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Article

# An Exogenous Auxin Indole-3-Butyric Acid (IBA) Induced Bean (*Phaseolus vulgaris*) Cultivars Reactions

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**Abstract:** Bean is a remarkable crop widely used as food worldwide, underscoring the significance of scientific research conducted on this plant. Developing a strong root system in the early stages of growth, which can spread wider and deeper, along with the consequent development of the shoot system crucial for photosynthesis, enhances the plant's productivity and resistance to diseases and pests. In the current study, the reactions of cultivars Önceler-98 and Topçu beans to different concentrations of IBA (indole-3-butyric acid) during the seedling stage were investigated. Three different concentrations, namely 0 (Control), 50, 100, and 150  $\mu\text{M}$  IBA, were applied, and their effects on root length (RL), root fresh weight (RFW), root dry weight (RDW), number of nodules on roots (RNN), shoot length (SL), shoot fresh weight (SFW), and shoot dry weight (SDW) parameters were measured. The pattern of the experiment was a randomized complete block factorial design with four replications and, statistically significant ( $p \leq 0.05$ ) and highly significant ( $p \leq 0.01$ ) differences were observed among the means of root and shoot traits. As a result, it was found that the cultivar Önceler-98 generally exhibited better responses to IBA solutions, and the optimal concentration range for both varieties was between 50-100  $\mu\text{M}$ , decreasing at 150  $\mu\text{M}$  and higher concentrations, yet still yielding superior means compared to the control.

**Keywords:** exogenous auxin; IBA; indole-3 butyric acid; root nodules; root/shoot; bean

## Introduction

Bean (*Phaseolus vulgaris* L.), belonging to the Fabaceae family is an annual plant of the Phaseolus genus (Xiong et al., 2023). They are one of the most widely cultivated legumes worldwide due to their importance as a food and income source for producers. Bean seeds are high in protein, low in fat, and low in calories. They are also rich in mineral elements such as iron, calcium, magnesium, manganese, and potassium (Rivera et al., 2018). Due to their rich nutritional content, affordability as a protein source, and long shelf life, beans are particularly popular in developing countries (Castro-Guerrero et al., 2016). However, their tolerance to various environmental conditions during growth directly affects yield.

Auxins are plant hormones that influence the growth, development, and stress tolerance of plants (Siposova et al., 2021). Indole-3-acetic acid (IAA), one of the most important hormones promoting growth, activates the ascorbate-glutathione cycle (Bashri and Prasad, 2016) and enhances the plant's defense against oxidative stress (Khan et al., 2019). Additionally, it is effective in root elongation and growth (Çakmakçı et al., 2020). Another hormone is indole-3-butyric acid (IBA). IBA converts to IAA, increasing nitric oxide production (Piacentini et al., 2020), and the increased nitric

oxide effectively reduces superoxide formation by facilitating peroxynitrite formation (Demecsova et al., 2020). There are numerous studies on the effects of IAA on plant growth and development. However, despite its theoretical functionality, IAA is unsuitable for practical use due to its low stability and rapid degradation (Nordstrom et al., 1991). While an IAA solution degrades after 2 days, the stability of an IBA solution can exceed 28 days (Kaczmarek et al., 2020a). However, the stability of the IBA solution is influenced by environmental factors such as heat and light, and it can even be used as a more stable fertilizer in combination with alkylated choline cations (Kaczmarek et al., 2020b).

Previous studies have observed that IBA, which has been intensively used, especially in rooting processes, converts to IAA through the HOMEBOX PROTEIN 24 (HB24) transcription factor and promotes the elongation of root hairs in plants (Zhao et al., 2021). The hair structures formed by protrusions of root epidermal cells increase the contact area between the root and the soil, thus enhancing water and nutrient uptake (Grierson et al., 2014). They also provide a rapid and effective response to environmental stimuli (Liu et al., 2018). Therefore, recent studies suggest improving the root system to increase yield, resilience, and tolerance to nutrient deficiencies (Ghanem et al., 2011; Colombi et al., 2018).

The known enhancement of root formation by IBA hormone, through the stimulation of IAA, GA3, and total phenolics (El-Banna et al., 2023), suggests that the exogenous application of natural and/or synthetic auxins could be used to promote adventitious root production (Wise et al., 2020; Chen et al., 2021). Based on their effectiveness in inducing adventitious roots, auxins, including IAA and IBA, have been reported by many researchers to be commercially used to stimulate root formation in various plant species, including *Cotinus coggygria* (Ilczuk and Jacygrad, 2016), *Zanthoxylum armatum* (Singh and Rawat, 2017), *Vitis vinifera* (Daskalakis et al., 2018), and *Magnolia wufengensis* (Wang et al., 2022). In this context, IBA has been noted to exhibit higher efficacy in root induction due to its higher performance in root induction and stability under light exposure (Lakehal and Bellini, 2019). However, it has also been determined that root formation and elongation vary depending on plant species and varieties (Ilczuk and Jacygrad, 2016). Therefore, this study aims to investigate the potential effects of exogenously applied IBA on root structure, nodule formation, and subsequently on seedling characteristics in bean plants.

## Materials and Methods

This trial was established on March 24, 2022, and terminated on April 28, 2022, at the Mersin University, Silifke School of Applied Technology and Business greenhouse. The experiment was set up in a randomized complete block design with a factorial arrangement with 4 replications. The first factor consisted of two bean varieties, 'Önceler-98' and 'Topçu', while the second consisted of 0, 50, 100, and 150 mM IBA solutions. To evaluate adaptability to field conditions, 5-liter pots were filled with field soil, and three bean seeds were sown in each pot.

After emergence, thinning was performed to leave only one plant per pot. Two weeks after emergence, the prepared solutions were applied to the respective pots as 200 ml of irrigation water. This process was repeated four times at three-day intervals. Observations were taken on the 8th day after the last application, as flowering had begun. During the period when solution application was not performed, the plant's water requirement was met with tap water. Concentrations used on cultivar Önceler-98 such as 0, 50, 100, and 150  $\mu$ M IBA were represented with Ö1, Ö2, Ö3, and Ö4 respectively, while T1, T2, T3, and T4 abbreviations indicated the concentration values for cultivar Topçu, respectively.

At the beginning of flowering, traits related to root and shoot systems such as Shoot Length (SL), Shoot Fresh Weight (SFW), Shoot Dry Weight (SDW), Root Length (RL), Root Fresh Weight (RFW), Root Dry Weight (RDW), and Root Number of Nodules (RNN) were measured. Root and shoot lengths were directly measured with a meter tool. Root and shoot fresh weights were measured using a scale with a sensitivity of 0.05 mg. Root and shoot dry weights were calculated by weighing them with the same sensitive scale after being kept in an oven at 60 degrees Celsius for 24 hours.

### Statistical Analysis

One-way ANOVA variance analysis was conducted using SAS 9.4 (SAS, 2023) version software. Additionally, ANOVA analyses of Bean cultivars X treatment interactions and Principal Component Analyses (PCA) were performed using JMP 17.0 (JMP, 2023) software to obtain relationships between bean cultivars's root and shoot traits. The significance differences between means were investigated ( $p < 0.05$ ). The least significant difference (LSD) was used to compare the means. Asterisks indicate the significance level between means. Accordingly, while  $** (p \leq 0.01)$  = highly significant and  $* (p \leq 0.05)$  = Significant, the non-significant differences are represented by "ns" ( $p > 0.05$ ).

### Results

In this study, different doses of IBA chemicals, such as 0  $\mu\text{M}$ , 50  $\mu\text{M}$ , 100  $\mu\text{M}$ , and 150  $\mu\text{M}$ , were applied to the "Topçu and Önceler-98" bean varieties belonging to the *Phaseolus vulgaris* L. species, and the effects of IBA on the root and shoot system characteristics of bean varieties were investigated. Additionally, the differences between Root/Shoot system traits and Genotypes X IBA applications statistically were established in Table 1. Accordingly, As indicated in Table 1, there is a significant difference in the mean root length among the varieties ( $p \leq 0.05$ ), however, the difference between the mean GXA interaction with IBA dosage applications is highly significant ( $p \leq 0.01$ ). Highly significant differences in means of Root Fresh Weight (RFW) were obtained between Genotypes, Applications, and GXA parameters. In the Root Dry weight (RDW) trait, highly significant ( $p \leq 0.01$ ) differences were between genotypes and Application doses, but a significant ( $p \leq 0.05$ ), difference was between GXA (Table 1).

**Table 1.** Two-way ANOVA of root/shoot system traits.

Mean Square								
SOV	df	RL	RFW	RDW	SL	SFW	SDW	RNN
Genotypes (G)	1	17.0017*	3.4656**	0.0620**	15.0417*	5.6551**	0.0368*	287.0417**
Application (A)	3	20.3517**	2.3872**	0.0235**	57.0139**	4.4229**	0.0933**	85.8194**
G x A	3	18.8850**	1.7987**	0.0017*	22.1806*	1.3505*	0.0805**	241.3750**
Error	16	0.8683	0.0265	0.0005	2.0521	0.2123	0.0057	2.0000

\*\*= highly significant, \*= significant, ns= not significant, df= degrees of freedom, SOV=Source of Variance, RNN= Root nodules number.

In the shoot system, The difference in mean shoot length among genotypes and G X A interactions is statistically significant ( $p \leq 0.05$ ), however, the difference between the doses of IBA application is highly significant ( $p \leq 0.01$ ), while highly significant ( $p \leq 0.01$ ) differences were between genotypes and applications, but significant difference between GXA ( $p \leq 0.05$ ). Similar results were obtained in third shoot trait SDW, accordingly, while the highly significant ( $p \leq 0.01$ ) differences were between application doses and GXA interaction, a significant difference ( $p \leq 0.05$ ) was between Bean cultivars genotypes means. In Root nodule numbers (RNN), highly significant ( $p \leq 0.01$ ) differences were obtained between Genotypes, Applications, and GXA interactions (Table 1).

traits

Findings associated with root system traits in the study are given in Table 2. Accordingly, the highest RL value (53.50 cm) in the cultivar Önceler-98 was observed in the Ö4 application, while the closest value of 53.07 cm was observed in Ö1 (control). In the RFW trait, the highest value (3.48 g) was observed in the Ö3 application, and the lowest value (1.03 g) was observed in the control group. Again, in the cultivar Önceler-98, the lowest RDW value (0.25 g) is observed in the Ö1 application, while the highest RDW value (0.42 g) was observed in the Ö3 application. The highest Root nodule number (RNN) in cultivar Önceler was seen in Ö1 concentration and the RNN in Ö3 concentration.

In Root length (RL), Root fresh weight (RFW), Root dry weight (RDW) and Root Nodule number (RNN) both Bean cultivars exhibited the best reaction (53.50 cm, 2.80 g, 0.37 g, 23.50) at 150, 50, 100, and 0 (control)  $\mu$ M IBA concentrations, respectively, while the lowest values were recorded in RL (49.17 cm) at 50  $\mu$ M IBA, in RFW (1.40g) at 0  $\mu$ M IBA, in RDW (0.22) at 0  $\mu$ M IBA and the 15.17 RNN value at 50  $\mu$ M IBA concentration.

**Table 2.** Different IBA concentrations' effects on Root system traits means.

		<b>Root length (cm)</b>	<b>Root fresh weight (g)</b>	<b>Root dry weight (g)</b>	<b>Root Nodule number</b>
Cultivars	Önceler-98	52.52 a	2.70 a	0.35 a	21.58 a
	Topçu	50.83 b	1.94 b	0.25 b	14.67 b
	<b>LSD</b>	19.58 **	130.98**	126.14**	143.52**
Concentrations	0 $\mu$ M IBA	51.62 b $\pm$ 0.96	1.40 c $\pm$ 0.41	0.22 c $\pm$ 0.04	23.50 a $\pm$ 8.26
	50 $\mu$ M IBA	49.17 c $\pm$ 1.92	2.80 a $\pm$ 0.38	0.30 b $\pm$ 0.07	15.17 c $\pm$ 0.98
	100 $\mu$ M IBA	52.42 ab $\pm$ 1.38	2.63 ab $\pm$ 0.96	0.37 a $\pm$ 0.05	15.83 bc $\pm$ 4.02
	150 $\mu$ M IBA	53.50 a $\pm$ 0.29	2.45 b $\pm$ 0.79	0.32 b $\pm$ 0.07	18.00 b $\pm$ 11.10
	<b>LSD</b>	23.44**	90.22**	47.80**	42.91**
Önceler-98	0 $\mu$ M IBA (Ö1)	<b>53.07 a</b> $\pm$ 0.60	1.03d $\pm$ 0.07	0.25c $\pm$ 0.02	<b>31.00a</b> $\pm$ 1.00
	50 $\mu$ M IBA (Ö2)	52.17 ab $\pm$ 0.58	<b>3.13a</b> $\pm$ 0.14	0.36ab $\pm$ 0.03	15.00c $\pm$ 1.00
	100 $\mu$ M IBA (Ö3)	51.33 b $\pm$ 1.15	<b>3.48a</b> $\pm$ 0.36	<b>0.42a</b> $\pm$ 0.02	12.33c $\pm$ 1.15
	150 $\mu$ M IBA (Ö4)	<b>53.50 a</b> $\pm$ 0.87	<b>3.16a</b> $\pm$ 0.13	0.38ab $\pm$ 0.03	<b>28.00a</b> $\pm$ 2.65
Topçu	0 $\mu$ M IBA (T1)	50.17 c $\pm$ 1.04	1.77c $\pm$ 0.09	<b>0.19c</b> $\pm$ 0.02	16.00bc $\pm$ 1.00
	50 $\mu$ M IBA (T2)	46.17 d $\pm$ 0.76	2.47b $\pm$ 0.10	0.24c $\pm$ 0.03	15.33bc $\pm$ 1.15
	100 $\mu$ M IBA (T3)	<b>53.50 a</b> $\pm$ 0.50	1.77c $\pm$ 0.03	0.33b $\pm$ 0.02	19.33b $\pm$ 1.53
	150 $\mu$ M IBA (T4)	<b>53.50 a</b> $\pm$ 1.50	1.74c $\pm$ 0.15	0.25c $\pm$ 0.02	<b>8.00d</b> $\pm$ 1.00
	<b>LSD</b>	21.75**	67.98**	3.36*	120.69**
	<b>Ortalama</b>	51.68	2.32	0.30	18.13
	<b>CV(%)</b>	1.8	7.01	7.35	7.80

Cultivar Topçu has the highest RL value (53.50 cm) in the T3 application and is significantly different from T1 (50.17 cm) and T2 (46.17 cm), but not significantly different from T4 (53.50 cm). T1 and T2 have significantly shorter root lengths compared to T3 and T4. In the RFW trait, the T2 application has the highest RFW (2.47 g), significantly different from T1 (1.77 g) and T4 (1.74 g), but not significantly different from T3 (1.77 g). T1, T3, and T4 have similar RFW values. In RDW, the best value was recorded in T3 (0.33 g), while the lowest value (0.19 g) was observed in the T1 application.

T1 (0.19 g) and T4 (0.25 g) have significantly lower RDW compared to T2 (0.24 g) and T3. In cultivar Topçu, RNN values varied between 8-16 number. The highest RNN value was recorded in T3 (19.33) while the lowest was in application T4 (8).

The best values (53.50 and 50.07 cm) of RL were observed in cultivar Topçu at 100 and 150  $\mu$ M IBA and in cultivar Önceler-98 at 150 and 0  $\mu$ M IBA, respectively, while the lowest RL value (46.17 cm) was recorded at 50  $\mu$ M IBA concentration. In RFW, 3.48, 3.16 and 3.13 g values were the highest RFW values in cultivar Önceler-98 at 100, 150, and 50  $\mu$ M IBA, respectively. The best RDW value (0.42 g) was recorded at 100  $\mu$ M IBA in cultivar Önceler-98, and followed by 0.36 g at 50  $\mu$ M IBA concentration. Among the two cultivars, the lowest RNN value (8) was recorded in cultivar Topçu at 150  $\mu$ M IBA, while the best RNN value was obtained in cultivar Önceler-98 at 0  $\mu$ M IBA, followed by 28 RNN value at 150  $\mu$ M IBA of cultivar Önceler-98 (Table 2)



**Figure 1.** Effects of different IBA doses on cultivars Önceler-98 and Topçu on root and shoot system.

When the effect of concentration differences on traits is examined, the highest value in the SL feature is obtained in the control group (25.17 cm). The closest value to it is obtained from the application of 100  $\mu$ M IBA (23.25 cm), while the lowest SL value is observed in the application of 150  $\mu$ M IBA (17.92 cm) (Table 3). Regarding the SFW feature, again the highest value is obtained from the control group (7.67 g), while the second closest value is obtained from 100  $\mu$ M IBA (6.85 g) (Table 3). However, the lowest value is recorded again in applying 150  $\mu$ M IBA (5.63 g) (Table 3). When SDW values are examined, it is observed that 0, 50, and 100  $\mu$ M IBA concentrations cause statistically insignificant differences with 1.28 g, 1.31 g, and 1.34 g, respectively, but 150  $\mu$ M IBA causes a significant decrease (1.07 g) (Table 3).

**Table 3.** Different IBA concentrations effect on Root system traits.

3		Shoot length (cm)	Shoot fresh weight (g)	Shoot fresh weight (g)
Cultivars	Önceler-98	22.75 a	7.11 a	1.29 a
	Topçu	21.17 b	6.14 b	1.21 b
LSD		7.33*	26.64**	6.46*
Concentrations	0 $\mu$ M IBA	25.17 a $\pm$ 3.67	7.67 a $\pm$ 0.39	1.28 a $\pm$ 0.09
	50 $\mu$ M IBA	21.50 b $\pm$ 1.82	6.35 bc $\pm$ 0.97	1.31 a $\pm$ 0.14
	100 $\mu$ M IBA	23.25 ab $\pm$ 0.99	6.85 b $\pm$ 0.64	1.34 a $\pm$ 0.10

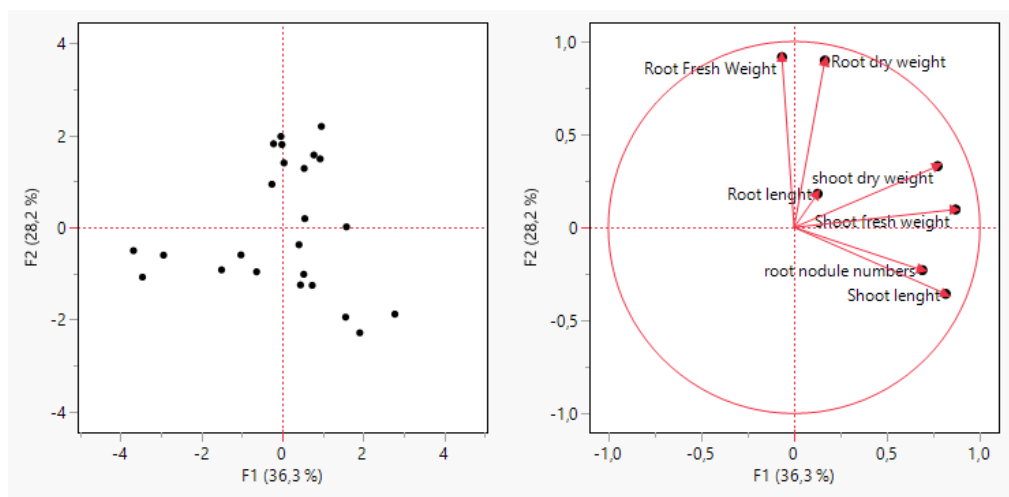
	150 $\mu$ M IBA	17.92 c $\pm$ 2.27	5.63 c $\pm$ 1.06	1.07 b $\pm$ 0.20
	<b>LSD</b>	27.78**	20.83**	16.37**
Önceler-98	0 $\mu$ M IBA (Ö1)	<b>28.17a</b> $\pm$ 1.76	7.55a $\pm$ 0.46	1.22ab $\pm$ 0.08
	50 $\mu$ M IBA (Ö2)	20.33bc $\pm$ 1.26	7.17a $\pm$ 0.45	<b>1.42a <math>\pm</math>0.01</b>
	100 $\mu$ M IBA (Ö3)	22.83b $\pm$ 1.26	7.18a $\pm$ 0.60	1.28ab $\pm$ 0.06
	150 $\mu$ M IBA (Ö4)	19.67bc $\pm$ 1.15	6.53ab $\pm$ 0.52	1.22ab $\pm$ 0.11
Topçu	0 $\mu$ M IBA (T1)	22.17b $\pm$ 1.89	<b>7.79a</b> $\pm$ 0.35	1.33ab $\pm$ 0.06
	50 $\mu$ M IBA (T2)	22.67b $\pm$ 1.61	5.53bc $\pm$ 0.38	1.19b $\pm$ 0.07
	100 $\mu$ M IBA (T3)	23.67b $\pm$ 0.58	6.52ab $\pm$ 0.57	<b>1.41a <math>\pm</math>0.09</b>
	150 $\mu$ M IBA (T4)	<b>16.17c</b> $\pm$ 1.53	<b>4.72c</b> $\pm$ 0.26	0.91c $\pm$ 0.09
	<b>LSD</b>	10.81**	6.36**	14.11**
	<b>Ortalama</b>	21.96	6.63	1.25
	<b>CV(%)</b>	6.52	6.95	6.05

When genotype  $\times$  IBA application interactions were evaluated; it was observed that SL values ranged from 16.17 cm to 28.17 cm, with the highest value obtained from the Ö1 application and the lowest value obtained from the T4 application (Table 3). When examining SFW values, it was found that the data ranged from 4.72 g to 7.79 g, with the highest value obtained from T1. However, the Ö1, Ö3, and Ö2 applications were statistically indistinguishable from the T1 application (Table 3). On the other hand, the T4 application resulted in the lowest value in the SFW feature. When SDW data were examined, it was determined that the values ranged from 0.91 g (T4) to 1.42 g (Ö2) (Table 3). The Ö2 application resulted in the highest SDW, with the closest value being 1.41 g from the T3 application. Except for the T2 application, the other applications were observed to be in the intermediate group and statistically indistinguishable from each other (Table 3).

**Table 4.** Correlation between root and shoot traits.

	RL	RFW	RDW	SL	SFW	SDW	RNC
RL	****	-0,1281	0,0768	-0,0077	-0,1345	0,3419	0,2515
RFW	-0,1281	****	0,5874	0,4718	-0,3288	-0,1154	0,5754
RDW	0,0768	0,5874	****	0,7064	0,0372	0,1316	0,4143
SL	-0,0077	0,4718	0,7064	****	0,2471	0,2793	0,236
SFW	-0,1345	-0,3288	0,0372	0,2471	****	0,7369	-0,2322
SDW	0,3419	-0,1154	0,1316	0,2793	0,7369	****	0,0345
RNC	0,2515	0,5754	0,4143	0,236	-0,2322	0,0345	****

The Biplot and Principal Component Analysis (PCA) shown in Figure 2A and 2B represent 64.5% of the phenotypic variation. Figure 2A demonstrates that the variation between the shoot and root system parameters is high. Figure 2B shows a very strong positive relationship between shoot length and the number of root nodules, and a similar relationship exists between root dry weight and root fresh weight, as well as between shoot fresh weight and shoot dry weight. Additionally, it indicates that root length has a positive relationship with both root and shoot fresh and dry weight. There is a positive relationship between shoot length, number of root nodules, shoot dry and fresh weight, and root length. Root dry and fresh weight show a negative correlation or no correlation at all with all other parameters except for root length and shoot dry weight. The conclusion from this is that there is a very significant relationship between root length and the development of the shoot system.



**Figure 2.** Principal Component Analysis (PCA) of root/shoot systems traits.

## Discussion

IBA, a widely recognized plant growth regulator, has found extensive application in agriculture for managing plant growth and development. It's particularly used for stimulating the growth of adventitious roots in plant cuttings (Pacurar et al., 2014). Shi et al. (2014) reported that similar to IBA, the application of another auxin called IAA regulates root architecture and enhances drought resistance in plants.

The IBA concentration initially led to a decrease in the RL feature compared to the control in both bean varieties, but as the concentration increased, it increased root length. Based on this, we can say that the IBA chemical increases and develops root length. Regarding the varieties, in the "Önceler-98" variety, the RL value decreased compared to the control, but it increased again at the 150  $\mu\text{M}$  IBA level, showing a performance above the control group. In the Topçu variety, a decrease in the RL feature was observed at the 50  $\mu\text{M}$  IBA level compared to the control (0  $\mu\text{M}$  IBA), while an increase was observed at the 100 and 150 concentrations, giving statistically insignificant concentration difference values for both (Table 2). Li et al. (2018) reported that 5  $\mu\text{M}$  CdCl<sub>2</sub> application on plant significantly inhibited the plant root system including adventitious roots, but adding 10  $\mu\text{M}$  IBA to CdCl<sub>2</sub> (cd) reduced the negative effects of Cadmium (Cd) on the root system. Additionally, IBA increased the tolerance level against drought and cd effects in beans at seedling on roots. Similar to our results, Jemaa et al. (2011) reported that salt stress reduced root growth in *Arabidopsis* plants by 38-68%, but the presence of auxins such as IBA and IAA lowered this rate to as low as 20%. Another similar approach in *Arabidopsis* plants: Ludwig-Müller et al. (2005) reported that the development of the adventitious root system is dependent on the concentration of auxins such as IBA and IAA. In their research on rice plants, Šípošová, Kristína et al. (2021) revealed findings similar to ours, indicating that increasing IBA concentration initially increased root length, as well as fresh and dry root weight in rice. However, they also found that beyond certain doses, IBA had a negative effect on the root system. Khadr et al. (2020) stated that with increasing the IBA concentration from 0 to 100, root length increased but significantly decreased between 100-150  $\mu\text{M}$  IBA concentration.

While the concentration at which all varieties showed the best reaction in terms of the RFW feature was 50  $\mu\text{M}$  IBA, the RFW value decreased inversely with the increase in concentration. This could be explained by the plant focusing its efforts to improve its root system, or it may have exhibited a toxic effect similar to after certain doses in the bean plant. Compared to the control, the "Önceler-98" variety recorded its highest RFW value at the 100  $\mu\text{M}$  IBA concentration, while recording its lowest value in the control group. In the Topçu variety, the highest RFW value was obtained at 50  $\mu\text{M}$  IBA, and increasing concentration reduced the RFW value. Li et al. (2018) stated that the increase in cadmium dosage resulted in a decrease in fresh root weight in beans, but adding the IBA chemical to the cadmium solution caused the root fresh weight (RFW) to increase. Khadr et



al. (2020) conducted a similar study on carrot taproot to determine the effect of four doses of exogenous indol-3 butyric acid (0, 50, 100, and 150  $\mu\text{M}$  IBA) and reported an increased number and area of xylem vessels but decreasing gene expression lignin genes. At a high concentration ( $10^{-7}$  M) of indole-3-butyric acid (IBA), maize root length was decreased while root diameter increased. Conversely, at lower concentrations ranging from  $10^{-9}$  M to  $10^{-12}$  M IBA, both root growth and root diameter were stimulated (Šípošová et al., 2019). Khadr et al. (2020) indicated that by increasing the IBA concentration from 0 to 100, Root fresh Weight (RFW) increased but significantly decreased between 100-150  $\mu\text{M}$  IBA concentration.

Both varieties recorded their highest RDW values at a concentration of 100  $\mu\text{M}$  IBA. Overall, an increase in IBA concentration led to an increase in RDW values. In this study, the "Önceler-98" variety exhibited higher RDW values compared to the Topçu variety, showing an increase compared to the control, with the highest increase recorded at a concentration of 100  $\mu\text{M}$  IBA. Despite producing lower RDW averages, the Topçu variety achieved its best RDW average at the same concentration of 100  $\mu\text{M}$  IBA as the "Önceler-98" variety. Both varieties showed a positive reaction to increasing IBA concentration, leading to increases in shoot dry weight. Khadr et al. (2020) concluded that increasing IBA concentration in increments of 0, 50, 100,  $\mu\text{M}$  increased root length, and consequently, it is inferred that it would also increase fresh root weight (FRW) and dry root weight (RDW). Orhan et al. (2006) reported root dry weight (RDW) varied between  $2.6 \pm 0.28$ ab (g) at 50  $\text{mg L}^{-1}$  IBA to  $2.34 \pm 0.08$ a at 100  $\text{mg L}^{-1}$  IBA in Pistacia Vera at seedling stage

The highest number of nodules in the roots was recorded in the "Önceler-98" variety. In this study, it was observed that IBA concentration reduced the number of nodules in bean roots, but high concentrations led to an increase in nodules compared to the control. This situation warrants a more detailed field trial, involving at least two years of repetition, with four replications, and the use of numerous varieties and parent seeds for testing. Similarly, in the "Önceler-98" variety, concentration increase led to an increase in the number of nodules, which had initially decreased compared to the control. In the Topçu variety, while the number of nodules initially decreased compared to the control, with increasing concentration, significant decreases in the number of nodules were observed at 150  $\mu\text{M}$  IBA. This situation in the Topçu variety suggests that the increase or decrease in the number of nodules in bean varieties may be related to their genetic structure, ancestors, and, if observed under uncontrolled environmental conditions, environmental interactions.

In Figure 3, the Bean cultivar "Önceler-98" generally exhibited higher performance in shoot system traits compared to the cultivar Topçu. The IBA concentration initially increased Shoot length (SL), Shoot Fresh Weight (SFW), and Shoot dry Weight (SDW) traits, but then, with the increase in concentration, it led to a decrease. The highest value in the SL feature was observed in the control group for both bean varieties. Similarly, the increase in IBA concentration increased SL up to 100  $\mu\text{M}$  IBA level, but beyond this (150  $\mu\text{M}$  IBA) concentration, the values of this feature decreased. Although the "Önceler-98" bean variety generally exhibits better performance, it shows lower values than the Topçu variety in some traits. As shown in Table 3, while the SL feature shows superior characteristics in the "Önceler-98" variety in the control group (0  $\mu\text{M}$  IBA), the Topçu variety has shown better response at 50 and 100  $\mu\text{M}$  IBA concentrations. Differently from our findings, Khadr et al. (2020) reported that increasing concentrations of IBA (0, 50, 100, 150  $\mu\text{M}$ ) increased both shoot length and total plant length in the Carrot plant. This differences may arise from the genetic makeup of the carrot vegetable, the intensity and duration of the light, the potting soil, the amount of irrigation water, the irrigation interval, and the presence of possible different additives in the IBA chemical. Zalt (2024) utilized three plant growth regulators, Naphthaleneacetic acid (NAA), Indole-3-butyric acid (IBA), and Indole-3-acetic acid (IAA), in invitro conditions for blueberry plants. They found that generally, increasing IBA concentration enhanced root length, root number, and shoot length in blueberry varieties.

A similar situation was observed in the SFW feature as well. With the increase in IBA concentration, an increase in SFW values was observed, but a decrease was recorded at 150  $\mu\text{M}$  IBA. In general, the "Önceler-98" variety has shown better results in this feature. The increase in IBA concentration has had a more negative effect on the Topçu variety (4.72 g) compared to the "Önceler-

98 " variety (6.53 g). The increase in shoot fresh weight is directly related to photosynthesis. As the concentration of IBA increases, the increased green parts of the bean plant will expand the area that will increase the speed and amount of photosynthesis, so it is seen that the use of IBA chemical at a level that will not have a toxic effect on the plant will increase the yield. While the RFW decreased with increasing the IBA from 100 to 150  $\mu\text{M}$  IBA, on the contrary, shoot fresh weight was significantly increased. Xu et al. (2012) indicated that using both plant grow regulators (IBA and IBAA) improves plant growth better than using one of them at early growth. Wiesman et al. (1988), Li et al. (2018), Ludwig-Müller (2000), Xi et al. (2009) and Sosnowski et al. (2019) used indole-3 butyric acid in bean plants and obtained similar findings related to root and shoot system.

Both varieties exhibited similar reactions in the SDW feature as the IBA concentration increased, similar to the other two features. The "Önceler-98 " variety showed a better positive reaction to increasing IBA concentration compared to the other bean variety and recorded less value decrease compared to the Topçu variety even in overdose IBA concentration. The "Önceler-98 " variety recorded the highest SDW average at 50  $\mu\text{M}$  IBA, while the Topçu variety recorded it at the 100  $\mu\text{M}$  IBA concentration level. Under salinity stress, the Application of IBA on Faba Bean (*Visia faba* L.) reduced the accumulation of  $\text{Na}^+$  and increased the growth, shoot, and root system parameters (Abdel Latef et al. 2021). In plants, there is generally a positive and strong relationship between fresh shoots and root weights and their dry weights for the same plant. Therefore, in many studies, dry root weight is not calculated, but its calculation is strongly recommended. However, observations of root growth have revealed that root length does not always correlate with an increase in fresh or dry root weight.

## Conclusion

Effective root and shoot development, leading to increased photosynthesis, is crucial in bean plants, as it is in other plants. In this study, the optimal dosage(s) of IBA (indole-3-butyric acid) where both varieties generally exhibited the best response ranged from 40-100  $\mu\text{M}$ . It was concluded that while IBA concentration is crucial for rapid root and shoot systems during the seedling stage, beyond certain doses, it exhibits side effects. The Önceler-98 -98 bean variety showed a better response to increasing IBA concentration, suggesting it could be recommended for planting to farmers under field conditions. Additionally, there was a linear and very strong correlation between shoot fresh weight and shoot dry weight, as well as between root fresh weight and root dry weight. However, such a strong relationship was not observed between RL and RFW/RDW, as well as between SL and SFW/SDW.

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