levels largely depend on the genotypic capacities, as well as the efficiency and amount of amino acids determine the efficiency of biochemical activities. Therefore, this situation explains the amino acid levels, differences of genotypes from each other to the great extent. On the other hand, plant genetic performance is formed by genotype x environment interactions. This explains that the amount of amino acids in different stages in genotypes is highly affected by environmental conditions and agronomic applications. These differences will shed light on future studies in selecting genotypes with high yield and quality in various breeding studies.

References

Basavarajappa, B.S. 2019. Endocannabinoid System and Alcohol Abuse Disorders. *In Recent Advances in Cannabinoid Physiology and Pathology* (pp. 89-127). Springer, Cham.

Bouis, H.E. 2003. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? *Proceedings of the Nutrition Society*, 62(2): 403-411.

Fagard, M., A. Launay, G. Clément, J. Courtial, A. Dellagi, M. Farjad, A. Krapp, M.C. Soulié and C. Masclaux-Daubresse. 2014. Nitrogen metabolism meets phytopathology. *J. Exp. Bot*., 65: 5643-5656.

Forde, B.G. and P.J. Lea. 2007. Glutamate in plants: metabolism, regulation, and signaling. *J. Exp. Bot.,* 58(9): 2339-2358.

Galili, G., T. Avin-Wittenberg, R. Angelovici and A.R. Fernie. 2014. The role of photosynthesis and amino acid metabolism in the energy status during seed development. *Front. Plant Sci*., 5: 447.

Ghelis, T. 2011. Signal processing by protein tyrosine phosphorylation in plants. *Plant Signaling & Behavior*, *6*(7): 942-951.

Hare, P.D., W.A. Cress and J. Van Staden. 2003. A regulatory role for proline metabolism in stimulating *Arabidopsis thaliana* seed germination. *Plant Growth Regul.*, 39(1): 41-50.

Haroun, S. A., W.M. Shukry and O. El-Sawy. 2010. Effect of asparagine or glutamine on growth and metabolic changes in Phaseolus vulgaris under in vitro conditions. *Biosci. Res.*, 7(1): 01-21.

Henderson, J.W., R.D. Ricker, B.A. Bidlingmeyer and C. Woodward. 2001. Rapid, accurate, sensitive and reproducible HPLC analysis of amino acids. Application of Agilent (US); Santa Clara (CA), Publication Part No. 5980-1193E.

Hildebrandt, T.M., A. Nunes Nesi, W.L. Arau´ jo and H.P. Braun. 2015. Amino acid catabolism in plants. *Molecular Plant*, 8(11): 1563-1579.

Kalefetoğlu, T. and Y. Ekmekçi. 2010. Bitkilerde Kuraklik Stresinin Etkileri ve Dayanıklılık Mekanizmaları (Compilation). *Gazi Uni. J. Sci.*, 18(4): 723-740.

Khalifa, Y.A., A. El-Naem, F. Gamal and M.A. Mahmoud. 2020. Effect of tryptophan and ascorbic acid on yield and some chemical constituents of lupine (*Lupines termis* L.) plants. *Egyptian J. Agron.*, 42(1): 47-61.

Khan, M.S., E. Ali, S. Ali, W.M. Khan, M.A. Sajjad and F. Hussain. 2014. Assessment of essential amino acids in wheat proteins. A case study. *J. Biodiversity and Environmental Sciences*, 4: 185-189.

Lebeau, J., J.P. Efromson and M.D. Lynch. 2020. A review of the biotechnological production of methacrylic acid. *Front. Bioeng. & Biotechnol.*, 8: 207.

Miflin, B.J. and D.Z. Habash. 2002. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. *J. Exp. Bot.*, 53(370): 979-987.

Mikkelsen, M.D. and B.A. Halkier. 2003. Metabolic engineering of valine-and isoleucine-derived glucosinolates in Arabidopsis expressing CYP79D2 from cassava. *Plant Physiol.*, 131(2): 773-779.

Moe, L.A. 2013. Amino acids in the rhizosphere: from plants to microbes. *Am. J. Bot*., 100: 1692-1705.

Pratelli, R. and G. Pilot. 2014. Regulation of amino acid metabolic enzymes and transporters in plants. *J. Exp. Bot*., 65: 5535-5556.

Ravichandran, S., R. Ragupathy, T. Edwards, M. Domaratzki and S. Cloutier. 2019. MicroRNA-guided regulation of heat stress response in wheat. *BMC Genom.*, 20(1): 488.

Ros, R., J. Muñoz-Bertomeu and S. Krueger. 2014. Serine in plants: biosynthesis, metabolism, and functions. *Trends in Plant Sci.*, 19(9): 564-569.

Sairam, R.K., K.V. Rao and G.C. Srivastava. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci.*, 163(5): 1037-1046.

Shewry, P.R. 2007. Improving the protein content and composition of cereal grain. *J. Cereal Sci.*, 46(3): 239-250.

Wronkowska, M., A. Troszynska, M. Soral-Smietana and A. Wolejszo. 2008. Effects of buckwheat flour (*Fagopyrum esculentum* Moench) on the quality of gluten-free bread. *Polish J. Food & Nutr. Sci.*, 58(2):

Yancey, P.H. 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.*, 208(15): 2819-2830.

Zeier, J. 2013. New insights into the regulation of plant immunity by amino acid metabolic pathways. *Plant Cell Environ*., 36: 2085-2103.

(Received for publication 22 June 2021)