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Research Article

Mutant Mürdümük (*Lathyrus sativus* L.) Genotiplerine ait Tohumların Bazı Serbest Amino Asit İçerikleri

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Öz

Bu araştırmada, EMS (etil metan sülfonat) mutajeni kullanılarak geliştirilen özel bir koleksiyon olan 40 mürdümük genotipi ve Gürbüz-2001 çeşidi, aspargin, L-homoarginin, arginin, aspartik asit, glutamik asit, prolin, metionin, tirozin, lösin + izolösin, fenilalanin gibi serbest amino asit içeriği açısından incelenmiştir. Mürdümük tohumlarında en az seviyede bulunan serbest amino asit prolindir ve 3.53 mg kg^{-1} olarak bulunmuştur. L-homoarginin, 12369.40 mg kg^{-1} düzeyinde elde edilen en yüksek serbest amino asit içeriğidir. Bu çalışmada elde edilen serbest amino asitlere ait değerler yeni çeşit geliştirme çalışmalarında kullanılabilir.

Anahtar kelimeler: *Lathyrus sativus,* serbest amino asitler, EMS, mutasyon ıslahı

Some Free Amino Acid Contents in the Seeds from Mutant Grass Pea Genotypes

Abstract

With this research, 40 genotypes, a special collection developed through the EMS (Ethyl Methane Sulfonate) mutagen, and released variety Gürbüz-2001 were examined for free amino acid content including aspargine, L-homoarginine, arginine, aspartic acid, glutamic acid, proline, methionine, tyrosine, leucine + isoleucine, phenylalanine. The least abundant free amino acid in grass pea seeds is proline and was found as 3.53 mg kg^{-1}. L-homoarginine gave the highest free amino acids contents was obtained at 12369.40 mg kg^{-1}. These variations can be useful and usable throughout product/variety development phase.

Key words: *Lathyrus sativus,* free amino acids, EMS, mutation breeding

Introduction

The *Lathyrus* genus belong to the legume family (Fabaceae) and containing 187 annual or perennial variety (Allkin et al., 1983; Jackson and Yunus,1984; Plitmann et al., 1995; Noto et al., 2001). Grass pea (*Lathyrus sativus* L.) is the most cultivated and economically important species of the genus *Lathyrus* (Campbell, 1997; Ochatt et al.,2004). Grass pea, like other legumes, is a very valuable crop and is grown because of the high protein content in its seeds. Furthermore, grass pea is a very popular among poor farmers as it can be successfully grown in marginal areas in many parts of the world in harsh climatic conditions without needing much production input (Vaz Patto et al., 2006; Arslan, 2019). Indeed, grass pea seeds are the only food that can be consumed during famine periods when harsh and difficult climatic conditions are experienced. (Zhao et al., 1999). Orphan legumes, such as grass pea, are grown primarily by resource-constrained farmers, mostly by women, who use seeds on minor plots and marginal lands in certain agro-ecological niches to provide with high-nutrient food to families (Diane, 2016).

It is potential source of important nutrients for animal feeding, both its grains, herbage and hay are preferred by animals (Chinnasamy et al., 2005). Compared to soybean, which is used extensively in the compound feed industry, the most important advantages of the plant contains high protein and can be produced with less input costs (Grusak and DellaPenna, 1999; Arslan, 2016).

Grass pea is a plant that is mostly self-pollinated and can be pollinated by 2-3%, especially as a result of bee activity. Therefore, genetic variation in the grass pea gene pool is very narrow and difficult to obtain new varieties using the existing this. It is observed that the fastest and most accurate way to do in this direction is mutation breeding. Mutations can be used directly or indirectly in plant breeding, and when compared to the traditional breeding method, if it is used to improve and change one or two characteristics on the plant, the result is achieved in a shorter time and more economically than the classical breeding methods. Even if undesirable traits occur in mutants resulting from mutation breeding, these mutant cultivars can be used as rootstocks thanks to their superior properties in traditional breeding methods (Sağel et al., 1994). The most preferred method in mutation breeding is chemical mutagens, is ethyl-methane-sulfanate (EMS). Seeds obtained by sowing seeds kept in EMS solution are M1s and gene mutations are observed in M2 progeny (Kodym and Afza, 2003). Unlike physical mutagens, EMS method causes changes in small chromosome segments (Anonymous, 1977; Shu et al., 2012).

Grass pea and other large-grain legumesare contain high amounts of protein. Protein content in seeds varies between 21 and 25 percent on average. Compared to cereals, legume seeds are richer in lysine, but generally lower in methionine and cysteine, which are the sulfur-containing amino acids. Large-grain legumes are an excellent supplemental protein source for cereals (Fikre et al., 2008; Aniszewski et al*.,* 2013). Proteins are made up of various combinations of up to 26 amino acids. Although some of the amino acids can be synthesized in the body, that is, they can be converted from another biomolecule and intermediate products during metabolic events, some of them cannot be synthesized. Therefore, classification can be made by considering what kind of sources amino acids should be provided in case of metabolic need. On the other hand, exogenic amino acids are essential, that is, they must be taken from the diet for life to continue (Harper et al., 2014). Mainly used as building blocks for the synthesis of proteins, the amino acids are the necessary and vital nutrients to maintain the physiological activities of humans and animals (Ates et al., 2010; Arslan, 2017b). Free amino acids are also important for keeping non-protein meals. Experiments with dextran show that it is not the quality of the meal that is critical but rather the ability to produce free amino acids. It is known that free amino acids play a key role as a signal used by the midgut to control retention of lumen content. Free amino acids in the midgut lumen can be sensed by midgut endocrine cells such as digestive cells or allatostatin cells, and these cells can directly or indirectly affect the activity of midgut muscles and thus food retention via the nervous system (Caroci and Noriega, 2003). Morever, free amino acids, normally found in proteins and various other non-proteinogenic amino acids such as, mimosine, canavanine, homoarginine and 3-N-oxalyl-2,3-diaminopropionic acid (ODAP), include 20 amino acids. (Bell, 2003). Although free amino acids, better known for their high concentrations in the leguminosae, are also found in algae and fungi as well as other nutritionally specific crop families such as *Brassicaceae*. (Megias et al., 2016a).

Free amino acids have also been shown to act as precursors or substrates for a variety of events. Comprehension the interaction and relationship between free amino acids and its processes necessitates a close examination of the seed itself in the gene pool under current conditions (Nimbalkar et al., 2012). Plant saps frequently contain significant amounts of free amino acids, differ from animal tissue fluids (Carratu et al., 2008). However, these studies either do not explain in detail the amount of free amino acid found in it or do not cover the entire amino acid spectrum (Nimbalkar et al., 2012; Arslan, 2017a).The goal of this study was to quantification of some free amino acid contents in seeds of mutant grass pea collection.

Materials and Methods

Grass pea seeds of 40 mutant genotypes and released variety Gürbüz-2001 were used as a plant materials in this experiment. The mutant genotypes were developed through the Ethyl Methane Sulfonate (EMS) mutagen. EMS mutagen was applied to grass pea seeds with the same procedure at 0.5% and 1% concentrations, as reported by Singh and Sadhukhan (2019). The seeds were obtained from Master Project supported by Akdeniz University Scientific Research Projects Coordination Unit with FYL-2020-5330 (Doğan Çetin, 2021). Free amino acids analyzed from the mutant seeds in SUDUM-Natural Products Application and Research Center Laboratory at Suleyman Demirel University.

Analysis of the amount of free amino acids in grass pea samples prepared as ground was carried out using UPLC-MS/MS device according to the method specified by Kıvrak et al. (2015). According to this method, seeds samples were homogenized of 0.5 g, solution containing 0.1% (v/v) formic acid was extracted with 10 ml of water:methanol (80:20) (v/v). Analysis and determination of free amino acid

Table 1. Proline, methionine, tyrosine, leucine+isoleucine and contents of grass pea seeds (mg kg^{-1})

Genotypes	Asp	L-harg	Arg	Asp acid	Glu acid	Pro	Met	Tyr	Leu+ Iso	Phe
GPM1	742.8	3437.1	17.13	69.52	319.76	17.51	13.39	17.21	10.44	77.94
GPM2	2038.0	2169.4	19.10	142.49	270.96	8.64	16.86	13.88	17.97	34.50
GPM3	2717.4	6483.2	25.12	125.15	292.82	5.87	18.03	11.46	10.24	23.74
GPM4	4459.2	11010.7	230.81	135.42	349.31	8.96	18.46	27.52	25.81	54.55
GPM5	2772.4	3138.7	79.57	144.63	308.77	5.57	10.83	14.83	10.49	23.12
GPM6	3366.5	8878.3	138.22	146.12	347.94	8.27	36.24	23.84	22.26	39.04
GPM7	3360.0	9034.9	132.08	146.68	343.17	7.88	35.30	22.96	21.58	36.52
GPM8	2483.5	10213.8	226.35	143.20	282.39	6.92	25.74	16.44	20.48	59.76
GPM9	2499.3	11259.1	234.40	150.25	292.68	7.09	30.76	16.63	19.97	58.28
GPM10	2380.7	10506.0	231.46	138.74	282.93	6.99	23.38	16.75	20.85	60.93
GPM11	2392.0	10847.3	215.77	125.75	264.45	6.34	21.68	15.81	19.14	54.89
GPM12	3175.2	5385.4	232.39	136.70	346.10	4.47	23.63	13.07	12.78	21.53
GPM13	2348.7	5302.0	134.90	159.81	300.53	5.21	10.60	9.79	7.50	28.35
GPM14	1755.5	6147.4	45.16	146.04	207.41	4.59	12.05	11.36	9.84	33.34
GPM15	2716.5	4107.5	69.01	124.16	413.31	5.61	16.53	16.00	15.50	25.06
GPM16	2129.0	8289.4	107.30	248.85	291.63	6.62	19.07	13.36	16.30	31.65
GPM17	2431.5	6398.8	82.79	141.58	262.50	4.62	14.46	10.45	12.80	30.78
GPM18	4580.2	6440.2	308.60	98.81	259.01	9.03	22.16	14.70	21.14	32.82
GPM19	2561.5	5684.8	52.99	126.97	295.98	6.87	20.95	22.39	16.79	31.86
GPM20	2685.6	6160.5	95.01	131.19	199.00	6.44	16.07	14.21	13.87	40.16
GPM21	574.8	2400.4	50.01	27.58	238.92	9.16	8.72	19.53	9.30	97.74
GPM22	3199.7	6472.0	79.86	133.16	403.83	8.86	14.53	21.70	12.90	36.26
GPM23	2142.4	6435.2	109.93	123.72	286.73	6.70	17.54	16.91	11.77	37.89
GPM24	4850.9	7047.4	190.79	135.52	348.55	6.69	18.20	11.85	17.93	30.31
GPM25	2121.7	3550.7	18.93	165.26	272.33	7.87	12.71	18.31	10.56	45.37
GPM26	2062.4	2169.3	43.07	134.40	335.25	5.04	17.37	11.20	7.79	11.45
GPM27	3290.9	8554.8	128.80	144.16	340.14	8.17	35.93	24.55	23.90	39.86
GPM28	2428.4	10307.9	242.08	144.56	291.32	7.36	24.57	17.58	20.98	61.00
GPM29	3120.2	8530.0	120.67	131.13	299.86	6.78	31.37	21.03	20.70	34.45
GPM30	3327.7	8999.2	133.83	151.86	349.56	7.98	45.37	22.55	21.20	37.29
GPM31	3184.8	8270.3	123.20	127.10	315.85	7.14	31.98	21.43	20.31	35.13
GPM32	2769.6	7796.3	128.09	168.01	343.07	6.61	22.67	19.68	14.79	24.71
GPM33	2304.9	5474.3	67.81	126.11	387.36	6.11	18.59	15.87	8.64	34.62
GPM34	4211.6	5553.6	150.68	112.08	326.80	9.70	22.37	16.31	16.91	35.49
GPM35	1621.8	3206.3	15.23	113.82	211.53	3.53	10.87	8.25	8.24	18.95
GPM36	5142.2	8736.6	239.83	148.95	355.64	7.57	16.09	13.62	18.20	26.45
GPM37	2234.9	12369.4	226.30	142.38	284.01	6.82	24.64	17.02	20.57	49.56
GPM38	2544.5	10719.8	227.64	144.63	273.46	9.84	25.70	14.93	30.47	60.42
GPM39	2883.6	5462.6	70.05	119.11	294.96	8.27	18.13	14.80	24.91	35.30
GPM40	3545.4	9964.5	508.21	175.57	286.25	9.56	24.73	19.53	15.92	29.34
Gürbüz-2001	**3440.5**	**7203.1**	**353.96**	**160.91**	**236.41**	**6.52**	**18.11**	**17.22**	**15.54**	**63.40**

contents were done with the Thermo Scientific Ultimate 3000 UPLC, Thermo Scientific- TSQ Fortis (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) system. Chromatographic evaluations were performed using Xcalibur software. Hypersil Gold RP C18 (1.9µm), 50x2.1 mm, (Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA) UHPLC column was used as analytical column for chromatographic separation. The seeds were screened for all free amino acids, but only 10 free amino acids could be detected in grass pea seeds. The other free amino acids were not evaluated because they were too low to be detected.

Statistical analyses

The values of the mutant grass pea genotypes in the study were compared with the population averages of the starting material Gürbüz-2001 variety with a sample t test (1-Sample t test) using the MINITAB Statistical Software program. Principal component analysis (PCA), based on the mean values of 40 genotypes for 10 free amino acids, was performed to determine the relationship and association between genotypes pattern of variation (Iezzoni and Pritts, 1991).

Result and Discussions

Seeds of totally 41 grass pea genotypes containing 10 free amino acids are shown in Table 1. Viewed Table 1, it is noteworthy that there is a large variation among genotypes in terms of free amino acids. In addition, the comparison of free amino acids’ minimum, maximum and mean values obtained in genotypes with Gürbüz-2001 is incidated in Table 2.

Table 2. Min, Max, and Mean Values of Content of Some Free Amino Acids in Grass Pea Genotypes (mg kg^{-1})

Free Amino Acids	Min (minimum)	Max (maximum)	Mean	Gürbüz-2001 (initial material)
Aspargine	574.77	5142.19	2798.03	3440.46
L-homoarginine	2169.30	12369.40	7085.05	7203.12
Arginine	15.23	508.21	149.55	353.96
Aspartic acid	27.58	248.85	136.94	160.91
Glutamic acid	199.00	413.31	302.90	236.41
Proline	3.53	17.51	7.46	6.52
Methionine	8.72	45.37	21.41	18.11
Tyrosine	8.25	27.52	16.80	17.22
Leucine+Isoleucine	7.50	30.47	16.63	15.54
Phenylalanine	11.45	97.74	40.77	63.40

For a general aspect, while minimum, maximum ve mean of aspargine in mutant genotypes were determined as 547.77, 5142.19 and 2798.03 mg kg^{-1}, respectively, 3440.46 mg g^{-1} in Gürbüz-2001. Another free amino acid most abundant in grass pea seeds is L-homoarginine. The lowest and highest contents were obtained at 2169.30 mg kg^{-1}, 12369.40 mg kg^{-1} and mean was 7085.05 mg kg^{-1}, respectively. Morever, the least abundant free amino acid in grass pea seeds is proline. The proline was found in amounts ranging from 3.53 to 17.51 mg kg^{-1} among genotypes. In additon, it was observed that Gürbüz-2001 also contains 6.52 mg kg^{-1} proline (Table 1). On the other hand, arginine, aspartic acid, glutamic acid, methionine, tyrosine, leucine+isoleucine and phenylalanine contents of grass pea seeds had values in wide variation range as shown in Table 1 and 2.

Arslan (2017a) reported that investigated the free amino acid variations in 173 grass pea genotypes and determined free amino acids, including arginine, aspartic acid, glutamic acid, proline, methionine, tyrosine, leucine + isoleucine and phenylalanine. As a result of the examination, determined that arginine was the most fluctuating free amino acid and the genotypes had contents ranged from 0.10 to 506.85 mg kg^{-1}. Besides, according to the mean values, Glutamic acid had the highest values with 311.61 mg kg^{-1}.

Megias et al. (2016b) reported that some free amino acids such as aspartic acid, glutamic acid, asparagine, arginine, proline, methionine, tyrosine, isoleucine + leucine, phenylalanine were determined as 21.98, 27.52, 19.31, 5.34, 0.00, 0.12, 0.55, 4.36 and 0.76 g/100 g, respectively, in *Vicia ervillia* seeds.

The relationships among the free amino acids of 40 grass pea mutants are given in Table 3.

While L-homoarginine had the highest significant positive correlation with Leucine + Isoleucine (0.706**), Aspartic acid had significant negative correlations with Phenylalanine (-0.444**). Aspargine that has the most association with other free amino acids. In most studies, it was determined that free amino acid levels related with concentration of the other free amino acids (Firke et al., 2007; Arslan, 2017a; Arslan, 2017b; Arslan et al., 2017). Nimbalkar et al. (2012) reported that the free amino acids also function as precursor or substrate for various treatments. It is also necessary to examine the gene pool in seed, to understand the relationship between apparently free amino acids and their processes. Based on this, the researchers, considering that data on free amino acid content of cereal amaranth is very inadequate, published the first report on use of LC-MS/MS method to analyze the free amino acid compound of this crop. The amount of legumes' free amino acids are greater than 10% by weight of the seeds and tend to be lost during cooking process. However, these free amino acids represent an tremendous potential nutrient store for man and animal. Furthermore, the free amino acids are not lost during animal feeding because it is directly consumed by them, which makes it important to identify the legumes' free amino acid contents (Bell, 2003). Therefore, selection of grass pea genotypes with high free amino acid content will be possible owing to the results of this study. Although the grass pea seeds have been extensively studied for neurotoxic contents such as ODAP (Hanbury et al., 1999; Fikre et al., 2008; Kumar et al., 2011; Arslan et al., 2017), the data on the determination of free amino acid content are very inadequate, thus limiting the scope of the current analysis to compare with other reports.

Table 3. Correlation of free amino acid contents in 40 grass pea mutants (mg kg^{-1})

	Asp	L-harg	Arg	Asp acid	Glu acid	Pro	Met	Tyr	Leu+ Iso
L-harg	0.365*								
Arg	0.514**	0.689**							
Asp acid	0.224	0.380*	0.239						
Glu acid	0.430**	0.059	0.034	0.111					
Pro	-0.024	0.041	0.109	-0.329*	0.137				
Met	0.351*	0.618**	0.369*	0.238	0.283	0.109			
Tyr	0.230	0.390*	0.173	-0.044	0.392*	0.419**	0.576**		
Leu+Iso	0.470**	0.706**	0.484**	0.164	0.119	0.274	0.663**	0.497**	
Phe	-0.393*	0.253	0.106	-0.444**	-0.242	0.571**	0.024	0.327*	0.254

*Significant at the 0.05 probability level.**Significant at the 0.01 probability level. (Aspargine: Asp, L-homoarginine: L-harg, Arginine:Arg, Aspartic acid: Asp acid, Glutamic acid: Glu acid, Proline: Pro, Methionine: Met, Tyrosine:Tyr, Leucine + Isoleucine: Leu+Iso, Phenylalanine: Phe)

Conclusion

Grass pea is one of the oldest cultivated crop worldwide with a long history of domestication especially in Turkey, Syria and Iraq. However, the importance of grass pea, which has been neglected for many years, has increased in recent days against climate change and hard environmental conditions.

This study was planned to determine free amino acid content in grass pea seeds improved with EMS mutagen. The free amino acid contents such as Aspargine, L-homoarginine, Arginine, Aspartic acid, Glutamic acid, Proline, Methionine, Tyrosine, Leucine + Isoleucine, Phenylalanine were investigated. As result of the study the data obtained indicate that there is a wide variation in free amino acid in this collection. Free amino acid content in mutant seeds is a very important criterion in ruminant nutrition. Some content values are high, indicating that promising seeds are obtained. This shows the quality of these mutants, which are planned to be used in feed rations. This variations can be useful and usable throughout product/variety development programs.

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