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Diversity for Fatty Acid and Organic Acid Contents in Mutant Grass Pea (*Lathyrus sativus* L.) Genotypes

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ABSTRACT

Background: Considering global warming and climate changes, grass pea (*Lathyrus sativus* L.) is becoming advantageous and popular with its characteristics such as resistance to biotic stresses. The objective of the present study was to determine the variations in fatty acid and organic acid content in induced grass pea populations.

Methods: Forty-one grass pea genotypes that include forty mutant genotypes and one released variety (Gürbüz-2001) were investigated in term of fatty acids using the GC-MS device and organic acids using UPLC-MS/MS device.

Result: Heptadecanoic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid were determined as major fatty acid constituent in forty mutant genotypes. In saturated fatty acids, the highest values were determined as 403.24, 401.39 and 398.87 mg 100 g^{-1} (in GPM5, GPM20, GPM34), respectively. Compared the control cultivar, the maximum values of GPM21 (18.77%), GPM5 (18.68%) and GPM33 (18.12%) were higher in PUFA. And in the similar, the values of GPM19 (11.52%), GPM28 (10.54%) and GPM4 (5.62%) were lower obtained in PUFA. As the maximum values of succinic, lactic, oxalic, citric and tartaric acid were obtained in GPM9, GPM37 and GPM11, the lowest values were found in GPM4, GPM13 and GPM18, respectively. The values of fatty acids and organic acids, important criteria for animal feeding and human nutrition, will guide further breeding studies for the development of these 40 mutant genotypes.

Key words: Fatty acid, *Lathyrus sativus*, Mutation breeding, Organic acid.

INTRODUCTION

Legumes are an excellent source of minerals and organic materials for both humans and animals (Grusak and DellaPenna, 1999). Moreover, legumes are a common food group and due to their utilization, is a significant source of protein, lipid and fatty acids for both animal and human nutrition (Yoshida *et al.*, 2009). Also, according to other research findings, consumption of legumes should be increased for better health and the management of chronic diseases such as cancer, diabetes and cardiovascular disease (Pirman and Stibilj, 2003).

Because of their usefulness for industrial usage, seed oils, that contain a significant number of very long-chain fatty acids, have attracted attention among the biochemical components (Bauman *et al.,* 1988). These substances may also have chemotaxonomic importance. As taxonomic markers in higher plants, the fatty acid content of seed lipids can be used (Bagci *et al.,* 2001).

Organic acids and their salts are substances that prevent the growth of mold in feed, prolong the storage of feed and feed raw materials and aid in digestion and absorption. These substances exhibit their antimicrobial properties by reducing the pH of the contents of the stomach and intestines or the feed they are given (Kocaoğlu Güçlü and Kara, 2010), which inhibits the growth of hazardous microbes that thrive in unfavorable circumstances (Dibner and Buttin, 2002; İpçak *et al.,* 2017). In addition, to boost feed digestibility and decrease methanogenesis and nitrogen loss in ruminants, organic acid and fatty acid are added to animal feed (Foley *et al.,* 2009).

Nowadays, many countries have focused on sustainable agricultural production in order to assure food security, cope with the increasing population's food demands and combat the effects of global climate change (Seydosoglu, 2019). In this process, the importance of researches focused on developing and improving plants that are underutilized or neglected is increasing (Talukdar, 2011). Grass pea (*Lathyrus sativus* L.) is an ideal and valuable legume for poor farmers, known by drought tolerance, thrives with minimal external input and insurance crop. Also, due to its high protein contents in mature leaves and seeds, the grass

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pea can be used for several purposes, including pasture crop, animal feed and fodder as well as human food (Rizvi *et al.*, 2016; Enneking, 2011). According to Lambein *et al.* (2019), grass pea is still an untouched source for composites that can contribute to human health, just like other orphan legumes.

In recent years, this crop gains importance, the place where the effects of global warming are felt most, with its low input cost and its ability to a certain amount of product in poor soil conditions. In addition to, it is known that the plant has a very high disease, pest and weed control power (Das, 2000). Grass pea has a large and significant expansion potential in its usage (Talukdar, 2009), due to increased salinity and exposure to biotic stress in arid and drought-prone areas (Almeida *et al.,* 2015). Furthermore, grass pea has a high protein content, their seeds are highly nutritious and are a cheap source of protein for a good human diet. The seeds of grass pea contain about 28% protein, 48% starch and less than 1% fat (Yan *et al.,* 2006).

Diversity characterization research in *Lathyrus* germplasm focused on nutrient content, phenology and yield, weed resistance, disease resistance or other quality traits (Almeida *et al.,* 2015). Additionally, grass pea is a fascinating source of dietary lipids, with a high percentage of polyunsaturated fatty acids (58%) and phenolic components that have strong antioxidant activity, such an average value of 68 mg 100 g^{-1} of gallic acid (Sellami *et al.,* 2022; Gonçalves *et al.,* 2022).

Plant breeding for crop productivity, high quality, richer nutrition compound and climate resilience is one of most important targets (Sarma *et al.,* 2022). Achievement in plant breeding also depends on a gene pool whose variation has been determined in terms of a desired character. Some species of family *Leguminosae* are a origin of economical protein for living beings. Fatty acids of extent and compound in seed lipids can fulfill as taxonomic indicators in higher plants. With this in mind, this study was conducted to determine the fatty acid and organic acid content of a total of 40 grass pea genotypes, improved with EMS mutagen.

MATERIALS AND METHODS

Forty-one grass pea genotypes that include forty mutant genotypes and one released variety (Gürbüz-2001) were investigated in this study. The seeds were obtained from Master Project supported by Akdeniz University Scientific Research Projects Coordination Unit with FYL-2020-5330 (Doğan Çetin, 2021). The 40 mutant grass pea genotypes (M2) improved by applying Ethyl Methane Sulfonate (EMS) mutagen. The seeds were analyzed for fatty acids and organic acids in SUDUM-Natural Products Application and Research Center Laboratory at Suleyman Demirel University.

Fatty acids analyses

The composition of fatty acids in the seeds of the grass pea plant was determined using the GC-MS device and determined acorrding to Arslan (2017).

Organic acids analysis

Quantification of organic acids in grass pea samples was made using UPLC-MS/MS device and analyzes were obtained according to Dincer *et al.* (2020).

RESULTS AND DISCUSSION

Fatty acid compositions

The histogram of some fatty acid compositions obtained from a total of 40 grass pea mutants M2 seed genotypes were in Fig 1.

Among the fatty acids in 40 grass pea mutants, the lauric acid contents were ranged from 1.94 (GPM12) to 0.44 (GPM38) mg 100 g^{-1} (Fig 1A). Whilst, GPM34 have maximum value for average stearic acid content with 82.37 mg 100 g^{-1}, that was higher than mean of all mutants (78.89 mg 100 g^{-1}) be 4.22%. The current research is similar to Bağcı and Şahin (2004) and Grela *et al.* (2017) study in terms of stearic acid (5.94% and 6.04%). This fatty acid is known to be auxiliary stabilize emulsions; its main significance is that it does not raise the level of cholesterol in the blood cause enzymes in the liver convert it to unsaturated fat during digestion.

There was also much variation in oleic acid content ranged from 158.34 and 109.31 mg 100 g^{-1} (Fig 1G). The nutritional value of seeds is determined not only by the amount of lipids they contain, but also by the quality of lipids they comprise (Chinnasamy *et al.*, 2005). As Grela *et al.* (2010) stated, the fatty acid oil content of grass pea seeds contributes to their high dietary value. Whilst all these scopes are combined, the fatty acid constituents and quality of grass pea seeds are evocative of and substantiate once again how valuable a product can be in animal feeding.

As the main fatty acid of the grass pea, the minimum linoleic acid mean was 224.63 mg 100 g^{-1} in GPM19, the highest of linoleic acid were detected in GPM34 found as 297.70, mg 100 g^{-1} (Fig 1H). In addition the γ-linolenic acid content was ranged 28.39 to 17.19 mg 100 g^{-1} (Fig 1I). The maximum γ-linolenic acid value was determined as 68.03 mg 100 g^{-1} and minimum was 44.93 mg 100 g^{-1} (Fig 1J). Heptadecanoic acid, stearic acid, oleic acid, linoleic acid and γ-linolenic acid were determined as major fatty acid constituent in forty mutant. The profiles of major fatty acid contents are similar to each researchers (Hanbury *et al.,* 2000; Chinnasamy *et al.,* 2005; Pastor-Cavada *et al.,* 2009). The ω6/ω3 ratio, play an important role impressing blood flow to the central nervous system, may influence the vascular system (Yehuda *et al.*, 2005; Sinn and Howe, 2008; Enneking, 2011). Proven by previous studies that the ω6/ω3 ratio differs for different genotypes (Swarup and Lal, 2000).

Whereas examined in terms of saturated fatty acids, the highest value was determined as 190.78 mg 100 g^{-1} (in GPM13); the lowest value was obtained as 131.90 mg 100 g^{-1}, respectively (in GPM19), (Table 1). Some experiments report that Fabaceae seed oils are similar in whole unsaturated fatty acid components and oleic and linoleic acid are the main components in the seed oil (Kokten *et al.,* 2015). The monounsaturated fatty acids (MUFA) content ranged from

114.28 to 162.87 mg 100 g^{-1}. The MUFA content was low in the GPM28 (121.59 mg 100 g^{-1}), GPM3 (118.28 mg 100 g^{-1}) and GPM29 (121.59 mg 100 g^{-1}) mutants. Meanwhile, the GPM20 (162.87 mg 100 g^{-1}), GPM21 (161.67 mg 100 g^{-1}) and GPM5 (154.27 mg 100 g^{-1}) mutants had high levels (Table 1). Giugliano and Esposito (2005) stated that monounsaturated fat from numerous sources may possess the advantageous impacts on blood lipids and oxidative stress (Sahin *et al.,* 2009).

As the control was taken as the reference, the maximum values of GPM5 18.64%, GPM34 18.55% and GPM20 18.46% were higher in polyunsaturated fatty acids (PUFA). And in the similar conditions compared to the control, the minimum values of GPM19 11.58%, GPM28 9.22% and GPM4 5.62% lower were obtained in PUFA. The ratio of saturated: monounsaturated: polyunsaturated was predictably 1:1.41:4.28 in current experiment, respectively (Table 1). The PUFA, a critical oil for cardiovascular diseases, as 18:2 and 18:3 have a significant role in the initial oxidative processes since fatty acids are turned into carbohydrates, whereat of their streamline of peroxidation (Bağcı and Şahin, 2004). Similar to many studies (Kokten *et al*., 2015; Sagan *et al*., 2016) it was explored that the PUFA values were higher in mutant grass pea seeds in this experiment. However, the PUFA contents were also significantly greater, only lower than the conclusion obtained in other experiments.

Contrary to this experiment, Grela *et al.* (1999) stated that grass pea had 14:0 fatty acids in the saturated class and 16:1 in MUFA, even at low levels. In addition, Chinnasamy *et al.* (2005); Grela *et al.* (2012) and Grela *et al.* (2017), in another experiment, the presence of C 14:1, C 20:0, C 20:1 and C 20:4 fatty acids were proved as a result of the analysis, even though in low proportions.

The relationships among the fatty acids of 40 grass pea mutants are given in Table 2. Lauric and tridecanoic acid with stearic and linoleic acid had the highest significant positive correlation (0.82**), in addition, lauric acid is positively correlated with pentadecanoic (0.67**), palmitic

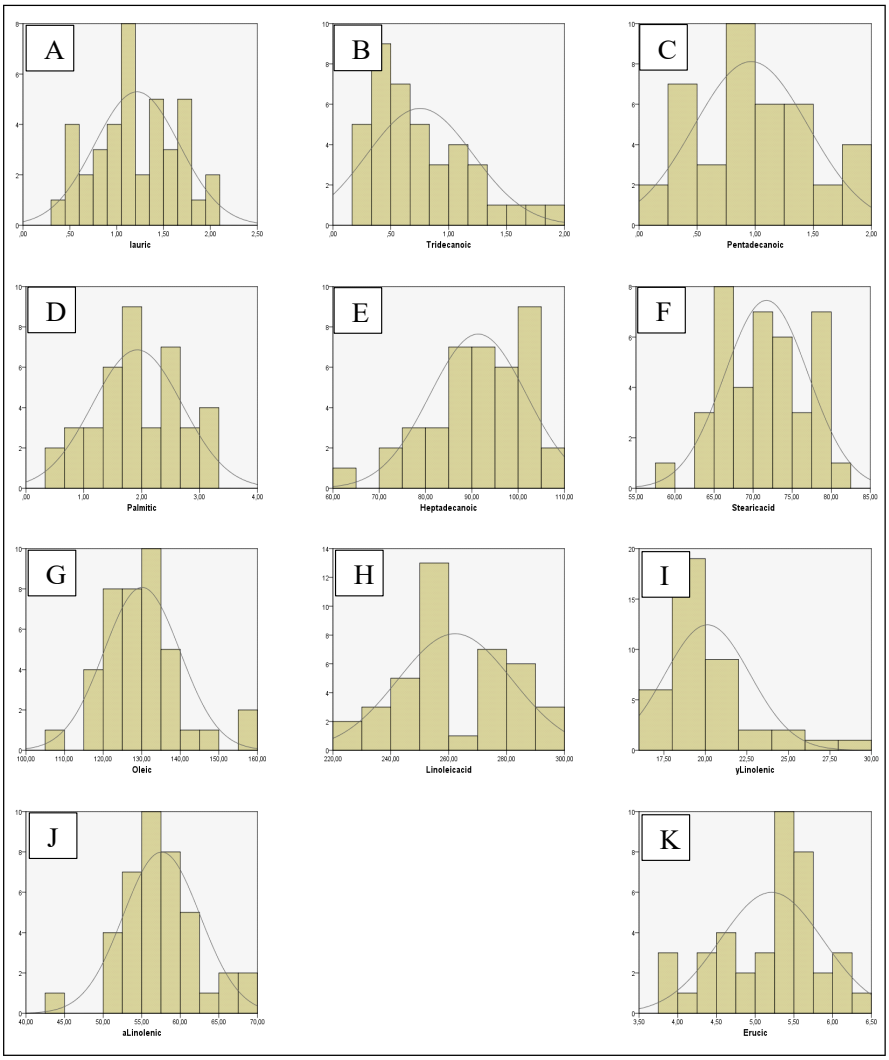

Fig 1: The histogram of fatty acid contents in grass pea mutant lines (mg 100 g^{-1}).

(0.58**) and γ-linolenic acid (0.36*). Heptadecanoic acid had significant negative correlations with erucic acid (-0.32*). γ-linolenic acid has the most association with other fatty acids, was found to be associated with tridecanoic, pentadecanoic, heptadecanoic, stearic, oleic, linoleic and α-linolenic acid. Besides, it was seen that there was a negative relationship between some fatty acids, but no significant relationship was found other than the one mentioned (Table 2).

Organic acid compositions

The large variation among mutant grass pea genotypes for organic acid contents are given as histogram in Fig 2.

The high variations were determined among mutant grass pea genotypes for all traits. There was also much variation in fumaric acid content ranged from 23.50 (GPM8) and 5.80 mg 100 g^{-1} (GPM4) (Fig 2D). While the maximum values of succinic, lactic, oxalic, citric and tartaric acid values were obtained in GPM9, GPM37 and GPM11. And in the similar conditions compared to the control, the lowest values of succinic, lactic, citric, oxalic and mallic acids were found in GPM4, GPM13 and GPM18 (Fig 2). When the control was taken as the reference, the GPM9 was the highest and GPM4 lowest in oxalic acid (Fig 2F). Thus, suggested that these mutants, are in wealty organic fatty acids, may also have huge nutritional value. Organic acids were symbolized mostly by malic (Krebs cycle) and threonic (ascorbic acid oxidation product) acids. Solovyeva *et al.* (2019) found the highest contents of malic acid, one of the main organic acids, in two grass pea varietiesas 495 and 505 mg 100 g^{-1}. Observed that organic acids can be a good preservative in roughage and silage, help in the digestion of cellulose and reduce the production of CH_4 gas, promote rumen development in calves, improve rumen microflora and use of feed (Baytok and Aksu, 2005; Selwet, 2006; Kato *et al.,* 2011; Ali *et al.,* 2013). Stated that organic acids added to the rations, in particular, increase the secretion of digestive enzymes secreted from the pancreas, maintain the electrolyte balance in the gastrointestinal tract and increase

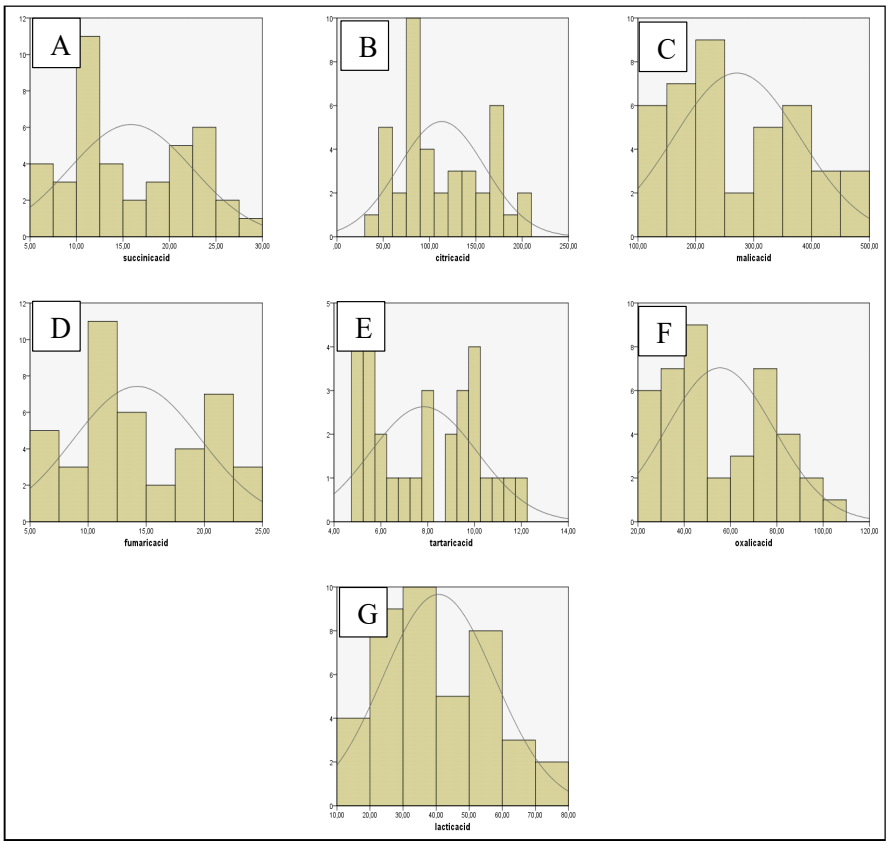

Fig 2: The histogram of organic acid contents in grass pea mutant lines (mg 100 g^{-1}).

Table 1: Minimum, maximum and average fatty acid values of grass pea genotypes (mg 100 g^{-1} seeds).

Fatty acids	Control	Maximum	Minimum	Mean
SFA	156.21	190.78	131.90	145.05
MUFA	135.71	162.87	114.28	135.45
PUFA	322.77	392.61	286.83	339.45
Total fatty acids	614.69	776.26	533.01	619.95

Table 2: Correlation of fatty acids contents in 40 grass pea mutants (mg 100 g^{-1}).

	C12:0	C13:0	C15:0	C16:0	C17:0	C18:0	C18:1n9c	C18:2n6c	C18:3n6	C18:3n3
C13:0	0.82**									
C15:0	0.67**	0.49**								
C16:0	0.58**	0.34*	0.67**							
C17:0	0.09	0.12	0.52**	0.24						
C18:0	0.32	0.12	0.22	0.05	0.59**					
C18:1n9c	0.16	0.25	0.27	0.03	0.45**	0.75**				
C18:2n6c	0.23	0.29	0.21	0.08	0.52**	0.82**	0.73**			
C18:3n6	0.36*	0.46**	0.37*	0.16	0.47**	0.66**	0.69**	0.76**		
C18:3n3	0.22	0.33*	0.15	-0.01	0.42**	0.61**	0.73**	0.75**	0.71**	
C22:1n9	0.18	0.36*	-0.03	-0.16	-0.32*	-0.02	0.09	0.07	0.05	0.10

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level.

Lauric acid (C 12:0), Tridecanoic acid (C 13:0), Pentadecanoic acid (C 15:0), Palmitic acid (C 16:0). Heptadecanoic acid (C 17:0), Stearic acid (C 18:0), Oleic acid (C 18:1n9c), Linoleic acid (C 18:2n6c). γ-Linolenic acid (C 18:3n6), α-Linolenic acid (C 18:3n3), Erucic acid (C 22:1n9).

Table 3: Correlation of organic acids contents in 40 grass pea mutants (mg 100 g^{-1}).

	Succinic acid	Lactic acid	Oxalic acid	Citric acid	Fumaric acid	Malic acid
Lactic acid	0.996**					
Oxalic acid	1.000**	0.996**				
Citric acid	0.996**	0.998**	0.996**			
Fumaric acid	0.904**	0.929**	0.903**	0.932**		
Malic acid	0.985**	0.985**	0.985**	0.983**	0.914**	
Tartaric acid	0.940**	0.945**	0.940**	0.946**	0.905**	0.937**

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level.

the digestion and absorption of minerals (Kocaoğlu Güclü and Kara, 2010; Gul *et al.,* 2013).

The correlation analysis among the organic acids of 40 grass pea mutants are in Table 3. Succinic and Oxalic acid had the maximum significant positive correlation (1.00**). All organic acid that were investigated in this experiment, had significant negative correlations with each other and all were had significant correlations higher than 91.40% (Table 3).

CONCLUSION

Forage crops especially legumes occupy a significant role in the feeding of ruminant animals with regard contributing energy (complex carbohydrates, lipid, fatty acids), vitamins, protein and minerals. Some grain legumes such as grass pea are neglected sources of energy and micronutrients and their utilization is still restricted owing to fact that unpredictability about the rate and the effect of antinutritional factors they can include. The studied legumes belonging to the 40 grass pea mutants genotypes' oil contents, indicated quantitative variations but the oil of seed represented same fatty acid compositions. Heptadecanoic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid were determined as major fatty acid constituent in forty mutant. While the maximum organic acid values of succinic, lactic, oxalic, citric and tartaric acid were obtained in GPM9, GPM37 and GPM11. Our research has redounded forty new data on the biochemical composition of grass pea. Since the fatty acid profiles of the seed oil have considerable systematic value and importance in plants, it is constantly used as a component in biochemical systematics and has proven to be precious in experiments of some plant groups. It is considerable to determine the properties of promising mutants with breeding experiments and to determine their suitability for future use in animal feeding rations and human nutrition.

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Conflict of interest: None.

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