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The Effect of Nitrogen and Humic Acid Treatments on Flower Yield and Its L-DOPA Content with Agronomic Characteristics of Faba Bean (*Vicia faba* L.)

Azot ve Hümik Asit Uygulamalarının (*Vicia faba* L.)'nın Çiçek Verimi ve L-DOPA İçeriği ile Tarımsal Özelliklerine Etkisi

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THE EFFECT OF NITROGEN AND HUMIC ACID TREATMENTS ON FLOWER YIELD AND ITS L-DOPA CONTENT WITH AGRONOMIC CHARACTERISTICS OF FABA BEAN (VICIA FABA L.)

ABSTRACT

In addition to its agricultural characteristics, the faba bean plant is remarkable due to its L-DOPA content. In this study, the effects of 3 different humic acid doses (H0: control, H1:2 l da-1, H2:6 l da-1) and nitrogen fertilizer (5 kg da-1) treatments on some characteristics of Lara and Maçka genotypes and L-DOPA content in its flowers were investigated. The trial was carried out in the Strip Plots Trial Design in Samsun for one year. According to the findings, the effect of HA treatments on the amylose content in the seed was found to be statistically significant, and the values obtained from the treatments were equivalent to the control values in other examined properties. It has been determined that there are statistically significant differences between genotypes in many of the characteristics examined; It was determined that the flower yield and the amount of L-DOPA transferred to the tea water were higher in Maçka genotype.

Keywords: Faba Bean, Nitrogen, Humic Acid, Flower, Flower Tea, L-Dopa.

AZOT VE HÜMİK ASİT UYGULAMALARININ (VİCİA FABA L.)'NIN ÇİÇEK VERİMİ VE L-DOPA İÇERİĞİ İLE TARIMSAL ÖZELLİKLERİNE ETKİSİ

ÖΖ

Bakla bitkisi tarımsal özelliklerinin yanısıra L-DOPA içeriği nedeniyle dikkat çekici bir bitkidir. Bu çalışmada, 3 farklı hümik asit dozu (H0: kontrol, H1:2 lt da-1, H2:6 lt da-1) ve azotlu gübre (5 kg da-1) uygulamasının Lara ve Maçka genotiplerinin bazı özellikleri ile çiçek, çiçekteki L-DOPA içeriğine etkisi araştırılmıştır. Deneme Samsun'da Şerit Parseller Deneme Deseninde bir yıl süre ile yürütülmüştür. Elde edilen bulgulara göre; H uygulamaların istatistiki açıdan tanede amiloz içeriğine etkisi önemli bulunmuş olup diğer incelenen özelliklerde uygulamalardan elde edilen değerler kontrol değerlerinle benzer bulunmuştur. İncelenen birçok özellikte genotipler arasında istatistiki açıdan önemli farklılıklar olduğu tespit edilmiş; çiçek verimi ve çay suyuna geçen L-DOPA miktarının Maçka genotipinde daha yüksek olduğu belirlenmiştir.

Anahtar Kelimeler: Bakla, Azot, Hümik Asit, Çiçek, Çiçek Çayı, L-DOPA.

1. INTRODUCTION

The faba bean plant, which is one of the cool climate legumes, is an important legume plant due to its strong growth ability, high nitrogen fixation ability, and its ability to be planted in the winter in Samsun conditions and grown without the need for irrigation. When planted in winter, the high L-DOPA content in its flowers and the fact that flower harvests can be made in mid-April and May (Bozoğlu and Bezmen, 2021; Oğuz 2022) allow summer crops to be planted in the region. In the region, the fresh harvest period coincides with May and the dry harvest at the end of June. After the dry seed harvest, it is possible to plant field crops such as corn and soybean as a second crop, and to grow many vegetables such as leek, spinach, and cabbage.

Bezmen and Bozoğlu (2021) reported that the flowers of the plant, which are poured because they cannot turn into pods and therefore considered as a loss of energy, can be dried and used based on our herbal tea culture, and L-DOPA is transferred to the tea water when the tea is brewed. Topal et al. (2016) determined the L-DOPA content of 4.23%, 0.98% and 0.076%, respectively, in flowers, pollen and honey obtained in a greenhouse environment where only pods were grown. Pollen and honey from bees feeding on both faba bean flower tea and flowers can be considered sources of L-DOPA. Therefore, in this study, although L-DOPA is found in leaves and fresh pods (Topal and Bozoğlu, 2016), especially flower yield and L-DO-PA content were emphasized, and yield and quality-enhancing practices were tried.

Humic acids (H) are components with high organic matter content used in agriculture as fertilizers, inhibitors against diseases and pests, and remedies that increase the cation exchange capacity of the soil and facilitate the uptake of nutrients by the plant. Humic acid is effective in the root development of plants, reproduction of soil microorganisms, decomposition of the stubble in the field in a short time, increasing the wate- holding power of light soils and germination of seeds in a short time. It is also known that humic acid facilitates the uptake of plant nutrients such as nitrogen, phosphorus, potassium, iron and zinc, and has positive effects on improving the structure of heavy clay soils, preventing salt accumulation in soils and aeration of soils (Kaya et al. 2005). In some studies on different types of plants, with different treatment methods and doses, humic acid treatments have a positive effect on nutrient intake (Sarwar et al. 2012, Demirtas et al. 2013, Gezgin et al. 2013) and in some studies, it provides an increase in yield (El-Bassiony et al. 2010, Khan et al. 2010, Kaptan and Aydın 2012, Gürsoy et al. 2016) are reported. Roudgarnejad et al. (2021), according to the research, H treatment to faba bean (Vicia faba L.) resulted in positive effects on plant height, seed yield, protein, moisture and nutrient content of the seed. According to some research results, the expected results for the increase in yield from H treatments in legumes could not be obtained (Öktem 2017, Çakmak 2019, Sarılar 2021). However, humic acids are substances with a complex structure and their effects should be evaluated from multiple perspectives. Therefore, even if it does not directly increase yield, it may have positive effects on soil biology, diseases and pests, and product quality. Dawood et al. (2019), reported that foliar treatment of humic acid to faba bean (*Vicia faba* L.) caused significant increases in carbohydrate content, total phenolic content, proline, and free amino acids compared to the control.

We have been working to increase the diversity of use of the faba bean, emphasizing that it is an important plant with its L-DOPA content and easy to grow for the region. In this study, it was aimed to investigate the effects of humic acid doses treated foliar and nitrogen fertilization which is the classical recommendation dose to the plant in the field, on some properties of faba bean and L-DOPA content in its flowers.

2. MATERIAL AND METHOD

The soil of the trial field was found to be clayey, pH neutral, salt-free, organic matter content of 1.88%, and high P content. In the trial period, the lowest temperature was in January and it was 0.8 °C higher than the long years. According to the data of many years, while the total amount of rainfall was 504.6 mm in the vegetation period, 478.3 mm of rainfall fell in the trial year. Before sowing, the precipitation that saturated the soil with water and coincided with the interval between the first emergence and the vegetative growth period of the plant was sufficient for plant growth.

In this study, Lara variety (G_1) and Macka genotype (G_2) , which varieties that were found to have high L-DOPA content in their flowers in a study conducted in the same region in previous years were used. Lara (G_1) is a registered variety developed for fresh consumption; it is purple and the Macka (G_2) is a cream-brown coloured line that we brought from this region and selected. The trial was carried out in strip plots trial design with 3 replications in the 2019-2020 growing season. In the trial, the genotypes were placed in vertical strips and the treatments (K: control, N: nitrogen at 5 kg da-1, H,: humic acid at 2 lt da-1, H,: humic acid at 6 lt da) in horizontal strips. Seeds were sown manually in 4 rows and in plots with 2 m row length and 60 cm row spacing on November 22, 2019. In March, when the rainfall decreased and the temperature increased, H were treated to the leaf, and ammonium sulfate with 21% N was treated from the soil around the root of the plant. As H, a liquid form commercial product of Leonardite origin (Black Strong HUM VET) was used at the doses recommended by the company for legumes. H content; total humic and fulvic acids are 18%, total organic matter is 13%, water-soluble potassium oxide is 3.9%, pH is 8-10. When necessary, weed control was done, and watering and any pesticide treatment were not considered necessary.

Variance analysis was performed with the MSTATC package program, DUN-CAN multiple comparison test was used to compare the means. The correlations between the characteristics to take into account the mean of all data were determined by using the SPSS21 program by correlation analysis.

3. RESULTS AND DISCUSSION

According to the results of the analysis of variance, the significance of the genotype difference and the effects of the treatments on the characteristics and the groupings are given in Table 1 and Table 2 and the relations between the characteristics are given in Table 3.

Faba bean is a tall and upright legume. In previous studies in the ecology where this research was conducted, the plant height of different faba bean genotypes was found to be 56-123 cm (Pekşen, 2007; Bezmen, 2019). Bezmen (2019) determined the plant height as 66.26 cm and 81.73 cm, respectively, in Lara and Maçka genotypes. In this study, the Lara mean was 80.3 cm, and the Maçka selected from a local material was 104.3 cm long, and this difference was found to be statistically significant (P<0.01) (Table 1).

Maçka had higher values than Lara in other investigated agromorphological characteristics except for seed yield per decare. Bezmen (2019), the mean seed yield per decare for Lara and Maçka was found to be respectively 743.58 and 891.97 kg genotypes. In the study, 872.78 kg for Lara and 746.12 kg for Maçka. Although the effect of HA and N treatments on seed yield was not found to be statistically significant, the responses of genotypes to treatments (P<0.01) differ. While Lara gave the highest value in control, Maçka was positively affected by N and H1 treatments (Table 1).

Biological yield includes all organic content produced by the plant. Pekşen (2007) reported the variation range of biological yield in 15 different genotypes of faba bean (*Vicia faba* L.) plant, 1620.33-173.33 kg da-1. Biological yield in Lara and Maçka genotypes, Bezmen (2019) determined 1150 and 1747 kg, respectively, and they are close to the values in this study. Although the difference between genotypes and the effect of treatments on biological yield were not statistically significant, it was determined that biological yield decreased in both genotypes in H₂ treatment (Table 1).

Genotype (G)		Treat	ments				0				
	N	H ₀	H ₁	H ₂	G _{MEANS}	N	H ₀	H	H ₂	G MEANS	
		Plant He	ight (cm)		**						
G ₁	78.30	75.60	79.90	87.40	80.3 b	1347.87	1445.10	1440.58	1250.03	1370.89	
G ₂	94.87	93.77	131.10	97.70	104.3 a	1710.52	1713.56	1598.28	1463.24	1621.40	
T _{means}	86.58	84.68	105.50	92.55		1529.20	1579.33	1519.43	1356.63		
	Nu	mber of Po	ods (per pl	ant)							
G ₁	12.67	16.00	14.67	12.67	14.00	755.91	872.78	843.98	728.65	800.33	
G ₂	16.33	13.67	15.00	13.00	14.50	824.92	746.12	793.82	707.91	768.19	
T _{means}	14.50	14.83	14.83	12.83		790.41	809.45	818.90	718.28		
		100 Seed	Weight (g)		**	C	**				
G ₁	105.68	101.51	106.01	100.84	103.5 b	19.86	21.64	20.47	21.41	20.84b	
G ₂	120.70	123.26	124.59	131.50	125.0 a	22.79	22.72	22.85	21.49	22.46a	
T _{means}	113.19	112.38	115.30	116.17		21.33	22.18	21.66	21.45		

 Table 1. Means of some agro-morphological characteristics of faba bean

 genotypes treated with nitrogen and different H doses

*P<0.05, **P<0.01

Flower harvests were made 3 times at one-week intervals, and the total dry flower yield (kg) per decare was calculated. The flower yield of Lara decreased in the 2nd harvest but increased in the 3rd harvest. On the other hand, Macka increased flowering after the 2nd and 3rd harvest (Table 2). According to the results of the correlation analysis, the 2nd and 3rd flower harvests are in an important and positive relationship with the previous harvests. Flower plucking stresses the plant and encourages re-bloom. According to the statistical analysis, while the treatments did not have a significant effect on the flower yield, a significant difference was found in terms of both the flower yield (P<0.01) and L-DOPA (P<0.05) content of the genotypes. In the study conducted under similar conditions and techniques, Bezmen (2019) reported the mean dry flower yield as 20.40 kg per decare. While the dry flower yield per decare was 36.29 kg in the Lara variety, it was 46.72 kg in Macka by a wide margin. Bezmen (2019) determined the highest L-DOPA amount in the Lara genotype (8.25%) among 18 genotypes. The researcher reported that the Macka genotype (7.73%) is one of the genotypes with the potential to be a candidate for registration due to its high flower yield and high L-DOPA content.

Based on the hypothesis that tea can be made from flowers due to the rich L-DOPA content in faba bean flowers, the effect of the treatments on the L-DOPA content in the tea prepared with the infusion method with different amounts of flowers was not found to be statistically significant. However, the difference between genotypes was significant (P<0.05) and the L-DOPA content of the Maçka was found to be higher (Table 2). Bozoğlu and Bezmen (2021) determined the amount

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of L-DOPA in tea to be 8.04% on the mean. In this study, when the number of flowers added to the water was 100 mg, the L-DOPA content was 8.33% on the mean in the control, which is similar to the value reported by Bozoğlu and Bezmen (2021). In this study, when the number of flowers used in tea was increased to 200 mg, it was determined that the L-DOPA diffision increased, and the amount of L-DOPA became 20.84% in tea water (Table 2).

Topal (2012), determined that the crude protein ratio in flowers varies between 27.08% and 62.17%, and the mean is 36.26% in 26 faba bean genotypes sown in winter. In this study, the difference between the proteins in the flowers of the genotypes was found to be statistically significant (P<0.05). This value was 26.05% for Lara and 27.50% for Maçka in the control, and the effect of the treatments was not found to be significant (Table 2). In addition, Topal (2012) determined that the relationship between protein and L-DOPA contents is positive and important. However, such a relationship could not be determined in this study. As a result of the correlation analysis, there was a positive correlation between the protein ratio in the seed and the protein in the flower (r= 0.605^{**}), L-DOPA in 100 mg flower tea (r= 0.516^{**}) and L-DOPA in 200 mg flower tea (r= 0.521^{**}), a significant relationship was determined (Table 3).

		Trea	tment		G		G						
Genotype	N	H ₀	H	H ₂	MEANS	N H ₀			H	H ₂	. G MEANS		
(G) ⁷¹	1st Ha	rvest Flow	ver Yield (g	g plant-1)		F	*						
G ₁	0.72	0.73	0.80	0.78	0.76	25.31		26.05	25.45	25.53	25.59 b		
G ₂	0.80	0.68	0.90	1.01	0.85	26.16		27.50	26.83	25.92	26.60 a		
T _{means}	0.76	0.71	0.85	0.90		25.73		26.78	26.14	25.73			
	2nd H	arvest Flov	ver Yield (g plant-1)	**		L-DOPA in 100 mg Tea (%)						
G ₁	0.40	0.51	0.47	0.37	0.44 b	8.15		8.13	7.53	8.48	8.13 b		
G ₂	0.89	0.71	0.92	0.95	0.87 a	8.43		8.54	9.05	8.78	8.54 a		
T MEANS	0.64	0.61	0.69	0.66		8.29		8.33	8.29	8.63			
	3rd Ha	arvest Flow	ver Yield (ş	g plant-1)	**	L-DOPA in 200 mg Tea (%)					*		
G_1	0.59	0.94	0.96	0.77	0.81 b	20.33		20.36	18.88	21.20	20.19 b		
G ₂	1.57	1.41	1.61	1.66	1.56 a	21.05		21.33	22.68	21.93	21.75 a		
T _{means}	1.08 1.17 1.28 1.21			20.	69	20.84	20.78	21.57					
	D	ry Flower	Yield (kg o	da-1)	**								
G ₁	28.41	36.29	37.13	31.94	33.44 b	_							
G ₂	54.28	46.72	57.02	60.35	54.59 a	-							
T MEANS	41.34	41.51	47.07	46.15		_							

Table 2. Means of flower yield and quality of faba bean genotypes treated with nitrogen and different H doses

*P<0.05, **P<0.01

The most important quality characteristics of legumes are the coat ratio of the seed, their water intake and swelling capacity, and protein, starch and amylose contents. As the seed coat ratio increases, the water intake rate decreases, thus delaying both cooking and germination. Genetic differences are an effective factor in coat ratios. According to the analysis of variance, it was determined that the coat ratio, crude protein ratio in the seed and amylose content in the seed showed statistical differences (P<0.05) in the genotypes. Maçka gave high values in the three characteristics. A previous study with similar techniques in the same ecology, Bezmen (2019) determined the mean protein and starch content in seeds to be 23.33% and 36.38%, respectively. In this study, the protein and starch contents of the seed were determined respectively as 20.84% and 38.40% in Lara, 22.46% and 37.99% in Macka. (Figure 1). Seed content is directly related to the genetic structure as well as the nutrient content obtained from the soil. Neither nitrogen nor humic acid treatments did not change the coat, protein and starch content of the seed. Differently, the effect of the treatments on the amylose content in the seed was found to be significant (P<0.05). While the amylose content was 8.42% in control, it increased to 10.05% with the N treatment (Figure 3). In order to reduce the risk of diabetes, cardiovascular diseases, insulin resistance and obesity, foods with a low glycemic index and high amylose content come to the fore. Considering the use of faba beans as a food, the content of amylose is considered important.

The water intake and swelling capacity of the seed were positively affected by the treatments, and the lowest water intake capacity value was seen in the control with 1.12 g seed-1. The highest value was given by H2 treatment with 1.29 g seeds-1 (Figure 2).



Figure 1. Means of some seed characteristics of Lara (G1) and Maçka (G2) genotypes



Figure 2. The effect of N and H treatments on the water intake and swelling capacity of the seed



Figure 3. The effect of N and H treatments on the coat, protein, starch and amylose ratios of seed

According to the correlation analysis we conducted to determine the relationships between characteristics, positive and very important relationships were found between seed yield and biological yield ($r=0.758^{**}$), similar to the results reported by Pekşen (2007) and Bezmen (2019). Again, a significant and positive relationship was determined between biological yield and the number of pods ($r=0.726^{**}$), and also between the number of pods and seed yield ($r=0.829^{**}$) (Table 3).

As the coat ratio decreases, which is one of the most important characteristics that will affect the cooking time, the swelling of the seed increases (Karayel, 2012). A positive and significant relationship was determined between hundred seed weight and water intake capacity ($r=0.657^{**}$) and swelling capacity ($r=0.752^{*}$), but a negative and significant correlation with seed coat ratio ($r=-0.652^{**}$) (Table 3).

The relationship between flower yield in the 2nd harvest ($r=0.646^{**}$), flower yield in the 3rd harvest ($r=0.644^{**}$) and total dry flower yield ($r=0.631^{**}$) with 100 seed weight was found to be positive and significant (Table 3).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2	206	1															
3	.000	.726**	1														
4	290	.829``	.758``	1													
5	.287	.064	.512`	.141	1												
6	.118	009	109	.067	.229	1		ĺ									
7	.362	.105	.276	024	.646**	.570**	1	ĺ									
8	.225	.372	.573``	.322	.644**	.489*	.853``	1									
9	.270	.253	.405*	.198	.631''	.672**	.935``	.961``	1								
10	.361	.117	.239	.022	.328	.237	.336	.313	.338	1							
11	.493*	.165	.256	.091	.189	.265	.355	.370	.385	.267	1						
12	.483*	.177	.257	.102	.191	.276	.354	.373	.389	.270	.999``	1					
13	.156	283	.030	057	.657``	.298	.389	.286	.352	046	.195	.196	1				
14	.247	153	.131	036	.752**	.332	.493*	.368	.441°	.054	.059	.063	.839**	1			
15	405*	054	398	.012	652**	143	669**	573**	578``	358	146	138	214	333	1		
16	.430`	.372	.412*	.175	.396	.108	.261	.304	.284	.605"	.516**	.521"	.007	.137	362	1	
17	179	406*	343	440*	132	427`	185	215	273	194	278	279	.128	133	.062	373	1
18	025	.066	.314	.140	.352	015	.316	.273	.260	168	.162	.152	.326	.326	170	.023	.011

Table 3. Relationships between some characteristics of faba bean

1: Plant Height. 2: Number of Pods. 3: Biological Yield. 4: Seed Yield. 5: 100 Seed Weight. 6:1st Harvest Flower Yield. 7: 2nd Harvest Flower Yield. 8: 3rt Harvest Flower Yield. 9: Dry Flower Yield. 10: Crude Protein Ratio in Flower. 11: L-DOPA in 1. Dose Flower Tea 12: L-DOPA in 2. Dose Flower Tea. 13: Water Intake Capacity. 14: Swelling Capacity. 15: Seed Coat Ratio. 16: Crude Protein Ratio in Seed. 17: Starch Ratio in Seed. 18: Amylose Ratio in Seed.

4. CONCLUSION

Thanks to their nitrogen fixation abilities, legumes not only meet their own needs, but also feed the soil, especially in terms of nitrogen. In this sense, the use of synthetic fertilizers is different from other plant groups. The economic and ecological disadvantages of fertilizers lead to the use of organic materials such as humic acids. Humic acids are used as both fertilizer and remedy. Therefore, in this study, the effects of N treatment, which is the recommended dose for legumes, and H doses on some characteristics of faba bean were investigated. According to the findings, N and H treatments showed similar results with the control in most of the

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characteristics examined in both genotypes. In other words, while the effect of the H doses we chose was not significant compared to the control, it was determined that the N given with the calculation of 5 kg da-1 did not make a difference either. Based on the studies on different legumes in the literature, it was concluded that humic acid and nitrogen fertilizer treatments should be evaluated by examining both the soil and plant protective-healing effects rather than the increase in yield. The faba bean plant can be easily grown in Samsun ecological conditions without the need for additional inputs. In addition to the use of the plant as food, research on the possibilities of using it as medicinal plant due to its L-DOPA content continues.

In this study, it was observed that the flower yields of the faba bean and the L-DOPA contents in the tea obtained from the flowers were remarkable. We think that cheap and natural methods that encourage flowering and L-DOPA synthesis should be studied in order to increase the L-DOPA yield per unit area in the case of using pod flowers due to their high L-DOPA content. In this study, H was treated once foliar with the onset of flowering in March, when the climatic conditions became favourable. Our observations indicate that different treatment times and doses should be investigated as the climate permits. The differences between the Lara variety used in the trial and the Maçka genotype were found to be significant in many of the characteristics examined. The biological yield of the Maçka genotype is higher, and the flower yield is remarkable due to its high L-DOPA content. With these characteristics, it stands out in this research that it is a genotype that can be nominated for a variety.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

Author Contribution Rates

Design of Study : ZO(%30), HB(%70)

Data Acquisition : ZO(%80), HB(%20)

Data Analysis : ZO(%80), HB(%20)

Writing Up : ZO(%50), HB(%50)

Submission and Revision : ZO(%50), HB(%50)

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