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## ORGANIC CHEMISTRY | RESEARCH ARTICLE

# Defining free amino acid contents of grass pea (*Lathyrus sativus*) genotypes in Turkey

Mehmet Arslan<sup>1\*</sup>

**Abstract:** Free amino acid contents, including Arginine (Arg), Aspartic acid (Asp), Glutamic acid (Glu), Proline (Pro), Methionine (Met), Tyrosine (Tyr), Leucine + Isoleucine (Leu + Ile) and Phenylalanine (Phe) of 173 different *Lathyrus sativus* (grass pea) genotypes were studied. Arg was the most fluctuated and at the same time it gave the highest and the lowest yielded free amino acids among the all genotypes, ranging from 0.10 to 506.85 mg g<sup>-1</sup>. However, regarding to the mean values, Glu is having the highest levels with 311.61 mg g<sup>-1</sup> among the all. This study provides an additional data for this field of work and seems to be unique.

**Subjects:** Crop Science; Agriculture and Food; Biodiversity

**Keywords:** grass pea; free amino acids; 173 accessions

### 1. Introduction

Regarding to global food demand and feed resources and modern cropping systems (Makoi & Ndakidemi, 2011), the legume genus *Lathyrus* is receiving increased attention by scientists (Biswas & Biswas, 1997). *Lathyrus sativus* L., commonly known as grass pea, is an annual plant widely grown as a pulse crop and its dried seeds are harvested and consumed as a human food since ancient times (Tamburino, Guida, Pacifico, Parente, & Di Maro, 2012). *Lathyrus* is recognized as many as 187 species (Allkin, Macfarfarlane, White, Bisby, & Adey, 1983). *L. sativus* L. (grass pea, mürdümük in Turkish), is an annual pulse crop belonging to the *Fabaceae* family and *Vicieae* tribe (Biswas & Biswas, 1997; Campbell, 1997).

### ABOUT THE AUTHOR

Mehmet Arslan graduated from Akdeniz University, Faculty of Agriculture, Department of Field Crops in 2000 with a bachelor's degree, in 2003 with his master's degree and in 2008 with his PhD in the field of Agronomy and Breeding of Forage Crops. He is still working as a lecturer in Akdeniz University, Faculty of Agriculture, Department of FieldCrops. He deals with species and varieties of forage plants, which tolerant to the effects of abiotic stress factors, such as global climate change, drought and salinity. In this respect, he is conducting researches which are based on agronomic, morphological, phenological in *Lathyrus sativus* genotypes, which is collected from nature. At the same time, he goes on quality-based research on silage, an important food source for animal nutrition continues.

### PUBLIC INTEREST STATEMENT

Grass pea has a number of advantages in respect to its properties, such as its high protein content, a high yield potential, low fertilization levels its tolerance to flood or salinity, drought survival. This plant is also commonly grown for animal feed and forage. Grass pea seeds may represent a potential source of several important nutrients for human and animal nutrition. On the other hand, the free amino acid constitutes of legumes are more than 10% of the weight of the legume seeds and are tend to be lost in cooking. These free amino acids, nevertheless, represent an enormous potential store of nourishment for man and animal. In this study, the free amino acids profile of 173 grass pea genotypes were determined. The results show that grass pea is rich in free amino acids and there is a wide variation between grass pea genotypes.

Grass pea has a high yielding potential (regarding a pea level) at a low fertilization level (Campbell et al., 1994; Patto et al., 2006). Currently, it is considered as a model crop for sustainable agriculture (Arslan, 2016; Patto et al., 2006). It is perhaps the most environmentally resistant legume crop with high tolerance to drought, flooding and insect attacks (Campbell, 1997; Talukdar, 2011). In addition, it is stated to be tolerant to the pest, insects and weeds (Das, 2000).

Grass pea is valued, like the other pulse crops, and be cultivated for its high protein content in the seeds which are not only rich in crude protein (24–31%) but also in amino acid profile to provide a balanced diet to poor people in its major production zones (Akalu, Johansson, & Nair, 1998; Aletor, Goodchild, & El Moneim, 1994; Hanbury, White, Mullan, & Siddique, 2000).

This work aims to reveal the free amino acid contents of 173 grass pea genotypes grown in Antalya course conditions. The plants were grown on the same conditions and free amino acids were determined to reveal the contents of them.

## 2. Material and methods

### 2.1. Plant materials

Totally 173 *L. sativus* L. genotypes were used as a plant material those of which 92 genotypes were collected from the natural habitat of Antalya by BATEM (Western Mediterranean Agricultural Research Centre); of 10 genotypes were by Ministry of Agriculture of Yemen; of 4 genotypes were by GAP International Agricultural Research and Education Centre; of 20 genotypes were by Aegean Agricultural Institute Management Gene Center; of 43 genotypes were by Bahri Dagdas International Agricultural Research Institute, of Ceora genotypes by Prof. Dr Kadambot Siddique (Centre for Legumes in Mediterranean Agriculture); of Iptas, Karadag and Eren varieties by Gaziosmanpaşa University Faculty of Agriculture-Field Crops Department. This work was conducted on the open field at BATEM in Antalya in Turkey (36°52' N, 30°50' E, and altitude 15 m) in 2016. All the genotypes were sown on 4 April 2015 and were harvested on 24 May 2016. The seeds were analyzed for free amino acids in which were conducted at Akdeniz University, Food and Agricultural Research Laboratory. Each sample was analyzed in five replication and results were given as mg g<sup>-1</sup> in the dry material.

### 2.2. Extraction

The seeds were grinded with a blender and homogenized. Homogeneous sample weighed 1 g into 50 ml sample tube. Twenty-five millilitres of extraction solution, 0.1% (v/v) formic acid in water:methanol (50:50) (v/v), was added to the sample tube. For recovery studies, spike of the standards was done at this stage. The mixture was extracted with ultra-turrax for 2 min at 10,000 rpm. To control the temperature changes, the sample tube placed in an ice bath during ultra-turrax extraction. Extracted sample centrifuged at 4°C at 4,000 rpm for 10 min. The supernatant was passed through 0.2 µm PTFE membrane filter. Filtered sample diluted with mobile phase and injected 10 µl to LC-MS/MS. Analytical procedure was carried out as reported by Stein and Moore (1963). All reagents and standards were of analytical grade. Individual stock standard solutions of chemicals (approximately 1,000 mg/L) were prepared in methanol and stored in –18°C.

### 2.3. Instrumentation and chromatographic conditions for UHPLC-MS/MS

The UHPLC-MS/MS system consisted of Thermo Accela UHPLC (pump, degasser, autosampler) coupled to Thermo Quantum Access Max triple quadruple mass spectrometer. Electro spray ionization (ESI) probe was used for atmospheric pressure ionization. Thermo Hypersil Gold (100 × 2.1 mm, 1.9 µm particle size) C18 column was performed for analytical separation of analysts. UHPLC column oven temperature was set at 40°C. Injection volume was 10 µl.

Reverse-phase chromatography was implemented for separation of the analytes. The mobile phase consisted of A was 0.1% (v/v) formic acid and 4 mM ammonium acetate in water:methanol (95:5) (eluent A) and methanol (eluent B). The analysis was started with 100% mobile phase A and was held 2 min at this composition. After 2 min, mobile phase A percentage was linearly decreased

to 0% in 1.5 min. Mobile phase A percentage was held at 0% for 3 min. Then, flow was changed to 100% mobile phase A and re-equilibration time takes 0.5 min. Total run time was 7 min. The flow rate was 0.4 ml/min. By means of a gradient programme, apolar characteristics of column and polar structure of the analytes, they were detected in short time.

#### 2.4. Data analyses

Pearson's correlation coefficient is a bivariate correlation procedure and it was used to measure associations among morphological characteristics. Principal component analysis (PCA), which was based on the mean values of 173 genotypes for 8 free amino acids, were performed to determine the relationship and pattern of variation between genotypes (Iezzoni & Pritts, 1991). Pearson's correlation coefficients and PCA were calculated using the statistical package, SAS 9.1 (SAS Institute, 2003).

### 3. Results and discussions

Table 1 gives the free amino acid contents of all the genotypes, including Arg, Asp, Glu, Pro, Met, Tyr, Leu + Ile and Phe contents of the samples. At a first glance, a great variation among the measured parameters in the genotypes has been determined. This is related to the genetic variations of the grass peas. For a general aspect, Arg content, which is most fluctuated among the all, ranged from

**Table 1. Content of some free amino acid contents in grass pea genotypes (mg g<sup>-1</sup>)**

Genotypes	Arg	Asp	Glu	Pro	Met	Tyr	Leu+Ile	Phe
LS-1	58.98	145.51	324.80	7.75	9.88	13.73	12.42	34.89
LS-2	38.77	125.50	274.64	5.13	9.57	10.73	12.01	16.80
LS-3	34.09	108.05	287.75	5.45	9.72	8.12	7.68	44.66
LS-4	15.61	120.36	214.19	3.55	10.45	7.72	7.77	18.22
LS-5	41.53	139.51	303.39	3.72	14.65	8.55	9.28	21.84
LS-6	363.80	168.11	326.56	5.96	14.73	13.90	10.58	25.82
LS-7	64.37	152.45	322.82	7.36	17.40	12.54	9.00	37.41
LS-8	66.98	121.97	292.58	7.07	11.27	17.75	12.16	25.98
LS-9	0.00	140.80	267.52	4.92	6.87	9.51	9.50	19.89
LS-10	19.27	148.38	287.25	7.04	10.88	9.13	10.67	23.56
LS-11	164.25	143.29	259.83	6.97	13.03	15.81	16.10	26.55
LS-12	26.05	142.84	234.63	6.14	7.83	10.88	9.56	20.37
LS-13	91.00	112.76	259.69	4.01	10.18	13.06	12.51	33.79
LS-14	42.42	127.91	262.03	3.15	8.60	6.41	10.61	29.82
LS-15	48.37	88.04	268.96	3.98	9.89	12.44	5.19	32.20
LS-16	24.75	170.41	279.17	5.47	7.77	12.24	7.94	18.18
LS-17	69.98	110.01	271.13	5.94	13.67	13.26	7.79	24.07
LS-18	10.68	150.71	267.25	5.14	7.63	12.36	9.37	19.08
LS-19	150.53	124.05	321.74	5.77	11.46	9.26	12.29	19.27
LS-20	58.22	127.01	227.41	6.97	9.38	8.60	9.70	24.09
LS-21	34.72	114.98	248.12	5.57	11.46	6.17	11.90	25.96
LS-22	111.42	149.17	303.70	7.57	11.48	15.16	12.92	26.54
LS-23	158.65	156.87	309.44	6.13	9.29	14.09	8.63	19.53
LS-24	144.63	156.55	296.23	7.13	15.90	12.54	8.13	36.46
LS-25	175.85	139.86	260.54	8.68	10.72	20.60	22.03	23.33
LS-26	506.85	120.04	272.07	6.64	7.26	9.04	9.25	24.16
LS-27	77.53	122.28	346.52	5.83	14.27	9.40	12.04	22.49

(Continued)

**Table 1. (Continued)**

<b>Genotypes</b>	<b>Arg</b>	<b>Asp</b>	<b>Glu</b>	<b>Pro</b>	<b>Met</b>	<b>Tyr</b>	<b>Leu+Ile</b>	<b>Phe</b>
LS-28	169.50	156.12	280.27	6.32	11.50	11.99	8.10	28.73
LS-29	156.00	145.06	413.10	9.77	13.60	27.57	31.05	49.78
LS-30	152.96	126.93	374.30	6.45	12.66	11.83	14.36	27.42
LS-31	90.38	175.31	354.70	7.04	10.42	11.84	17.82	21.83
LS-32	247.33	163.72	309.55	5.87	11.57	8.47	9.72	19.28
LS-33	94.47	156.68	295.45	8.36	18.60	20.96	17.89	29.33
LS-34	29.14	122.57	269.86	6.03	16.00	14.22	13.36	22.90
LS-35	481.13	150.81	259.36	6.23	10.89	7.66	10.54	24.28
LS-36	141.16	163.80	320.54	5.51	10.39	9.34	6.94	27.80
LS-37	30.90	102.70	227.89	5.96	11.90	12.04	7.72	18.06
LS-38	83.96	145.63	233.40	6.51	11.86	18.42	10.26	26.37
LS-39	82.22	121.38	251.29	4.53	9.98	12.50	9.64	17.67
LS-40	147.74	152.46	228.80	3.69	12.61	14.18	13.52	25.85
LS-41	174.57	129.33	315.66	4.81	10.08	9.59	8.97	16.52
LS-42	52.41	136.48	342.18	5.15	12.00	11.79	7.44	16.71
LS-43	84.28	157.57	290.91	4.64	10.84	9.15	9.18	25.08
LS-44	41.88	157.03	323.13	5.42	13.80	12.95	10.66	19.77
LS-45	20.93	126.31	245.23	3.86	13.54	11.23	9.38	26.26
LS-46	38.66	130.68	380.61	6.12	10.79	12.44	12.22	34.93
LS-47	49.70	166.72	345.78	6.73	19.64	14.22	19.83	86.34
LS-48	60.97	142.72	303.58	4.97	13.53	10.44	10.39	30.51
LS-49	0.00	97.21	223.47	4.32	8.81	9.82	5.58	23.25
LS-50	121.63	103.00	260.68	4.79	8.71	10.98	11.22	21.33
LS-51	184.97	162.46	272.17	6.59	18.84	10.24	14.44	26.65
LS-52	46.29	154.43	210.02	4.61	11.59	10.63	9.28	32.06
LS-53	125.58	153.33	274.98	4.99	19.84	17.39	16.15	48.85
LS-54	48.70	148.00	252.37	5.73	18.96	13.61	12.39	21.30
LS-55	55.34	196.98	304.76	5.51	14.14	8.84	9.38	18.12
LS-56	0.00	110.60	287.03	3.99	14.06	11.08	10.13	38.93
LS-57	16.52	146.97	290.36	5.97	16.10	13.54	26.88	47.21
LS-58	83.45	143.39	282.93	4.36	10.91	9.74	13.57	26.98
LS-59	58.28	119.86	285.38	4.52	13.90	14.17	12.11	35.54
LS-60	97.51	126.73	248.57	5.37	18.23	4.55	10.12	16.41
LS-61	62.22	189.09	243.96	5.08	12.82	10.86	9.68	21.06
LS-62	41.61	120.51	283.59	2.41	14.05	7.48	10.53	38.62
LS-63	46.13	109.60	285.81	5.15	13.40	14.81	7.07	25.30
LS-64	101.70	185.13	275.72	5.89	19.91	16.25	19.40	28.57
LS-65	19.40	174.76	275.76	7.91	12.23	17.14	9.96	43.63
LS-66	150.89	157.70	352.26	5.06	14.21	10.79	8.42	24.83
LS-67	81.55	152.95	312.66	5.60	10.41	13.88	9.90	22.23
LS-68	51.54	201.73	344.05	5.51	25.96	10.93	12.56	35.97
LS-69	69.13	132.51	366.48	5.26	24.84	15.91	12.48	34.52
LS-70	83.39	190.05	288.02	4.60	19.27	9.51	9.08	18.99

(Continued)

**Table 1. (Continued)**

<b>Genotypes</b>	<b>Arg</b>	<b>Asp</b>	<b>Glu</b>	<b>Pro</b>	<b>Met</b>	<b>Tyr</b>	<b>Leu+Ile</b>	<b>Phe</b>
LS-71	46.05	161.95	268.28	5.64	18.98	9.65	8.31	30.67
LS-72	119.48	163.21	304.64	4.74	14.80	9.97	12.19	23.38
LS-73	29.70	146.22	338.61	5.51	13.05	14.88	8.66	17.74
LS-74	18.13	84.89	300.48	4.96	15.42	12.58	10.54	19.66
LS-75	15.12	102.39	215.28	3.98	9.74	13.25	13.53	38.13
LS-76	45.07	137.76	357.58	5.33	17.04	10.69	7.21	11.22
LS-77	0.00	112.52	271.07	5.80	13.35	12.17	9.40	33.79
LS-78	31.34	178.66	253.55	5.14	14.05	13.44	11.08	37.48
LS-79	55.45	130.14	315.69	7.27	20.55	21.35	15.55	31.24
LS-80	37.83	118.56	274.28	3.00	15.44	13.24	10.81	33.14
LS-81	459.96	141.28	240.81	5.29	9.72	12.65	13.14	28.28
LS-82	12.24	159.05	282.01	4.28	10.68	9.54	8.95	30.44
LS-83	0.00	160.50	277.55	3.81	9.57	15.90	15.03	53.10
LS-84	109.22	154.75	357.34	7.38	16.52	18.44	24.84	50.22
LS-85	188.71	161.55	321.59	4.22	16.54	9.56	9.00	24.60
LS-86	35.68	126.81	321.25	4.59	20.35	16.80	16.42	37.70
LS-87	362.80	170.16	239.39	6.55	17.42	16.12	14.66	60.97
LS-88	359.80	157.75	339.49	14.08	18.24	30.87	48.90	45.45
LS-89	180.61	107.64	271.26	4.39	11.20	12.85	8.64	35.66
LS-90	347.33	135.83	298.06	5.15	19.32	9.78	8.47	31.56
LS-91	479.51	155.39	261.90	8.59	16.77	17.37	14.40	26.57
LS-92	487.29	138.39	275.52	6.88	19.14	10.70	15.13	36.52
LS-93	87.95	111.56	347.49	6.05	19.38	16.00	11.25	20.95
LS-94	140.64	122.63	364.68	5.95	18.30	9.76	8.88	42.33
LS-95	69.34	130.43	362.04	6.31	15.35	12.99	12.26	32.76
LS-96	178.90	121.33	383.42	6.23	16.70	11.81	7.23	17.89
LS-97	0.00	28.56	244.41	9.59	8.23	18.96	8.45	90.51
LS-98	58.02	81.58	423.16	5.03	17.27	11.92	9.98	18.51
LS-99	70.37	133.59	413.77	6.55	19.05	15.17	12.08	35.33
LS-100	72.49	160.86	414.95	8.16	24.34	14.31	11.60	16.67
LS-101	85.99	101.71	390.02	6.67	17.70	7.75	9.96	22.77
LS-102	10.73	177.66	257.48	3.86	7.58	6.36	7.56	25.83
LS-103	42.92	189.46	269.92	5.93	16.61	9.48	10.65	24.52
LS-104	59.25	161.77	299.69	5.22	10.25	7.65	12.31	26.03
LS-105	81.52	198.10	405.66	6.52	12.56	13.32	12.03	25.52
LS-106	221.16	132.98	267.78	6.37	20.85	14.79	18.06	52.79
LS-107	80.71	145.82	351.11	6.84	18.99	13.09	12.11	20.12
LS-108	19.57	150.68	274.37	8.67	16.22	12.99	16.96	33.18
LS-109	66.09	105.42	269.86	7.43	12.29	13.16	22.52	31.98
LS-110	87.65	189.30	405.32	5.63	11.12	17.74	15.07	24.48
LS-111	66.36	166.97	426.86	7.86	17.54	20.53	20.33	23.34

(Continued)

**Table 1. (Continued)**

<b>Genotypes</b>	<b>Arg</b>	<b>Asp</b>	<b>Glu</b>	<b>Pro</b>	<b>Met</b>	<b>Tyr</b>	<b>Leu+Ile</b>	<b>Phe</b>
LS-112	88.28	155.74	287.95	8.56	14.79	18.51	19.27	43.40
LS-113	86.48	175.30	367.17	7.76	18.02	9.08	9.81	17.94
LS-114	27.88	171.80	289.04	5.18	21.59	12.87	8.52	28.20
LS-115	47.59	160.42	354.61	5.98	13.36	12.03	10.99	16.00
LS-116	116.25	179.97	336.22	6.29	23.62	12.69	10.01	23.07
LS-117	116.49	174.38	346.36	7.26	23.29	18.77	14.48	26.68
LS-118	30.21	139.40	267.16	4.47	17.00	12.37	11.21	49.96
LS-119	184.25	104.43	302.68	6.88	17.22	17.12	22.71	27.02
LS-120	77.45	144.90	371.38	7.05	19.83	18.77	20.80	60.11
LS-121	71.66	174.07	324.46	5.27	15.55	13.01	10.42	24.75
LS-122	243.17	140.12	369.15	4.72	23.17	12.47	11.84	21.11
LS-123	0.00	127.87	316.60	6.36	14.84	13.67	14.85	31.43
LS-124	17.38	72.00	327.10	18.32	12.64	16.71	9.49	72.18
LS-125	80.99	157.54	343.80	7.76	18.15	11.24	11.12	30.57
LS-126	55.67	122.73	318.76	7.04	18.01	16.26	15.86	27.29
LS-127	62.22	174.27	317.03	6.11	19.12	9.62	11.89	30.30
LS-128	73.20	129.70	313.46	5.33	15.67	14.51	7.89	22.60
LS-129	77.97	167.51	310.07	6.58	18.93	16.66	12.35	36.49
LS-130	99.72	131.23	373.73	5.14	21.63	15.62	18.22	26.81
LS-131	53.83	138.29	324.97	5.87	20.17	8.95	12.06	37.84
LS-132	53.87	132.97	366.26	5.81	14.05	10.26	11.03	32.33
LS-133	86.63	145.11	279.98	4.89	14.18	9.97	11.85	30.18
LS-134	94.83	180.13	238.89	5.92	15.64	11.02	11.79	30.94
LS-135	63.10	108.45	306.20	7.67	20.09	19.37	17.99	39.97
LS-136	26.28	128.27	312.32	6.21	17.67	10.93	9.48	23.28
LS-137	115.03	126.81	305.83	7.08	17.20	16.13	10.90	37.15
LS-138	57.32	145.18	357.51	8.05	18.44	14.73	14.45	21.83
LS-139	148.39	149.08	420.73	7.41	18.51	12.77	15.20	34.48
LS-140	80.00	153.43	366.12	5.26	18.63	10.91	13.24	34.44
LS-141	70.96	129.26	413.16	6.46	18.23	15.13	8.00	33.95
LS-142	56.97	192.63	373.11	8.41	18.89	19.29	14.20	48.86
LS-143	199.65	138.90	371.76	7.08	17.85	11.30	16.60	29.72
LS-144	150.42	139.23	369.23	6.79	14.76	13.77	16.71	32.32
LS-145	71.26	127.48	350.80	7.14	21.19	23.37	17.32	37.07
LS-146	154.44	118.52	330.91	9.74	21.51	15.26	15.95	34.12
LS-147	234.17	140.24	357.33	9.38	17.42	26.73	23.47	50.51
LS-148	316.30	104.49	262.27	9.07	21.31	13.76	19.95	31.56
LS-149	200.72	163.38	269.01	7.98	16.28	16.46	10.41	27.11
LS-150	201.90	137.57	312.92	7.75	16.06	14.43	14.22	33.11
LS-151	245.82	157.51	360.12	7.60	15.47	12.75	17.17	25.44
LS-152	81.85	140.81	408.92	8.89	13.97	20.31	12.17	34.87
LS-153	82.10	122.96	347.69	6.99	21.10	15.40	13.19	32.74
LS-154	158.90	129.67	450.45	6.46	13.26	13.71	20.76	21.74

(Continued)

**Table 1. (Continued)**

Genotypes	Arg	Asp	Glu	Pro	Met	Tyr	Leu+Ile	Phe
LS-155	160.79	128.32	361.87	6.07	15.90	16.57	12.75	30.11
LS-156	158.99	162.27	323.84	7.93	23.13	12.16	16.10	26.85
LS-157	110.51	120.21	292.22	5.77	18.86	14.68	15.48	32.77
LS-158	11.92	199.36	338.95	6.61	16.85	14.14	18.44	21.25
LS-159	170.96	130.66	343.39	9.68	17.24	19.99	18.13	119.84
LS-160	114.72	137.56	273.46	5.08	13.62	16.67	13.07	28.53
LS-161	104.38	121.95	319.66	7.79	16.85	14.33	18.46	51.95
LS-162	97.39	138.73	201.51	6.47	15.45	13.30	13.09	38.62
LS-163	112.28	255.06	311.05	7.00	18.70	12.75	15.09	31.03
LS-164	49.11	174.50	319.78	4.12	23.55	18.94	14.57	105.42
LS-165	126.28	134.40	319.83	7.17	30.76	20.06	19.17	33.78
LS-166	131.29	177.67	347.39	6.63	21.80	18.42	13.96	23.76
LS-167	130.59	165.16	315.36	6.85	20.18	20.31	10.47	17.50
LS-168	76.09	128.27	266.81	6.98	23.07	17.36	14.65	40.76
LS-169	28.22	123.90	355.53	8.06	12.81	21.35	12.23	96.34
Ceora	72.21	127.26	440.83	5.93	16.21	15.26	14.35	24.57
Eren	97.17	121.10	356.17	10.32	20.62	16.22	13.49	26.93
İptaş	36.65	142.55	398.25	9.48	20.24	19.29	14.06	29.23
Karadağ	35.84	117.73	394.34	7.56	20.78	16.03	11.16	20.36

0.10 to 506.85 mg g<sup>-1</sup>; however, Glu gave close values ranging from 201.51 to 450.45 and the mean was 311.61 as seen in Table 1. Another aspect, Arg gave the lowest and the highest levels among the studied free amino acids, interestingly. These results could be easily attributed to the genetic variation. Arg is mentioned by Aragão et al. (2015) that increased Arg contents promote the synthesis of gibberellins, which are germination promoters. Therefore, Arg could be linked to the germination potential of the genotypes as well. Under the drought stress, plants are known to accumulate osmolytes and Pro is one of the most common compatible osmolytes in drought-stressed plants. Giving an example, the Pro contents increased under drought stress in pea (Alexieva, Sergiev, Mapelli, & Karanov, 2001; Sánchez, Manzanares, de Andres, Tenorio, & Ayerbe, 1998). Pro accumulation can also be observed with other stresses, such as high temperature and under starvation (Sairam, Rao, & Srivastava, 2002). Regarding to these studies, higher Pro content could be linked to the potential stress tolerance of the genotypes, which can be used under this condition if it is necessary. These genotypes were grown on the same conditions; hence, the differences are mostly related to the genetically variations and this could be a good indicator for further studies (Arslan, 2017; Arslan et al., 2017).

The free amino acid constitutes of legumes are more than 10% of the weight of the legume seeds and are tend to be lost in cooking. These free amino acids, nevertheless, represent an enormous potential store of nourishment for man and animal. These free amino acids are not lost for animal feeding because it is directly consumed by the animals, which makes the important to determine the free amino acid contents of the legumes (Bell, Qureshi, Charlwood, Pilbeam, & Evans, 1976).

As seen in the Table 1, scrutiny of this large number containing values are not easy to evaluate, thus PCA is preferred. This is the reason why the results are given in Table 2 as results of PCA analysis. The other statistical methods are not adequate to assess the relationships among traits and their optimization, and grouping because the traits related to morphology and chemical structure could show more than quantitative variability; assessment of differences is difficult when data of dissimilar nature are involved. The PCA gives the simple evaluation possibility with reduced dimensions as seen in Table 2.

**Table 2 Principal component (PC) analysis of characteristics associated with 173 grass pea genotypes**

	PC axis		
	1	2	3
Eigenvalues	2.7705	1.2844	1.0727
Explained proportion of variation (%)	34.6	16.1	13.4
Cumulative proportion of variation (%)	34.6	50.7	64.1
Characteristics	Eigenvectors		
Arg	0.159	-0.135	-0.817
Asp	0.045	-0.649	-0.152
Glu	0.297	-0.378	0.432
Pro	0.453	0.154	-0.077
Met	0.339	-0.396	0.236
Tyr	0.501	0.156	0.049
Leu + Ile	0.471	0.023	-0.209
Phe	0.303	0.460	0.123

**Table 3. Correlation coefficients of free amino acid contents (mg g<sup>-1</sup>) in 173 grass pea genotypes**

	Arg	Asp	Glu	Pro	Met	Tyr	Leu+Ile
Asp	0.079*						
Glu	-0.011*	0.096*					
Pro	0.205**	-0.058*	0.301**				
Met	0.092*	0.184*	0.386**	0.239**			
Tyr	0.093*	-0.013*	0.285**	0.564**	0.336**		
Leu+Ile	0.245**	0.098*	0.228**	0.476**	0.300**	0.628**	
Phe	-0.014*	-0.121*	0.023*	0.311**	0.150*	0.408**	0.298**

\*Significant at the 0.05 probability level.

\*\*Significant at the 0.01 probability level.

In our PCA analysis (Table 2), three of the eight principal component axes had eigenvalues >1. Therefore, these three components can be interpreted as reflecting the general variability in the data-set without much loss of information. This is a usual finding that a few dominant PCs explain a high percentage of variance. The first three components explained 64.1% of the variability among the 173 grass pea genotypes (Table 2). The PCA1 explained 34.60% of the total variance and was positively correlated with Tyr, Leu + Ile and Pro. The PCA2 explained 16.10% of the total variance and was mainly correlated with Phe, Tyr and Pro. The PCA3 explained 13.40% of the total variance and was mainly correlated with Glu, Met and Phe.

In addition, correlation analyses were performed in order to evaluate correlation of the measured parameters. Table 3 shows the correlation among the parameters.

For simply, Tyr and Leu + Ile ( $r = 0.628$ ,  $p < 0.01$ ) are given the strong relationship all the free amino acids and the further assesment are left to the readers. The others showed negative or positive correlative relationships as seen in the Table 3.



#### 4. Conclusions

This research is a unique study revealing the free amino acid contents of grass pea seeds of 173 different genotypes. Every accession reacted differently in terms of free amino acids. Finding similar results or to cite former studies seem to be impossible for this research which made this study to be important and the rare. Therefore, this work will easily give a way to the researcher who wishes to use this genetic pool for further breeding studies.

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