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Determination of L-Dopa (L-3, 4-dihydroxyphenylalanine) Content of Some Faba Bean (*Vicia faba* L.) Genotypes

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ABSTRACT

Faba bean, reflecting all properties of legume crops such as soil improvement and benefit of human health, also contains L-dopa which can be used to cure Parkinson's disease, is a rare plant. The study was conducted in Samsun province to determine the L-dopa content of leaf, flower and pod of faba bean genotypes. Four cultivars and 18 lines were sown on November in randomized complete blocks design with 3 replications. Samples were taken from leaves at vegetative period, flowers and fresh pods at generative period of faba bean. The L-dopa content was determined by HPLC. According to the result of variance analysis, the differences among genotypes for L-dopa content of flowers and fresh pods were found significant ($P<0.01$). L-dopa content of leaves, flowers and fresh pods varied between 10.88-33.41, 40.95-96.37; 4.16-52.28 mg kg⁻¹, respectively. L-dopa content of flowers of 20 genotypes, except a cultivar and a line, were found in same group statistically. Average L-dopa content of leaf, flower and pod of faba bean were 19.36, 75.87 and 25.27 mg kg⁻¹, respectively. The most common form of consumption of faba bean in Turkey and treatment for Parkinson's disease in some countries is fresh pod. However this study showed that L-dopa amount in flower is higher ($P<0.01$) than that of leaf and pod. Results of this study revealed that L-dopa content of 7 lines were richer than that of others. If agronomic characters of 7 lines are well, their potential to become a new variety should be investigated.

Keywords: Faba bean; L-dopa; Flower; Pod; Leaf

Bakla (*Vicia faba* L.) Genotiplerinin L-Dopa (L-3, 4-dihydroxyphenylalanine) İçeriklerinin Tespiti

ESER BİLGİSİ

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ÖZET

Bakla bitkisi baklagil bitkilerinin toprak ıslahı ve besleyicilik açısından tüm özelliklerini taşıyan ayrıca yaşlı toplumlarda sıkça görülen Parkinson hastalığının tedavisinde kullanılabilen L-dopa içeren nadir bitkilerdendir. Bu çalışma, Samsun

ilinde bakla genotiplerinin yaprak, çiçek ve taze meyvelerindein L-dopa ieriklerini belirlemek iin yrtlmřtr. alıřmada, 4 eřit ve 18 hat 3 tekrarlamalı olarak tesadf blokları deneme desenine gre Kasım ayında ekilmiřtir. Denemede bitkinin vejetatif dnemde yaprakları, generatif dnemlerde çiçek ve taze meyvelerinden rnekler alınarak Yksek Basınlı Sıvı Kromatografi sistemi (HPLC) ile L-dopa ierikleri belirlenmiřtir. Yapılan varyans analizi sonucu L-dopa ierikleri bakımından genotipler arasında çiçek ve meyvede ($P<0.01$) istatistiki farklılıklar bulunmuřtur. Genotiplerin L-dopa ieriklerinin; yaprakta 10.88-33.41, çiekte 40.95-96.37 ve meyvede 4.16-54.29 mg kg⁻¹ arasında deđiřtiđi tespit edilmiřtir. Bir eřit ve bir hat hari, geri kalan hatların çieklerindeki L-dopa ieriđinin istatistiki olarak farklı olmadığı tespit edilmiřtir. Ortalama L-dopa ieriđi sırasıyla yaprak, çiek ve meyvede 19.36, 75.87 ve 25.27 mg kg⁻¹ olmuřtur. Trkiye’de halk arasında Parkinson iin baklanın en yaygın kullanım řekli meyvesidir. Ancak bu alıřma da çiekdeki L-dopa ieriđinin yaprak ve meyvedeki L-dopa ieriđinden daha yksek olduđu ($P<0.01$) tespit edilmiřtir. Bu alıřma sonucunda, L-dopa ieriđi bakımından 7 hattın diđerlerinden zengin olduđu belirlenmiřtir. Bu hatların agronomik zellikleri de dikkate alınarak eřide aday olabilme olasılıkları arařtırılmalıdır.

Anahtar Kelimeler: Bakla; L-dopa; iek; Meyve; Yaprak

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1. Introduction

In recent years, quality factor has been taken into account in plant breeding studies due to increased demand healthy balanced diet by consumers. Public opinion of a lot of countries in the world agree that measures must to be taken to avoid sickness is less expensive than the cost of treating a disease. Hence, one of the most important step to be healty is nutrition with healty nutrient. The production and consumption of food which promotes a healty community is widely preferred. Therefore, to reveal the role of these products in prevention of diseases has become an important area of research nowadays.

Grain legume are protein plants. Mean of nutritional values and quality of legume crops for human and animals are high protein and amino acids content and low antinutritional factors. Turkey is a homeland of the most of legume crops and one of these plants is faba bean (řehirali 1988). In recent years, the importance of these food for human health has been increasing. Faba bean can be grown in temperate climatic conditions and it is an important legume plant that should be included in crop rotation for soil improvement. Faba bean that has L-DOPA (L-3,4-dihydroxyphenylalanine) content is one of the rare plants and this substance used medically in the treatment of Parkinson’s disease (PD).

PD, the second most common neurodegenerative disease in the world, is characterized by progressive

motor symptoms marked by slowness of movement, gait problems, rigidity and tremors (Chowdhury et al 2014). Later symptoms can include thinking, sleep, emotional and behavioral problems including dementia (Fox 2013). After the loss of 80 percent or more of the nerve cells in the substantia nigra, symptoms of PD will develop (Fox 2013). PD is a common disease of unknown etiology in elderly people and one of the major causes of disability among elderly (Lucas-Carrasco et al 2014). Over age 50 in Western Europe’s 5 most and the world’s 10 most populous countries PD patients were between 4.1 and 4.6 million in 2005 and will double to between 8.7 and 9.3 million by 2030 (Dorsey et al 2007). Higher rates were reported for Caucasians in Europe and North America, intermediate rates for Asians in China and Japan, and the lowest rates for Blacks in Africa (WHO 2006). The number of patients with PD in Turkey are approximately 100 thousand and 10,000 new patients are diagnosed every year (PHD 2016).

Acetylcholine is the principal neurotransmitter in brain so there is a balance between acetylcholine and dopamine. This balance is distrupted in favour of dopamine in Parkinson’s. Unlike dopamine, L-dopa is able to cross the barrier between the blood and brain. It is converted into dopamine by an enzyme called dopa-decarboxylase in the brain, and can replace some of the lost dopamine (Musacchio 2013). Thus, L-dopa can be used to

increase dopamine concentrations in the treatment of PD. L-dopa is a chemical that is made and used as part of the normal biology of some animals and plants as legume crops.

A total of 724 species from 447 genera of 135 families have been screened and has been reported in species of legumes as *Baptisia*, *Lupinus*, *Mucuna* and *Vicia* at levels up to 1.9% (Daxenbichler et al 1971). It was analyzed that contain L-dopa in some species from *Euphorbiaceae*, *Gramineae* and *Leguminosae* families and reported that *Vicia faba* contained 0.15-0.18%, *Mucuna deringiana* 2.3% and in spring and autumn shoot of *Robinia pseudoacacia* and *Sarothamnus scoparius* (Kohlmunzer et al 1975). In a study that was investigated to amino acid and L-dopa content in 10 legume plants as chickpea, bean, lentil, lupen, faba bean were determined that *V. faba* var. minor had been the most rich in L-dopa (Longo et al 1974). Shetty et al (2003) reported that faba bean can be important food in diet of Parkinson's patient because of L-dopa content.

The amount of L-dopa or levodopa can vary greatly, depending on the species of faba, the area where it is grown, soil conditions, rainfall, and other factors. It appears that the young pod and the immature (green) beans inside the pod contain the greatest amount of levodopa, and the mature or dried bean contain the least. 84 g of fresh green fava beans or canned green faba beans, drained may contain about 50-100 mg of levodopa (Holden 2006).

Cenarruzabeitia et al (1978) investigated exchange of L-dopa content in vegetative period of faba bean and reported that L-dopa increased with growing period and the highest level of L-dopa is in flower.

Vered et al (1997) suggested that faba bean might be of value in treating conditions such as hypertension, heart failure, renal failure, and liver cirrhosis in which natriuresis and diuresis are medically beneficial.

We believe that the importance and consumption of faba bean will increase when the availability of complementary medicine of faba bean has been

proved by scientific studies as well as used to food and role of soil improvement. Aims of this study are to determine different faba bean genotypes which have high level L-dopa and also to find out level of L-dopa in different plant parts of them.

2. Material and Methods

Material of this study were composed of 22 genotypes, four of which were registered faba bean cultivars (Eresen-87, Filiz-99, Kıtıkı-2003, Lara) and 16 lines obtained from ICARDA and 2 lines collected from study region.

Experimental area was approximately 197 m elevation from the sea in Samsun province located between 41°21'49.79" N and 36°11'24.51" E in Middle Blacksea region coastal section, north of Turkey. Samsun province has warm and humid climate. In the experiment period, total rainfall was 589 mm. In this period, average monthly temperature varied from 6.10 to 24.8 °C and relative humidity ranged from 57.04 to 81.87%.

According to soil analysis result, the soil texture of the experimental area clay which was rich in phosphorus (64.3 mg kg⁻¹ P), medium in organic matter (2.7%), and non-saline (0.523 dS m⁻¹), and soil pH was neutral (6.9).

Experiment was conducted in a randomized complete block design with 3 replications. Plots were organized 8 m² and spacing 50 cm x 10 cm. Seeds were sown on November 9 in 2010.

Samples for L-dopa analysis were collected from 5 different plants at 3 different period which are the beginning of flowering for leaf sample, middle of full flowering time for flower sample and pod setting periods for green pods that took place in mid part of plant. These samples were initially dried in air after than dried 24 h of oven dry at 45 °C, ground in a grinder and stored in +4 °C refrigerator. Sample preparation, extraction and determination of L-dopa were conducted according to Shivananda et al (2003). L-dopa concentration of samples extract was determined by thermo separation HPLC (High Performance Liquid Chromatography) that included

autosampler on a C 18 column (4.6x250 mm) at 2 mL min⁻¹ and 30 °C with a sodium dihydrogen orthophosphate buffer (pH 2.8) as mobile phase and at 280 nm wavelength of UV detector.

To determine L-dopa content, 6 different standart solution were prepared. Pure chemical L-dopa oven dried at 45 °C for 72 hours, was weighed (S₁: 2, S₂: 4, S₃: 8, S₄: 16, S₅: 32, S₆: 64 mg) and dissolved in 0.1 M orthophosphoric acid and volume was made up to 100 mL using double distilled water to give a concentration range of 20 to 500 mg L⁻¹ solution. Prepared standarts being read on HPLC Thermo SP and determined L-dopa's coming duration was at third minute. These were used to plot a standart graf and regression formula after than were estimated L-dopa amount.

Analysis of variance using the SPSS-13 program was performed after L-dopa data were transformed. DUNCAN's multiple range test was used at the statistical grouping.

3. Results and Discussion

Commonly grown faba bean varieties is capable of indeterminate growth (Sepetoğlu 1994). For this

reason flowering potential of faba bean is high. In Samsun ecological conditions, in our 2-year study to determine flowering and pod setting in 12 different genotypes, the total number of flowers per plant varied between 9-279 and 10.2-27.1% of flowers blossomed have been found to set pod (Bozoğlu 2005). This is a significant loss of energy. Perhaps these flowers can be evaluated as a crude of medicine for PD. Cenarruzabeitia et al (1978) examined the L-dopa change during the vegetative period of faba bean and reported that flower is the organ which contains the highest L-dopa.

In this study it was determined the content of L-dopa in different components of 22 bean genotypes which were sown in winter under the conditions of Samsun. The L-dopa content of faba bean genotypes of different plant parts are shown in Table 1 and HPLC chromatogram examples are shown in Figure 1. According to the analysis of variance, the contents of L-dopa is not statistically different in the leaves of faba bean genotypes, but the content of L-dopa of flower and fruit is highly significant (P<0.01).

Table 1- L-Dopa amount in the different parts of faba bean (*Vicia faba* L.) genotypes

Çizelge 1- Bakla (Vicia faba L.) genotiplerinin farklı kısımlarındaki L-dopa miktarı

Genotypes	L-Dopa content (mg kg ⁻¹)			Genotypes	L-Dopa content (mg kg ⁻¹)		
	Leaf	Flower**	Pod**		Leaf	Flower**	Pod**
V _{lara}	11.85	40.95 b	16.34 c-g	V ₁₂	21.53	75.40 a	32.13 a-d
V _{filiz}	15.79	79.59 a	40.81 abc	V ₁₃	23.93	96.37 a	25.55 b-f
V _{kutuk}	17.83	73.55 a	5.04 g	V ₁₄	26.62	69.46 a	26.77 a-d
V _{eresen}	17.95	85.74 a	39.39 a-d	V ₁₅	10.88	83.42 a	15.24 d-g
V ₅	24.41	85.02 a	54.29 a	V ₁₆	18.47	79.06 a	7.79 fg
V ₆	20.04	88.29 a	50.09 ab	V ₁₇	18.88	88.24 a	50.14 ab
V ₇	13.23	46.47 b	4.16 g	V ₁₈	33.41	83.13 a	33.13 a-d
V ₈	22.82	90.17 a	5.18 g	V ₁₉	16.75	72.49 a	6.70 fg
V ₉	12.71	79.65 a	8.55 efg	V ₂₀	16.29	71.35 a	17.24 c-g
V ₁₀	25.99	66.57 a	24.66 b-f	V ₂₁	16.96	72.39 a	30.33 a-d
V ₁₁	16.83	74.70 a	15.66 c-g	V ₂₂	22.88	85.18 a	46.70 ab

** , P<0.01

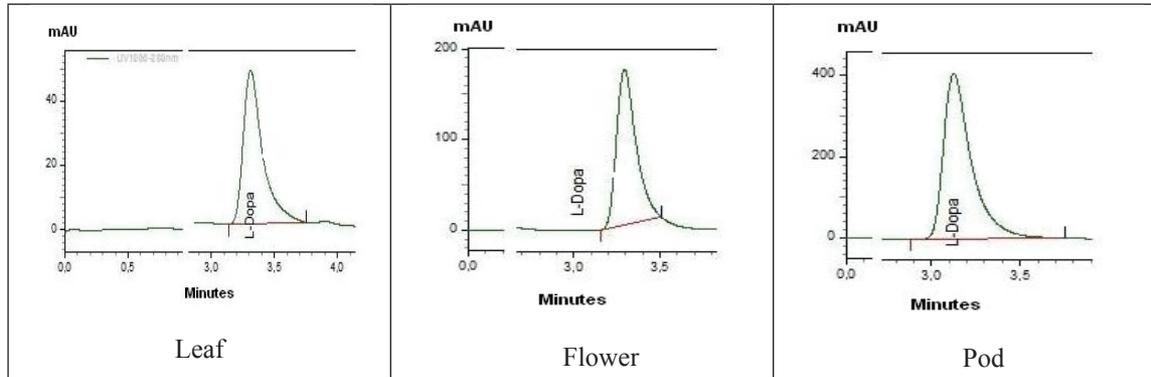


Figure 1- Some examples from HPLC chromatogram of L-DOPA

Şekil 1- L-dopa HPLC kromotogramlarından bazı örnekler

The content of L-dopa varied in the leaves 10.88-33.41 mg kg⁻¹, in flower 40.95-96.37 mg kg⁻¹ and in green pod 4.16-54.29 mg kg⁻¹ for faba bean genotypes. The use of faba bean against shaking, rigidity and slowness of movement caused by Parkinson's disease is known among the people in Turkey. Therefore fresh pods of faba bean is consumed. But it can be seen from the average rate of L-dopa content in leaf, flower and fruit of broad bean genotypes, flower has the most L-dopa content organ of plant. Ingle (2003) reported that L-dopa ratio was 0.2-0.75% in green peel of pod, 0.09% flowering green plant and 0.006-0.01% in green seed. Mohseni Mehran & Golshani (2013) obtained L-dopa from fava beans which were in the fresh and dry sprouted form, whose concentrations were 1.4, 1.5 and 2.6, 2.4 mg mL⁻¹ respectively. The results of this study indicate that faba beans are a good source of natural L-dopa.

In our study, concerning the amount of L-dopa in faba bean genotypes, whereas 22 genotypes is in the group that content highest L-dopa, 2 genotypes remained outside of this group. 10 genotypes (V₂, V₄, V₅, V₆, V₁₂, V₁₄, V₁₇, V₁₈, V₂₁, V₂₂) which consists the highest content of L-dopa in fruit also remained in the group which content the highest in flower. Also it has been found that the content of L-dopa in flower and fruit of lines is higher than the registered varieties (Figure 2). It can be seen in figure 2 content of L-dopa in leaf and flower of faba bean lines that were used in this study were higher than cultivars. L-dopa

level in fresh pods were closed. According to t-test, L-dopa amount in flower is higher (P<0.01) than leaf and pod. In our study, the highest L-dopa rate was 0.0096% and this rate was lower than scanned literature. It could be many reasons as kind of seed and soil conditions. Longo et al (1974) reported that *V. faba* var: minor had been the most content of L-dopa; Huang & Chen (1998) reported that if potassium nitrate and ammonium nitrate are used together L-dopa content increases and phosphate are negatively correlated with calcium but positively correlated with zinc content of soil.

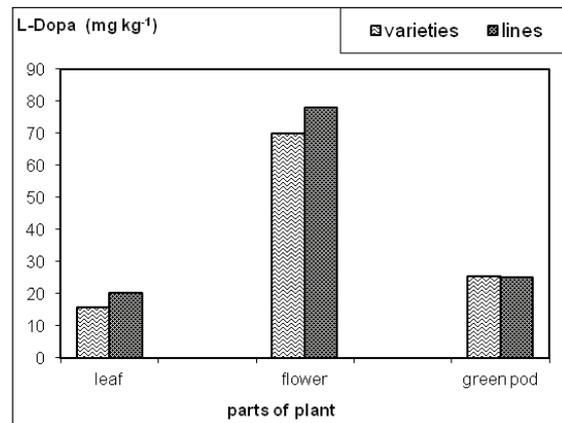


Figure 2- L-Dopa amount (mg kg⁻¹) in leaf, flower and pod of faba bean varieties and lines

Şekil 2- Bakla hat ve çeşitlerinin yaprak, çiçek ve bakladaki L-dopa miktarları (mg kg⁻¹)

4. Conclusions

In today's nutrition concept, health protective properties of food is important as well as their use as a food source. In all over world there is an increasing demand for preventive medicine, alternative medicine and natural ways of treatment. This has increased the popularity in the use of plants. The faba bean can be used as it is a plant contains L-dopa. With this feature, it was the subject of our study.

In this study it is aimed to determine the content of L-dopa in different parts of 22 faba bean genotypes which are sown in winter under the conditions of Samsun. Hence it is aimed to find a feature-rich genotype and discover whether the faba beans can be used for medical purposes rather than a food source.

Faba bean (*Vicia faba* L.), due to content of L-dopa are used as supportive therapy in addition to being a food product. After the result of variance analysis, the content of L-dopa in flowers and green pod were found to be statistically different in between the genotypes. Flowers of faba bean are not used as a food or tea. But L-dopa content of flower was founded more than pod and leaf. How can flowers of faba bean be used as a food, this situation should be investigated.

Using these results, it is essential to select L-dopa-rich genotypes and analyze samples that will collected more frequently during the development of the plant.

The fact that this metabolite content of plant does not mean that it can be suggested in direct medical purposes. It would not be scientific. Also these metabolites are considered blockers. Receiving less creates a drug effect (positive) when receiving in high dose it creates some health problems. For this reason, studies with a multidisciplinary infrastructure that could be used for medical purposes should be laid out. The most important step for the sustainability of life is the sustainability of agriculture. In order to this, the sustain of soil and biodiversity should be preserved. In this respect, we consider legumes significant and faba bean among

them is not widely used in our country. However, our country is one of the homeland of faba bean. To determine the use of the plant against PD will increase its popularity.

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