# Effects of Polyamines and Iron on Iron Absorption, Yield, and Enzymatic Activity in Soybean

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#### Abstract

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Soybean, a globally vital legume prized for its high protein and oil content, is essential for meeting human nutritional demands. This study evaluated the influence of polyamines (PAs), applied both individually and in combination with iron, on soybean yield and yield components over the 2021 and 2022 growing seasons. The field experiment was conducted at the research farm of Azad University using a randomized complete block design with three replications. The experimental treatments consisted of foliar applications of 2 mM spermine, spermidine, and putrescine, each with and without 2 mM iron, as well as combinations of 1 mM of polyamine with 3 mM iron were included to assess synergistic effects. Additionally, the iron sulfate and a control treatment were included. The results indicated that the treatments significantly affected the growth and yield components (such as plant height, pod number, seed weight, harvest index, seed protein and oil content) and the nutrient contents including Zn, Fe, B, N and Mg. The use of spermidine with iron treatment increased the seed weight by 71% compared to the control. As well as, the application of polyamines combined with Fe resulted in a significant increase in the activities of superoxide dismutase (44%), peroxidase (63%), and catalase (43%) enzymes compared to control. Notably, among the polyamines, spermidine combined with iron had the most significant effects on the studied traits and enzyme activity. These findings suggest that the application of polyamines with iron can significantly improve the soybean yield and yield components.

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#### **INTRODUCTION**

Despite an annual production of 271 thousand tons of oilseeds in Iran, the majority of edible oil in the country is imported. Over 90% of domestic oil consumption relies on the foreign sources, highlighting the urgent need for strategic planning to achieve the self-sufficiency in edible oil production. Soybean (*Glycine max* L.) is a critical crop for addressing the human nutritional needs

**Keywords:** Enzyme activity; Nutrients; Putrescine; Spermidine; Spermine.

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30	because its seeds contain approximately 40% protein content and 20% oil content (Sheikhzadeh et
31	al., 2022). Globally, soybean is one of six primary oilseed crops, along with palm oil, canola,
32	sunflower, cottonseed, and peanuts, which collectively account for 84% of the edible oil
33	production of the world (Ghorbani et al., 2019).
34	Iron is an essential micronutrient for plant growth. Iron deficiency impairs chlorophyll
35	synthesis, leading to chlorosis, which initially appears between the leaf veins and then gradually
36	causes the entire leaf surface to turn yellow, except for the veins. These symptoms typically appear
37	in the young leaves and upper stems, eventually affecting the entire plant (Askary et al., 2018;
38	Briat et al., 2015). Proper management of iron application is crucial, as it significantly influences
39	both plant yield and soil health (Shoaei et al., 2009). One way to manage the iron in soil and plants
40	is the use of polyamines.
41	Plant growth regulators, particularly polyamines (PAs), play a vital role in enhancing plant yield
42	and productivity. Polyamines are often regarded as the pseudo-hormonal compounds due to their
43	high concentrations in plant cells compared to traditional plant hormones like abscisic acid,
44	cytokinin, gibberellic acid, and auxin (Alet et al., 2012). Even at millimolar concentrations, PAs
45	can elicit the significant biological responses. They are involved in critical processes such as cell
46	division, gene expression, growth and development, leaf senescence, fruit ripening, DNA and
47	RNA synthesis, protein synthesis, membrane stability, enzymatic regulation, and stress tolerance
48	(Shi and Chen, 2014, Rangan et al., 2014). The primary PAs in plants include the putrescine (Put),
49	spermidine (Spd), and spermine (Spm), which exist in free or bound forms (Pal et al., 2021). The
50	application of PAs during various growth stages, including flowering, root formation, and cell
51	proliferation, has demonstrated the beneficial effects on plant metabolism (Rahdari, 2019).
52	However, the limited information is available on the role of PAs in mineral nutrient uptake and
53	their impact on the quantitative and qualitative yield of soybeans.
54	The regulation of plant iron (Fe) deficiency responses involves multiple signaling molecules,
55	such as nitric oxide (NO) (Graziano et al., 2002; Graziano and Lamattina, 2007; Chen et al., 2010),
56	hydrogen sulfide (H <sub>2</sub> S) (Chen et al., 2015), carbon monoxide (CO) (Kong et al., 2010), and carbon
57	dioxide (CO2) (Jin et al., 2009). Among these, NO has been shown to play a critical role in
58	modulating Fe uptake and homeostasis (Graziano et al., 2002; Graziano and Lamattina, 2007;
59	Chen et al., 2010). Notably, exogenous application of polyamines—putrescine (Put), spermidine
60	(Spd), and spermine (Spm)—triggers a rapid and marked increase in NO production in Arabidopsis

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(Ton et al., 2006), indicating that NO acts as a key intermediary in polyamine-dependent signaling cascades. Further supporting this notion, recent evidence demonstrates that exogenous putrescine enhances NO accumulation, which in turn activates Fe deficiency responses and facilitates the remobilization of Fe from the cell wall (Zhu et al., 2016). The effects of PAs on plant growth vary depending on the type of PA. For instance, the putrescine promotes the growth through its antioxidant properties and role in cation-anion balance (Tang and Newton, 2005), while the spermidine mitigates the osmotic stress damage in soybeans, improving the growth parameters such as height, fresh weight, and dry weight (Rahdari et al., 2012).

Given the importance of soybean in agricultural sector of Iran and the challenges associated with iron uptake in high-pH soils, the objective of this research is to investigate the effect of polyamines application on soybean morphological and phytochemical characteristics. Thus, this study explores the role of polyamines in facilitating iron availability and their impact on soybean enzyme activity, yield and nutrient absorption.

#### MATERIALS AND METHODS

## **Experimental Site**

The study was conducted during 2021 and 2022 crop seasons at the Agricultural and Medicinal Plants Research Center of Imam Khomeini Azad University. The experimental site is located at an elevation of 825 meters above sea level, with an average annual temperature of 15.3°C and precipitation of 168.2 mm. Some weather data are shown in Table 1. Soil samples collected from a depth of 0–30 cm revealed a texture of 38% sand, 34% silt, and 28% clay (Table 1).

**Table 1.** Chemical characteristics of soil at the experimental farm.

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EC (dS m <sup>-1</sup> )	pН	OC (%)	TNV (%)	Total N (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )
3.54	7.21	0.61	16.51	0.056	8.37	217.54	0.69	4.62	0.53	3.23
Year	Min. temperature (°C) mean ter		mean tem	perature (°C)	Max.	temperature	(°C)	Rainfall (1	mm)	
2021	9			14.7 20.3			191.3			
2022	9.4			15.5		21.5		236.7		

Note. EC, electrical conductivity; OC, organic carbon; TNV, total neutralizing value; Total N, total nitrogen; P, phosphorus; K, potassium; Cu, Copper; Mn, Manganese; Zn, Zinc; Fe, iron.

#### **Experimental Design and Treatments**

The experiment followed a completely randomized block design (CRBD) with three replications and include 27 plots. Treatments included:

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Treatments	Code	Polyamines (mM)	Iron (mM)
Spermine	Spm	2	-
Spermine with iron	Spm+Fe	2	2
Spermine	Spd	2	-
Spermidine with iron	Spd+Fe	2	2
Putrescine	Put	2	-
Putrescine with iron	Put+Fe	2	2
Spermine Spermine Putrescine with iron	PAs+Fe	1	3
Iron sulfate	Sulfate Fe	-	2
Control	Control	-	-

### **Crop Management**

Tillage and seedbed preparation were conducted before planting. Fertilizers, including 200 kg ha<sup>-1</sup> of triple superphosphate and 100 kg ha<sup>-1</sup> of potassium sulfate, were applied based on soil analysis. Soybean seeds (Williams cultivar) were sown on May 6, 2021, and May 8, 2022, in plots measuring 8 m × 3 m, with a row spacing of 60 cm and on-row spacing of 5 cm. Foliar applications of PAs were administered at the 8-leaf stage in the morning (before sunrise) using a handheld sprayer. One liter of solution with pH 7 was prepared and used for each plot.

#### **Data Collection and Analysis**

Morphological traits, yield components, leaf elemental composition, chlorophyll content, and enzyme activities were measured. To determine the leaf area index (LAI), in each plot, five plants were chosen at random for leaf area analysis. The Digimizer software was used to photograph and calculate the area of a single leaf from each selected plant. LAI was then derived by taking the leaf area (LA) of an individual plant and dividing it by the plot area (P) that the plant occupied.

$$LAI = \frac{LA}{P} \tag{1}$$

#### **Enzyme Extractions and Assays**

Fully expanded young leaves (0.5 g) from soybean plants were sampled and immediately frozen in liquid nitrogen. The frozen samples were ground in 5 mL of Tris buffer solution containing 0.25 M sucrose, 10 mM Tris, and 1 mM EDTA at pH 7.4. The homogenate was then subjected to centrifugation at 4800 rpm for 15 minutes at 4 °C. The resulting supernatant was collected for enzyme assays. The activity of superoxide dismutase (SOD) was determined using the SOD Assay Kit-WST. The reaction plate was incubated in a microplate reader at 37 °C for 20 minutes, and the absorbance of each reaction mixture was measured at 450 nm. For the peroxidase activity (POD)

assay, leaf tissues (0.5 g) were ground in liquid nitrogen and homogenized in 5 mL of Tris extraction buffer. The homogenate was then centrifuged at 10,000× g for 10 minutes at 4 °C. The POD activity was measured at 290 nm for 15 seconds (A1), followed by incubation of the reaction solution at 37 °C and measurement for 135 seconds (A2) using a spectrophotometer. CAT activity assay: The supernatant was mixed with sodium phosphate buffer (100 mM, pH 7.0) and H2O2 (1 M), and the CAT activity was measured at 240 nm. One unit of CAT activity was defined as the amount of CAT required to decompose 1 mole of H2O2 per minute (Hande alici and Arabaci, 2016).

After confirming the normality of data (using the Kolmogorov-Smirnov test) and assessing the residual normality with a Bartlett's test, a combined analysis of variance (ANOVA) was conducted for the data from two seasons. The significance level was set at P < 0.05. The analysis was performed by using SAS 9.2 and SPSS software. To determine the significant differences, the means were assessed using Duncan's multiple range test.

#### **RESULTS AND DISCUSSION**

The results of the combined ANOVA indicated that the treatment had a significant impact on all studied traits, and the year had a significance effect only on the height and LAI. Hovewer, the interaction of year and treatment was not significant.

### **Morphological Traits**

The results of Duncan's multiple range test for the mean comparison showed that the combination of Fe and polyamines (PAs) treatments resulted in the highest plant height (p>0.01). However, no significant difference was observed between this treatment and spermidine with iron (Spd+Fe). The control treatment showed the lowest plant height. Regarding the shoot fresh weight, the Spd+Fe treatment resulted in the highest mean values (p>0.01). The control treatment, with no iron supplementation, exhibited the lowest shoot fresh weight. Similarly, for shoot dry weight, the Spd+Fe treatment had the highest mean values, significantly outperforming other treatments involving PAs, which showed no significant differences among themselves. Additionally, the treatments containing iron exhibited higher shoot dry weights compared to those without iron (Table 2).

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## Yield and yield components

In terms of mean weight of pods, both spermine with iron (Spm+Fe) and Spd+Fe treatments resulted in the highest mean weight of pods (p>0.01). The control and sulfate Fe treatments had the lowest values. When considering the pod weight with seeds, the Spd+Fe treatment demonstrated the highest values (2161.8 g per plot). The sulfate Fe and control treatments exhibited the lowest mean values (703.5 g and 599 g per plot, respectively). The highest mean number of pods per plot was in the Spd+Fe treatment (p>0.01), significantly outperforming other treatments. The control treatment exhibited the lowest pod number. For mean number of seeds, the Spd+Fe treatment exhibited the highest values (p>0.01). The control, sulfate Fe and spm treatments, however, showed the lowest mean number of seeds. The mean weight of seeds followed a similar trend, with Spd+Fe showing the highest values (457.9 g per plot), while the control and sulfate Fe treatments again exhibited the lowest values. In terms of 100 seed weight, the treatments of Spd with iron had the highest value (p>0.01) and had no significance difference with Spd and PAs+Fe treatments. Hovewer, the Spm treatment had the lowest value of 100 seeds weight (Table 2).

## Physiological traits

The Spd treatment showed the highest mean leaf area, significantly differing from all other treatments except Spd+Fe. The control and Put treatments had the lowest mean for leaf area. For leaf area index, the Spd treatments had the highest mean values (p>0.01), which were significantly different from all other treatments. Conversely, the control and Spm treatments exhibited the lowest mean leaf area index. In terms of harvest index, the treatments of Spd with iron had the highest value (p>0.01), Spm, Spd and putrescine with iron (Put+Fe) had no significant difference with Spd+Fe. The control treatment had the lowest value of harvest index (Table 2).

The control treatment exhibited the lowest protein content (25.15%). The Spd+Fe treatment showed the highest protein content (43.92%). Spm+Fe, Spd, Put, Put+Fe and Sulfate Fe treatments did not show the significant differences. For seed oil content, the Spd+Fe treatment exhibited the highest mean values (28.29%), while outperforming all other treatments. The control treatment had the lowest oil content (12.59%) (Table 2).

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## **Nutrient Absorption**

For Zn content, the Spd+Fe treatment showed the highest mean values. The control treatment exhibited the lowest Zn content. Regarding Fe content, Spd+Fe exhibited the highest mean value (498.4 mg kg<sup>-1</sup>), the Put and Put+Fe treatments had no significance difference. The control treatment had the lowest Fe content and had no significance difference with Sulfate Fe treatment. In terms of Mg content, Spd+Fe exhibited the highest values, while the control treatment had the lowest Mg content. For nitrogen and boron, the control treatment had the lowest value and had significance difference with all other treatment (Table 2).

**Table 2.** Mean comparison of height, shoot fresh weight, shoot dry weight, pod weight and mean number of leaves.

	Traits	Plant height	Shoot fresh weight (kg ha <sup>-1</sup> )	Shoot dry weight	Mean weight of pods	Mean number of leaves (m <sup>-2</sup> )
Treatment	2021	(cm)		(kg ha <sup>-1</sup> )	(g m <sup>-2</sup> )	10001
	2021	59.9 b	<mark>4288.5 a</mark>	<mark>962.1 a</mark>	<mark>6.13 a</mark>	<mark>136.91 a</mark>
Year	2022	67.6 a	4361.1 a	<mark>996.9 a</mark>	<mark>6.44 a</mark>	142.59 a
	Significance	**	<mark>ns</mark>	<mark>ns</mark>	<mark>ns</mark>	<mark>ns</mark>
	Spm	51.9 e	3698.7 b	849.0 bc	<mark>4.33 e</mark>	113.62 de
	Spm+Fe	70.1 bc	<mark>4459.5 b</mark>	1025.7 bc	<mark>9.05 b</mark>	169.19 ab
	Spd	63.8 cd	<mark>4471.2 b</mark>	<mark>921.6 bc</mark>	<mark>7.28 c</mark>	132.71 cd
	Spd+Fe	78.4 ab	<mark>6627.9 a</mark>	1536.3 a	<mark>11.01 a</mark>	188.06 a
Polyamines	Put	62.9 cd	<mark>3936.3 b</mark>	<mark>897.9 bc</mark>	5.10 e	140.47 c
•	Put+Fe	63.0 cd	<mark>4367.1 b</mark>	1110.6 b	<mark>7.40 c</mark>	117.22 cde
	PAs+Fe	79.7 a	<mark>4470.6 b</mark>	809.7 c	<mark>6.08 d</mark>	162.95 b
	Sulfate Fe	55.5 de	4294.8 b	808.2 c	3.03 f	136.06 cd
	Control	48.4 e	2596.5 c	855.0 bc	3.30 f	97.44 e
	Significance	**	**	**	**	**

**Table 2 continued**. Mean comparison of pod weight with seed, mean number of pods, mean number of seeds, mean weight of seeds and 100 seed weight

Trait	S	Pod Weight	Mean number	Mean number	Mean weight	100 Seeds
		with Seed	<mark>of pods</mark>	of seeds	of seeds	<mark>Weight</mark>
Treatment		(g m <sup>-2</sup> )	(m <sup>-2</sup> )	(m <sup>-2</sup> )	(kg ha <sup>-1</sup> )	(g)
	2021	<mark>270.19 a</mark>	<mark>20.50 a</mark>	<mark>242.25 a</mark>	<mark>2637.2 a</mark>	124.8 a
Year	2022	<mark>285.56 a</mark>	21.03 a	<mark>246.69 a</mark>	2815.5 a	128.0 a
	Significance	ns ns	ns ns	<u>ns</u>	<u>ns</u>	<mark>ns</mark>
	Spm	<mark>249.6 e</mark>	10.88 f	149.28 e	2374.5 d	<mark>98.6 d</mark>
	Spm+Fe	458.8 d	25.19 c	265.56 bc	3553.5 b	115.2 c
	Spd	596.3 b	25.08 c	266.40 bc	3184.0 bc	142.8 ab
	Spd+Fe	900.8 a	35.09 a	436.68 a	<mark>4487.4 a</mark>	148.0 a
Polyamines	Put	460.0 d	17.79 e	214.92 d	2421.6 d	132.9 b
	Put+Fe	519.2 cd	19.50 d	249.00 c	2518.6 d	134.5 b
	PAs+Fe	581.0 bc	26.36 b	284.55 b	3095.8 c	144.4 ab
	Sulfate Fe	293.1 e	17.03 e	172.89 e	1575.8 e	113.0 c
	Control	<mark>24.96 e</mark>	<mark>9.94 f</mark>	158.6 e	1322.0 e	108.0 cd
	Significance	**	**	**	**	**

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**Table 2 continued.** Mean comparison of leaf area, leaf area index, harvest index, protein and oil of soybean.

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Treatment	Fraits	Leaf Area (cm <sup>2</sup> )	Leaf Area Index	Harvest Index	Protin (%)	Oil (%)
***	2021	94.74 a	1.476 b	86.9 a	37.12 a	18.18 a
Year	2022	<mark>96.65 a</mark>	1.874 a	87.0 a	38.53 a	18.38 a
	Significance	ns	**	ns	ns	ns
	Spm	80.24 cd	1.246 d	89.5 ab	37.35 bc	16.4 b
	Spm+Fe	97.42 bcd	1.828 b	88.9 b	40.01 ab	16.29 b
	Spd	152.38 a	2.249 a	89.5 ab	40.72 ab	18.71 b
	Spd+Fe	135.35 ab	1.504 c	90.9 a	43.92 a	28.29 a
Polyamines	Put	62.48 d	1.836 b	87.4 c	40.47 ab	19.04 b
<i>y</i>	Put+Fe	86.65 cd	1.933 b	89.8 ab	39.86 ab	17.42 b
	PAs+Fe	118.62 abc	1.782 b	88.7 bc	34.39 c	17.72 b
	Sulfate Fe	73.90 cd	1.514 c	80.4 d	38.58 abc	18.05 b
	Control	53.72 d	1.186 d	78.2 e	25.12 d	12.59 c
	Significance	**	**	**	**	**

**Table 2 continued.** Mean comparison of zinc, iron, magnesium, nitrogen and boron leaf elements

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,	Traits		Fe	Mg	N	В
Treatment		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(%)	(mg kg <sup>-1</sup> )
V	2021	46.6 a	319.4 a	43.5 a	1.75 a	109.9 a
Year	2022	47.9 a	322.0 a	45.1 a	1.75 a	111.2 a
	Significance	ns	ns	ns	ns	ns
	Spm	42.3 cd	269.6 cd	45.9 ab	1.97 a	130.4 a
	Spm+Fe	60.8 b	362.6 bc	48.2 ab	1.83 a	109.7 a
	Spd	34.3 d	255.6 cd	42.8 bc	1.84 a	110.4 a
	Spd+Fe	76.0 a	498.4 a	51.0 a	1.87 a	116.3 a
Polyamines	Put	35.9 d	407.5 ab	43.9 bc	1.84 a	119.1 a
	Put+Fe	55.9 bc	458.1 ab	46.1 ab	1.84 a	133.9 a
	PAs+Fe	42.8 cd	254.4 cd	40.1 cd	1.61 a	97.7 ab
	Sulfate Fe	45.0 cd	217.5 d	43.9 bc	1.91 a	114.6 a
	Control	32.3 d	162.4 d	36.8 d	1.05 b	62.9 b
	Significance	**	**	**	*	*

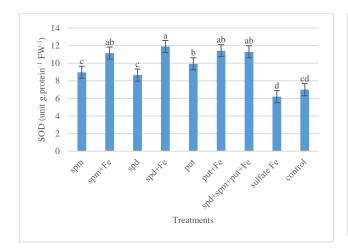
Within columns, means followed by the same letter are not significantly different according to Duncan's multiple range test (0.05). \*\*, \* and ns: significance at level 0.01, 0.05 and not significance, respectively.

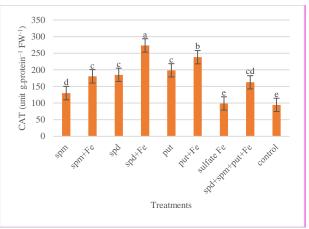
Spd: Spermidine, Spm: Spermine, Put: Putrescine, Fe: Iron, PAs: Polyamines.

#### **Antioxidant Enzymes**

A significant (p< 0.01) upregulation in the activity of the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) was observed in soybean plants treated with polyamines (PAs) and iron (Fe) in combination. The Spd+Fe treatment showed the highest SOD and CAT activity, while Put+Fe and Spm+Fe treatments exhibited the highest POD activities (Fig. 1).

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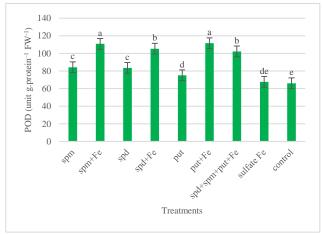


Fig. 1. Enzyme activity of soybean under polyamine and iron application.

#### **DISCUSSION**

This study revealed that the application of PAs in combination with iron significantly influenced several growth parameters and yield-related traits. The positive effects of PAs, particularly Spd and Put, in promoting growth are consistent with the previous studies that highlighted their role in enhancing plant development through hormonal regulation and stress alleviation. Furthermore, the application of iron was shown to be beneficial in improving pod and seed development, which supports the findings of previous studies emphasizing the importance of Fe in leguminous crops for optimal growth and yield.

#### **Growth parameters**

A study conducted by Amani (2020) investigated the effect of PAs on the plant height and obtained the similar observations. The study revealed that foliar application of nutrient solutions

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containing PAs at different times in sovbean plants led to a significant increase in plant height compared to the non-foliar application treatment. This finding highlights the positive effect of nutritional elements in promoting the vegetative growth and enhancing the plant height. PAs, being a nitrogen-containing signaling molecules, play a role in cell division and enlargement, thereby stimulating the plant growth (Rawia et al. 2011) and contributing to increase the plant height, leaf number, and fresh and dry leaf weight in plants (Mahqob et al. 2011). These findings are consistent with the results obtained in the current research. Similar outcomes were reported in a study conducted by Mirzashahi et al. (2016), where no significant difference in plant height was observed between different treatments of iron application from two sources: iron sulfate and iron folate. Considering the involvement of iron compounds in cell division and growth-related processes, an increase in plant height compared to the control group is not unexpected (Zidan et al. 2010). Heidarian et al. (2011) reported a 36% increase in soybean seed yield as a result of foliar application of iron. Gulser et al. (2019) emphasized that the source and dosage of iron intake, along with their interaction, significantly affected the various traits in soybean plants including plant height, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, and leaf number.

The increase in fresh and dry weight observed in plants treated with Put and Spd can be attributed to the effects of PAs on improving the growth indices, antioxidant properties, and their protective role in maintaining cell stability (Nohepisheh and Kalantari, 2011; Kausano *et al.* 2008; Hussein *et al.* 2006). Similarly, the external application of Spd and Put resulted in increased fresh and dry weight of aerial parts in strawberries (Mohadeseh *et al.* 2012), which is consistent with the findings of the present research.

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## **Nutrient uptakes**

In this study, the application of PAs was found to increase the nutrient uptake in soybean plants. The increased uptake of elements such as Zn, B, Fe, Mg, and N can have several beneficial effects, including enhancing the pigment content, improving the photosynthetic capacity, and promoting overall plant yield. Previous research has also demonstrated the positive effect of foliar spraying with PA hormones on crop yield. For instance, the foliar spraying of saffron leaves with PA hormones resulted in increased branch yield (Ehsanfar *et al.* 2018). In this study, the increased levels of elements such as K, P, Mg, and Zn observed in plants treated with Spd and foliar sprayed

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with Put can be another factor contributing to the enhanced growth and seed yield in these treatments, which is consistent with the findings of the present study. Similar outcomes have been reported, where the foliar spraying of Put has led to an increase in specific internal nutrients, such as N, K, P, Fe, and Mg, in marjoram plants (El-Abagy *et al.* 2010).

### **Seed quality**

In this study, oil and protein contents of soybean seeds are affected by PAs application. Studies on soybean composition have reported variable mean protein and oil contents across different varieties, with values of 39.8% and 20.5% (Krishna *et al.*, 2003) and 40.2% and 18.3% (Ramteke *et al.*, 2010), respectively. Beyond genetic variation, seed composition is also influenced by positional effects on the plant. Guleria *et al.* (2007) demonstrated that both oil and protein content vary significantly with seed location, finding that seeds at apical positions on the stem axis exhibited higher protein but lower oil content compared to those at basal positions. Consequently, plant architectural traits, such as height, which determines the number and distribution of seed positions, can influence the overall oil and protein content in soybean.

#### **Antioxidant enzyme activity**

Under stress conditions, plants generate reactive oxygen species (ROS), which can induce oxidative damage. To mitigate this, plants employ a coordinated antioxidant system comprising both non-enzymatic components and enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Abbas *et al.*, 2021; Rashid *et al.*, 2018; Abbas *et al.*, 2022). Evidence indicates that ROS detoxification in response to diverse environmental stresses occurs through a synchronized mechanism (Abbas *et al.*, 2021; Rashid *et al.*, 2018). Within this system, SOD plays a pivotal role by catalyzing the dismutation of superoxide (O2<sup>-</sup>) radicals into hydrogen peroxide (H2O2) and oxygen (O2) (Iftikhar *et al.*, 2022). Nitric oxide (NO) is a well-established regulator of iron (Fe) uptake and homeostasis in plants (Graziano *et al.*, 2002; Graziano and Lamattina, 2007; Chen *et al.*, 2010). Intriguingly, exogenous application of polyamines such as Put, Spd, and Spm rapidly stimulates significant NO production in *Arabidopsis* (Ton *et al.*, 2006), positioning NO as a central signaling intermediate in polyamine-mediated pathways. Corroborating this, recent findings show that exogenous putrescine induces NO accumulation, which subsequently activates Fe deficiency responses and enhances the remobilization of Fe from

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the cell wall (Zhu *et al.*, 2016). In the present study, the soybean showed the significant increase in activities of CAT, SOD, and POD that helped to availability of Fe to plant and improved the yield and yield-related traits of soybean.

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### **CONCLUSIONS**

The results of this study demonstrate that the combined application of polyamines (PAs) and iron significantly enhanced morphological traits, yield-related parameters, nutrient uptake, and enzyme activity. Consequently, the synergistic treatment positively influenced all measured traits, leading to improved yield and yield components. Notably, the treatments involving Spd+Fe, Spm+Fe, and Put+Fe demonstrated the significant effects on the studied traits. Therefore, the combination of PAs with iron can be utilized as foliar application to enhance the crop morphological traits, yield and yield components and enzyme activity of soybean.

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## **In Press, Pre-Proof Version**

چکیده

433	تاثیر پلی آمین و آهن بر جذب آهن، عملکرد و فعالیت آنزیمی سویا
434	
435	فاطمهسادات غمخوار، سیدمصطفی حسینیمزینانی، علیرضا پازکی، و محمد یوسفی
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سویا یک محصول حیاتی برای رفع نیاز های تغذیه ای انسان است و دانه ها به دلیل محتوای بالای پروتئین و روغن خود مشهور هستند. این مطالعه به بررسی اثر پلی آمین ها، در ترکیب با آهن و بدون آهن، بر عملکرد و اجزای عملکرد سویا در طول فصول زراعی 1400 و 1401 پرداخت. این آزمایش در مزرعه دانشگاه آزاد اسلامی با استفاده از طرح بلوک های کامل تصادفی با سه تکرار انجام شد. تیمار ها شامل 2 میلی مولار اسپر مین، اسپر میدین و پوترسین، با 2 میلی مولار آهن و بدون آهن، و همچنین ترکیبی از 1 میلی مولار پلی آمین با 3 میلی مولار آهن بودند. علاوه بر این، 2 میلی مولار سولفات آهن و یک تیمار شاهد نیز در نظر گرفته شد. نتایج نشان داد که تیمار ها به طور قابل توجهی بر عملکرد و اجزای عملکرد (مانند ارتفاع گیاه، تعداد غلاف، وزن غلاف (با و بدون دانه)، وزن دانه، شاخص برداشت، محتوای پروتئین و روغن دانه، شاخص سطح گیاه، تعداد غلاف، وزن غلاف روی، آهن، بور، نیتروژن و منیزیم تأثیر گذاشتند. استفاده از تیمار اسپر میدین همراه با آهن، و رن دانه را در مقایسه با شاهد 7 درصد افز ایش داد. همچنین، کاربرد پلی آمین ها همراه با آهن منجر به افز ایش قابل توجه فعالیت آنزیم های سوپر اکسید دیسموتاز (44%)، پراکسیداز (63%) و کاتالاز (43%) در مقایسه با شاهد شد. نکته قابل توجه فعالیت آنزیم ها نشان می دهد که کاربرد پلی آمین ها همراه با آهن می تواند عملکرد و اجزای عملکرد سویا را به طور قابل داشت. این یافته ها نشان می دهد که کاربرد پلی آمین ها همراه با آهن می تواند عملکرد و اجزای عملکرد سویا را به طور قابل توجهی بهبود بخشد.