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Original article

The Effect of Some Treatments on Seed and Plant on The Agricultural Characteristics of Faba Bean (*Vicia faba* L.) and The L-DOPA (L-3, 4-Dihydroxyphenylalanine) Content

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Abstract

In this research, 2 different doses of acetic acid (AA: 4%, 40%) were treated the seed before sowing; 2 different doses of humic acid (HA1 :2 L da-1 , HA2 :6 L da-1) and nitrogen (N: 5 kg da-1) treatments to the plant in the field were carried out in Strip-Plots Design in Samsun ecological conditions to determine the effects of faba bean agro-morphological, seed quality and L-DOPA content in flowers. According to the findings obtained, ıt was determined that the seed treatment had a statistically significant effect on the number of branches, pods, biological yield, seed yield and flower yield, and the 4% AA treatment gave better results compared to the control. HA and N as plant treatments showed a statistically significant and negative effect on the number of pods, biological yield and seed yield. The statistical effects of the treatments on the L-DOPA transferred to the herbal tea obtained from the flower were not found significant. However, it was determined that the AA treatment to the seed increased the ratio of L-DOPA in flower compared to the control. Therewithal, when the ratio of flowers was increased from 100 mg to 200 mg while preparing tea, there was an increase in the ratio of L-DOPA transferred to the tea water.

Keywords: Faba bean, Acetic Acid, Humic Acid, Flower Tea, L-DOPA.

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INTRODUCTION

In addition to being a food, feed and green manuring plant, faba bean (*Vicia faba L.*) hasrecently attracted attention with its medicinal potential. In Parkinson treatment, L-DOPA can be obtained from plants and used as medicine. Previous researches, it has been determined that there is a high level of L-DOPA in flowers of faba bean (*Vicia faba L.*). Cenarruzabeitiaet al. (1978), examined the L-DOPA exchange during the 6 vegetative development period of the plant and reported that the amount increased in parallel with the development and that the organ containing the highest L-DOPA was the its flowers. Topal (2012) determined that the L-DOPA content was 7.64-34.94 mg per kilogram of leaf, 10.95-117.39 mg per flower, and 1.52- 79.90 mg L-DOPA in fruit, from dry matter in different genotypes under different growing conditions. With following researches, Topal et al. (2020), determined that the L- DOPA content of flowers, pollen and honey obtained only in *Vicia faba* grown greenhouse was 4.23%, 0.98% and 0.076%, respectively. Based on this result, it was concluded that the pollen and honey produced by the bees fed with bees can be used as a source of L-DOPA. Bozoğlu and Bezmen (2021) reported that L-DOPA is transferred to the tea water when the flowers of the plant, which are shed because they cannot turn into pods and therefore considered as a loss of energy, based on our herbal tea culture are dried and brewed by brewing method.

The faba bean, which is one of the cool climate legumes, can be sown in winter or summer. In regions with a temperate climate such as Samsun, when sown in winter, the fresh harvest period coincides with May and the dry harvest at the end of June. Considering the possibility of medicinal use due to the high L-DOPA content in its flowers, flower harvests can be made between mid-April and May (Bozoğlu and Bezmen, 2021). As a second crop after harvest, it is possible to grow field crops such as corn, soybean, that is the most essential products of the region, and many vegetables such as leek, spinach and cabbage.

Seed-borne diseases can be a serious problem in organic agriculture and syntheticpesticides are not used. Alternatively, it is recommended to treat the seeds with vinegar beforesowing to prevent various factors that may occur during germination and emergence and adversely affect yield, to increase seed vigour and the effects of acetic acid in vinegar have been demonstrated by research. Some researchers reported that acetic acid treated to different types of seeds weakens the seed vigour proportionally with increasing concentrations, even though it has positive results to removal of infection (Borgen and Nielsen 2001; Kopp et al. 2008; Jin-Cheng et al. 2012; Dorna et al. 2018). It is also necessary to determine the possible effects of acetic acid treatment, which can be an alternative to prevent seed-borne diseases, on parameters such as seed germination ability, plant growth, agro-morphological characteristics and quality characteristics. Acetic acid can be a low-cost, nature-friendly and sufficient seed treatment with other treatments with proven positive effects in organic agriculture.

Humic acids are one of the active components of humus, the main content of organic substances in the soil, and they facilitate the uptake of nutrients by the plant. The researchers reported that humic acid-containing substances have significant effects on characteristics in their research with different types of plants and different treatment methods. However, according to the results of the some researchs (Öktem 2017; Çakmak 2019; Sarılar 2021), it isthought that the expected positive results from HA may be due to the nodule forming abilityof leguminous providing nitrogen and organic matter to the soil. HA treatments in different plants gave positive results in mostly dry matter and nutrient intake (Çimrin et al. 2001;Yetim and Yalçın 2008; Selçuk 2009; El-bassiony et al. 2010; Sarward et al. 2012b; Demirtaş et al. 2013).

Thanks to their nitrogen fixation abilities, legumes need nitrogen fertilization less than other plants, and sometimes even more than meeting their own needs, they also feed the soil especially with nitrogen. In such cases, the use of synthetic fertilizers is both economicallyand ecologically harmful rather than beneficial.

We are contradicting research to increase the variety of use of faba bean, to emphasize that it is an important plant due to its ease of cultivation and L-DOPA content for the region. In this research, it was aimed to investigate the effect of AA treatment to the seeds before sowing with the thought of effecting the fast germination of the seeds and providing disinfection in the environment in order to prevent seed diseases, HA treatment to the plant in the field, on the L-DOPA content in the flowers, the agro-morphological and quality characteristics of the faba bean.

MATERIALS and METHOD

The soil of the research and trial area of OMU Faculty of Agriculture, where the trial was carried out, was determined to be clayey, pH neutral (6.30), salt-free (EC=376.00 ms/cm), organic matter 1.88%, P content high (56.52 ppm). Other components were determined as Ca 33.3 mEq, Mg 12.6 mEq, K 1.15 mEq, Na 1.64 mEq per 100 g.

In the period in which the trial was carried out, the lowest temperature was experienced in January and was 0.8 ° C higher than in long years. While the highest precipitation amount was 83.5 mm in November according to long-term data, 62.8 mm of precipitation fell in this month in the trial year, and the precipitation that saturates the soil with water before sowing and coincided with the interval between the emergence and vegetative growth period was sufficient for plant growth.

Lara variety was used in the trial. The trial was conducted in a 3-replication Split Blocks(Strip Plots) trialal design in the 2019-2020 growing season. In the trial, pre-sowing seed treatments (0%: control: soaking in water, 4% AA and 40% AA) were placed on vertical stripes and plant treatments (C: control, N: 5 kg da^{-1}, HA1: 2 lt da^{-1}, HA2: 6 lt da^{-1}) on horizontal stripes with the appearance of the first flowers. HA was used in dose selection, considering the recommendation of TKI for legumes.

Ammonium sulphate with 21% Ncontent was used as N fertilizer, and a liquid commercial company product (Black Strong HUM VET) of Leonardite origin was used as HA. The content of HA used; total organic matter 13%, total humic and fulvic acids 18%, water-soluble potassium oxide 3.9% and the pH is 8-10. The seeds are kept in the mentioned AA doses for half an hour; were sown by hand, 20 in each row, in 4-row plots with a row length of 2 m and a row spacing of 60 cm. In March, when the precipitation decreased and the temperature increased, HA was treated to theplant leaves and N was treated to plant root zone to the soil when the plants began to bloom. Weed control was carried out by hoeing at 8-10 leaf stage, hand picking at flowering and mowing with a sickle during fruit set. Irrigation and any pesticide treatment was not done.

Analysis of variance was performed using the MSTAC package program and DUNCANmultiple comparison tests were used to compare the means.

RESULTS and DISCUSSIONS

According to the variance analysis results, the importance of seed and plant treatments on the characteristics of faba bean and groupings are given collectively in Table 1. The interactive effects of different seed and plant treatments were not found to be statistically significant, but it was determined that interactions were effective on some characteristics in the data in Table 2 and Table 3.

Table 1. Means of agricultural characteristics of Lara faba bean cultivar with different seed and plant treatments

	Seed Treatments		
Agricultural Characteristics	**0% AA**	**4%AA**	**40%AA**
Plant Height (cm) *	83. 83 a	80.30 ab	78.13 b
Number of Branches (per plant) **	2.83 b	3.25 a	2.75 b
Number of Pods (per plant) **	9.67b	14.00 a	8.75 b
Biological Yield (kg da^{-1}) **	996.20 b	1370.89 a	920.21b
Seed Yield (kg da^{-1}) **	531.82 b	800. 33 a	510.90b
100 Seed Weight Seed (g)	97.44	103.51	103.82
Total Dried Flower Yield (kg da^{-1}) **	28.75 b	33. 42 a	27.28 b
Crude Protein Ratio in Flower (%)	24.78	25.53	24.81
Ratio of L- Dopa in 100 mg Flower Tea (%)	7.37	8.07	7.63
Ratio of L- Dopa in 200 mg Flower Tea (%)	18.35	20.16	19.04
Coat Ratio in Seed (%)	14.03	15.08	11.91
Crude Protein Ratio in Seed (%)	22.04	20.79	21.08
Starch Ratio in Seed (%)	37.95	38.33	37.98
Amylose Ratio in Seed (%) *	10.10 a	8.50 b	9.66 ab

	Plant Treatments			
Agricultural Characteristics	**C**	**N**	**HA_1**	**HA_2 _**
Plant Height (cm)	80.57	77.28	84.52	80.66
Number of Branches (per plant) *	3.00ab	3.22a	2.67b	2.89b
Number of Pods (per plant) *	12. 67 a	10.11b	10.78b	9.67b
Biological Yield (kg da^{-1}) *	1227.91 a	1049.06 b	1140.12b	965.99 b
Seed Yield (kg da^{-1}) *	693. 22 a	568.77 bc	651.85 ab	543.57 c
100 Seed Weight Seed (g)	101.38	100.04	103.57	101.38
Total Dried Flower Yield (kg da^{-1})	31.41	27.77	32.28	27.80
Crude Protein Ratio in Flower (%)	25.52	24.66	25.36	24.63
Ratio of L- Dopa in 100 mg Flower Tea (%)	7.98	7.60	7.52	7.69
Ratio of L- Dopa in 200 mg Flower Tea (%)	19.84	18.99	18.76	19.14
Coat Ratio in Seed (%)	12.55	13.86	15.39	12.84
Crude Protein Ratio in Seed (%)	21.40	20.39	21.26	22.17
Starch Ratio in Seed (%)	37.74	37.29	38.60	38.71
Amylose Ratio in Seed (%)	9.22	9.87	9.58	8.99

*P<0.05, **P<0.01

The combined effects of different seed and plant treatments were not statistically significant. The effect of AA treatments on plant height was found to be statistically significant, while 4% AA means with the control were in the same group statistically, the height was shortened by 40% at the dose. The effect of HA treatments on plant height was notfound to be statistically significant (Table 1).

However, while the mean plant height was 84.90 cm in the control groups, it increased to 92.17 cm at the HA1 (Table 2).

Topal (2005) reported that the most appropriate value was 3.3 branches in his research, in where he tested the effects of the apex and branch removal on flower, pod retention and seed yield. A result of the research of Topal and Bozoğlu (2006) stated that 44-61% of the flowers and 47-63% of the pods are located on the main branch, but although the main branch is very important, it is not sufficient for productivity alone and they have concluded the number of branches in the faba bean plant should be more than one. In this research, 2-4 branches were observed in the plant. As mentioned in the researches above, the number of branches will be an important characteristic both agronomic characteristics and when considering flower production due to L-DOPA.. Although the number of branches in the faba bean plant did not change in a wide range, it was observed due to this characteristic, and it was determined that the 4% AA dose increased the number of branches compared to the control (Table 2).

The effect of seed treatments on the number of pods was found to be statistically significant ($P<0.01$) (Table 1); The number of pods was 11.67 per plant in the control groups and 16.00 in the highest 4% AA dose. The effect of HA2 was strong with 40% dose, which weconsidered as an overdose, and it reduced the number of pods to 7.33 (Table 2). The effect of plant treatments on the number of pods was statistically significant ($P<0.05$) and negative (Table 1).

The effects of both seed treatments ($P<0.01$) and plant treatments ($P<0.05$) on biological yield and seed yield are statistically significant (Table 1). While the mean biological yield in the control groups was 1136.46 kg per decare, it increased to 1370.1 kg in 4% AA treatment, and the highest value 1440.58 kg was obtained from 4% AA x HA1 treatment. While the mean seed yield in the control groups was at 587.19 kg per decare, it reached the highest value with at 872.78 kg at 4% AA dose. Although it was not statistically significant, it was determined that the seed treatments slightly increased the hundred-seed weight value compared to the control (Table 2).

Table 2. Means of agro-morphological characteristics of Lara faba bean cultivar with seed and plant treatments

Seed Treatments	Plant Treatments	Plant Length (cm)	Branch Number (per plant)	Pod Number (per plant)	BiologicalYield (kg da^{-1})	Seed Yield (kg da^{-1})	100 Seed Weight (g)
0% AA	C	84.90	3.00	11.67	1136.46	587.19	95.38
	N	78.33	3.00	8.67	896.75	452.01	96.98
	HA_1	92.17	2.33	9.33	1035.24	589.27	99.62
	HA_2	79.93	3.00	9.00	916.37	498.82	97.79
4% AA	C	75.60	3.33	16.00	1445.10	872.78	101.51
	N	78.30	3.67	12.67	1347.87	755.91	105.68
	HA_1	79.90	3.00	14.67	1440.58	843.98	106.01
	HA_2	87.40	3.00	12.67	1250.03	728.65	100.84
40% AA	C	81.20	2.67	10.33	1102.16	619.68	107.24
	N	75.20	3.00	9.00	902.57	498.39	97.45
	HA_1	81.50	2.67	8.33	944.54	522.29	105.07
	HA_2	74.63	2.67	7.33	731.58	403.25	105.51

Removing the flower or fruit encourages the plant to give these organs again. While the trial mean was 0.71 g in the first harvest, it decreased to 0.43 in the second harvest and increased to 0.64 by recovering again. In previous researches, the high amount of L-DOPA in the flower, the fact that L-DOPA passes into the flower tea and the honey obtained from the faba bean flowers also contains L-DOPA makes the flower yield of the faba bean plant important.

Flower harvests were made 3 times, but since there was no statistical difference betweenthe treatments made in each harvest, flower yield (kg) per decare was given. The mean total dry flower yield was found to be 1.77 g per plant in the control groups and the dry flower yield per decare was calculated as 29.49 kg. According to statistical analysis; plant treatments did not have a significant effect on flower yield, among seed treatments that were significant ($P<0.01$), 4% AA dose increased the total amount of dried flowers by a wide margin compared to control and 40% AA treatment (Table 1). The highest flower yield was obtained from 4% AA x HA1 treatment with 36.26 kg per decare (Table 3).

The protein content of faba bean flowers, which are considered as consumable plant parts, was also investigated. Topal (2012) found that the relationship between protein and L- Dopa contents was positive and significant. In this research, the crude protein ratio in the dried flowers was found to be 25.60% on mean in the control groups, and there was no significant effect of the treatments. The protein ratio in flowers is higher than the protein ratio in dry seed (Table 3).

Ratio of L-DOPA in the Flower Tea

Bozoğlu and Bezmen (2021) reported in their research that L-DOPA was transferred to tea made from faba bean flowers. The same researchers determined the amount of L-DOPA inflower tea prepared with 100 mg of sample on mean 8.04% in all genotypes; They calculatedit as 8.25% in Lara variety. In this research, the amount of L-DOPA in tea was investigated bychanging the amount of dried flowers added to the water. Based on these data, tea was prepared with 100 and 200 mg sample doses and the L-DOPA content was determined. There was no statistically significant effect of seed and plant treatments on L-DOPA content in tea (Table 1). However, L-DOPA content was calculated as 7.88% when the amount of flowers added to the tea was 100 mg, and 19.74% at 200 mg. While the AA treatment to the seed provided a slight increase in L-DOPA ratios compared to the control, the plant treatments showed a negative effect compared to the control (Table 3).

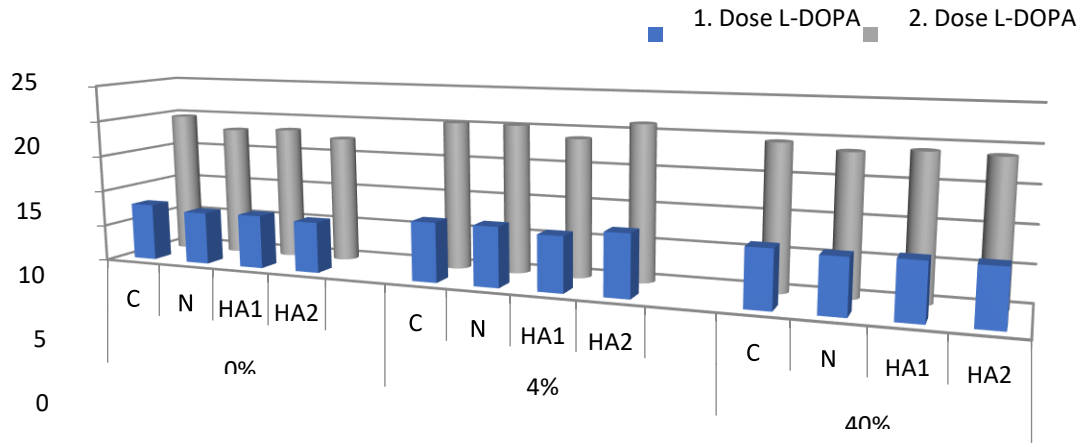

Figure 1. Change of L-DOPA content of Lara faba bean cultivar with different seed and plant treatments

The coat ratio, which is one of the important quality characteristics of the seed, varied between 10.29-16.13% in different treatments, but a statistical effect of the treatments was notdetected, but the coat ratio decreased by 40% at the AA dose from the seed treatments (Table1).

The protein content in the dry seed of the faba bean, which is one of the protein plants, was obtained from the HA2 treatment with the highest 23.7% in all treatments. However, this value did not differ statistically from the lowest value of 19.83%. The effect of different seed and plant treatments on the protein ratio (%) was not found significant (Table 1). Protein content is directly related to soil nutrient and genetic structure. The amounts of N and HA treated did not differ in protein since they were equivalent to nitrogen fixation of legumes.The effect of plant treatments on the amylose ratio (%) was found to be insignificant, the effect of seed treatments on the seed was significant ($P<0.05$).

While control and 40% AA were in the same statistical group, 4% AA treatment decreased the amylose content (Table 1). Amylose content in the trial varied between 7.676-10.22% (Table 3).

Table 3. Means of flower yield and quality of Lara faba bean cultivar with different seed and plant treatments

Seed Treatment	Plant Treatment	Total Dried Flowers(kg da^{-1})	Protein Ratio in Flower (%)	1.Dose L-DOPA (%)	2.Dose L-DOPA (%)	Coat Ratio in Seed (%)	Protein Ratio in Seed (%)	Starch Ratio in Seed (%)	Amylose Ratio in Seed (%)
0% AA	**C**	29.50	25.62	7.88	19.74	11.83	21.92	37.73	10.28
	N	25.24	24.54	7.23	18.06	13.56	20.93	37.26	10.13
	HA1	32.26	25.23	7.36	18.37	17.43	21.72	39.17	10.02
	HA2	28.09	23.86	6.99	17.42	13.39	23.73	37.80	10.04
4% AA	**C**	36.29	26.05	8.13	20.36	14.65	21.64	37.19	7.70
	N	28.41	25.31	8.15	20.33	16.18	19.86	38.66	9.44
	HA1	37.13	25.45	7.53	18.88	14.70	20.47	38.62	9.29
	HA2	31.94	25.53	8.48	21.20	14.94	21.41	39.13	7.70
40% AA	**C**	28.48	24.99	7.83	19.54	11.30	20.78	38.49	9.78
	N	29.72	24.23	7.43	18.70	11.96	20.48	36.06	10.11
	HA1	27.52	25.55	7.65	19.17	14.18	21.72	38.18	9.49
	HA2	23.42	24.65	7.61	18.94	10.32	21.47	39.34	9.29

As there are few researches on AA in the literature, they are generally on seed germination and protection against diseases (Borgen and Nielsen, 2001; Dorna et al., 2018) and there is not enough data on the effect on the morphological characteristics of the plant. However, it can beassociated with other characteristics, considering that it promotes germination at low doses in different plants and increases stature due to the prolongation of emergence and vegetation period. In this research, it is thought that AA treated to the seed prolongs the growing period by providing earliness in the germination and spreading of the seed. Bozoglu et al. (2004) in peas, El-Bassiony et al. (2010) reported that HA treatment increased the number of pods in mung beans. Waqas et al. (2014) found that HA treated to seed (0.5, 1, 1.5 and 2), leaf (0.05% and 0.1) and soil (1, 2, 3 kg ha $^{-1}$) did not affect the biological yield of mung bean in their research in Indonesia. , HA treatment determined that keeping it in soil, leaves or water does not make a difference. Different results have been obtained in various plants and at different HA doses, but what has been observed is that HA treatment affects the mineral substance uptake of the plant and the accumulation of these substances in the plant parts (Sözüdogru et al. 1996; Güneş et al. 1997; Kaya et al. 2005; Selçuk 2009). However, we believe that the source of HA used, the method and time of treatment and the nutrient content of the soil grown are important. Since the faba bean is a cool climate plant, it blooms early depending onthe weather conditions and when the temperature does not rise, flowering and vegetative development continue together. In this research, HA and N fertilizers were treated in March, when the winter cold and precipitation decreased. In addition to changing the value of the characteristics of the use of HA, the effects of the plant resistance to diseases and facilitating nutrient uptake should also be evaluated.

Conclusions

The effects of AA treatments to the seeds before planting and N fertilizer and HA treatments to the plants at the beginning of flowering on the agro-morphologicalcharacteristics of the faba bean and the amount of L-DOPA in the tea obtained from the flower were investigated. According to the findings, the effect of acetic acid treated to the seed before planting on plant height, number of branches, number of pods, biological yield, seedseed yield and flower yield was very important, and 4% AA dose showed positive effects compared to the control. Among plant treatments, HA doses and N fertilization had statistically significant effects on the number of branches, pods, biological yield and seed yield; The effects on flowers were not found to be significant.

Due to the high L-DOPA content of faba bean flowers, when tea is made like medicinal plants, L-DOPA passes into the water, and in this research, it was tested whether the amountof flowers to be added to the tea would have an effect. According to the results obtained from the tea prepared using 100 and 200 mg flowers, when the amount of dried flowers was doubled, the L-DOPA ratio in tea also increased. Although not statistically significant, it was determined that the doses of acetic acid treated to the seed increased the amount of L-DOPAin flower compared to the control.

Seeds treated with more ecologically toxic fungicides pose a danger to living things and traditional fungicides are not used in organic farming. This is an important constraint on organic production. Acetic acid treatment is easy and cost-effective, and the remaining acetic acid-treated seeds can be washed with water and continued to be used as animal feed or human food.

The results clearly show that the 5 kg da^{-1} nitrogen fertilizer and humic acid treatments we apply are equivalent to the faba bean's nitrogen fixation level to provide its own needs.The faba bean plant's superior nodule forming ability and strong growth characteristicsreduce the need for fertilizer. The fact that there is no difference in the characteristicsinvestigated in this research does not mean that HA treatment does not work at all. Since the faba bean is a cool climate plant, it blooms early depending on the weather conditions and when the temperature does not rise, flowering and vegetative development continue together. In the research, HA and N fertilizers were treated in March, when the winter cold and precipitation decreased. In our research, it was expected that the precipitation conditions would allow the treatment, especially since the HA treatment was made by spraying the plant.This coincided with the generative period of our plants. The results of HA treatment in previous periods should also be investigated. Considering the effects of HA use not only to change the value of the characteristics but also on the resistance of plant to diseases and facilitating nutrient uptake, HA1 treatment with 4% AA can be recommended. However, it would be appropriate to make a decision by evaluating it from an economic point of view with the control process.

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