

Effects of Salicylic Acid on Barley Growth and Productivity under Cadmium Stress Conditions in Rania, Kurdistan Region, Iraq



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ABSTRACT

Plant health and agricultural productivity are seriously threatened by heavy metal contamination. Due to the expansion of cereal agricultural lands into marginal areas close to urban areas and unpaved roads that are polluted by many pollutants, especially that of cadmium (Cd), this study examined the effects of Cd and salicylic acid (SA) separately and in combination on the growth, physiological, biochemical, and reproductive responses of barley (*Hordeum vulgare* L.). Three SA treatments (0, 86, and 172 mg/kg soil) and four Cd concentrations (0, 10, 20, and 30 mg/kg soil) were used in a factorial pot experiment. The findings showed that high Cd (Cd30) reduced vegetative growth but increased spike number and harvest index, indicating reproductive compensation, moderate Cd levels (Cd20) improved some growth traits, including plant height by 4.00% and flag leaf area by 13.11% compared to the control treatment, suggesting a possible hormetic effect. Particularly under moderate Cd stress, SA at 86 mg/kg markedly enhanced plant height, yield components, and antioxidant balance. On the other hand, SA at 172 mg/kg increased grain number and spike length but decreased overall yield, as a result of metabolic effects and hormonal interferences. Under extreme stress, endogenous defense mechanisms might be sufficient, and external SA could upset homeostasis, according to the interaction effects, which showed that Cd20 × SA86 maximized growth and productivity while Cd30 × SA0 produced an increase in grain and biological yields by an amount of 10.95 and 9.73%, respectively, compared to the control treatment. These results show that the performance of growth and yield components of *H. vulgare* L. was significantly and variably affected by both Cd and SA, both separately and in combination, depending on the concentration and the interaction. Data suggest that SA is most effective at moderate Cd stress levels.

Index Terms: Antioxidant, Cadmium Stress, Heavy Metal, *Hordeum Vulgar* L, Salicylic Acid

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) which belongs to the family Poaceae is one of the most important cereal crops cultivated in increasing numbers of areas in the world [1]. It belongs

to the *Hordeum* genus, which has 32 species and 45 taxa. Barley is one of the top ten crop plants in the world and comes in fourth place among cereal crops, after rice, wheat, and maize, according to Abdullah *et al.* [2]. In 2018, the global average barley production was 2.91 tons/ha [3]. Iraq produced 6,238,392 tons of barley in 2020, with 3,469,646 hectares under cultivation [4]. In Kurdistan region, rainy conditions give the highest yield of grain and straw. The area cultivated by barley in Kurdistan region governorates during the winter season 2013 was only 84170 hectares with production of only 135,183 tons with an average yield of 1,606 kg/ha [5].

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In ancient Kurdish communities, barley was used as a staple for bread-making and as a vital source of grain and straw for animals. Barley was also seen as an essential feed grain that was less expensive for low-income families [5]. Numerous studies have shown how it might enhance human health by reducing blood sugar and cholesterol levels, thus averting diabetes and cardiovascular ailments [6]. One of the most significant cereal crops in Iraq's Kurdistan area is barley which thrives in a variety of climates and grows better than other cereals during the dry winter months [2]. However, barley productivity is threatened by heavy metal contamination, especially cadmium (Cd), which is a non-essential element with no biological function. It is a highly toxic element for plants and animals and it is fixed in the environment, causing poisonous to plants even at low doses [7]. The two categories of sources that release heavy metals into soil, air, and water are natural and man-made sources [8]. The morphological, physiological, biochemical, and molecular levels of plants are all impacted by Cd poisoning. As Cd dosages rise, barley biomass and leaf area decline [7]. The mobility of Cd in soil is mostly determined by the soil's pH, mineral content, and organic matter, where Cd is more mobile in sandy, acidic soils with low organic matter, but it tends to bind strongly to iron oxides and organic matter, decreasing its availability. In plants, Cd is absorbed mainly as Cd^{2+} ions through root cell membranes through transporters normally utilized for essential metals such as calcium, iron, and zinc. Plant roots can readily absorb Cd, which then travels to the higher portions of the plant [9]. It enters roots through apoplastic diffusion and symplastic uptake, competes with Ca^{2+} at calcium channels, and is often sequestered in root cell vacuoles by chelation with phytochelatin to reduce toxicity. Some Cd is then loaded into the xylem for translocation to shoots, driven by transpiration, and may further move to seeds through the phloem [10].

Salicylic acid (SA) is considered one of the most important plant growth regulators that play a major role in plant growth and development. Many metabolic and vital physiological processes in plants including thermogenesis, stomata closure, and flowering are dependent on SA [11]. Previously, it was reported that SA can alleviate growth inhibition caused by Cd toxicity in various plant species, including barley [12]. At low to moderate concentrations (0.5–1.5 mM), SA enhanced lettuce's resistance to Cd stress. SA improved the plants' ability to tolerate oxidative stress by increasing root growth, proline, catalase activity, and carbohydrate content; in addition, it raised levels of chlorophyll a, which aided in photosynthesis. Higher SA levels (2 mM), however, decreased carotenoids, chlorophyll b, and leaf area, suggesting potential toxicity at high concentrations [13].

The aims of this study are to assess the impacts of different Cd and SA concentrations on some growth, physiological, biochemical, and yield components properties of barley and to recognize the ability of SA to reduce the toxicity of Cd on this plant.

2. MATERIALS AND METHODS

2.1. Plant Materials, Cultivation, and Treatments

The study included carrying out a factorial experiment at the University of Raparin, Rania city, Sulaimani, Kurdistan Region, Iraq, during the growing season from December 2024 to May 2025 based on a completely randomized design (CRD) with three replications on barley local variety brought from the University of Sulaimani, College of Agricultural Engineering Sciences, which was cultivated in a soil mixed with the heavy metal Cd and the plant hormone SA. A silty loam soil with pH 7.41, EC 0.26 dS/m, and O.M 2.29% was ground well and sieved to 4 mm. Plastic pots were filled with 32 kg of soil, and 15 seeds were sown in each pot (diameter of 42 cm and a height of 36 cm). Before sowing, a light irrigation was applied to ensure uniform germination, and other agricultural practices were carried out as needed. Nitrogen fertilizer was used at a rate of 126 kg/ha, half was in the form of DAP ($n = 18\%$ and $P = 46\%$), applied before the time of sowing, and the remaining was added in the form of urea ($n = 46\%$), separated into two parts, the first added at the beginning of the stem elongation stage and the other at the heading stage.

The study consisted of two factors, the first was Cd chloride ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$) as a source of Cd, with three concentrations of Cd (10, 20, and 30 mg/kg soil) (write the reference of Cd I sent), in addition to the no Cd treatment, mentioned as Cd0, Cd10, Cd20, and Cd30, and the second factor was SA ($\text{C}_7\text{H}_6\text{O}_3$) (2-hydroxy benzoic acid), with two concentrations (1 and 2 mM) converted to (86 and 172 mg/kg soil), similar concentrations have been used for barely as foliar application or soaking seeds before sowing, but there is no researches on using SA with soil (write the two reference I sent), in addition to the no SA treatment mentioned as SA0, SA86 and SA172. The soil of each experimental unit was mixed well with the added Cd and SA at the required concentrations according to the treatments (Fig 1).

2.2. Meteorological Data

Monthly averages of minimum and maximum of air and soil temperature and relative humidity, in addition to wind speed, are included in Table 1.

TABLE 1: Some meteorological data throughout the study period*

Year	Month	Air temperature (°C)		Soil temperature (°C)		Relative humidity (%)		Wind speed (km/h)
		Max.	Min.	Max.	Min.	Max.	Min.	
2024	December	9.52	7.81	9.45	8.89	72.18	65.71	1.22
2025	January	9.04	7.59	8.74	8.07	65.87	59.18	1.48
	February	6.71	5.42	7.79	7.72	63.22	56.23	1.51
	March	15.30	13.30	15.42	12.62	51.39	44.59	1.41
	April	19.91	18.24	19.84	14.28	53.41	15.35	1.53
	May	27.80	25.97	26.85	21.00	34.11	16.40	1.85

*Rania-Grdjan Station, Bakrajo Agronomy, General Directorate of Agriculture, Sulaimani

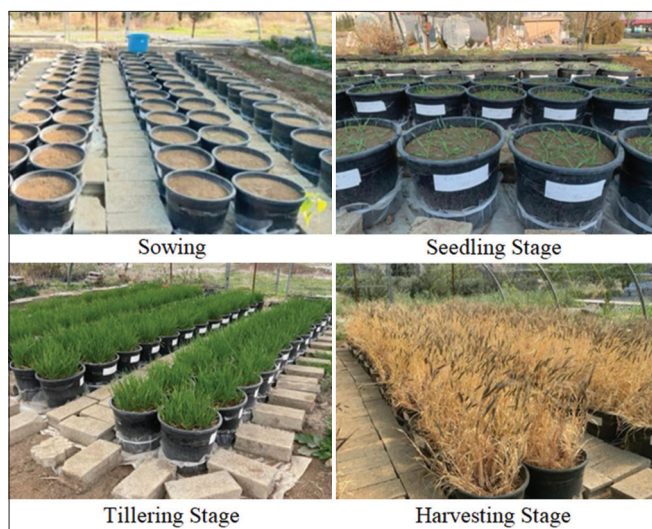


Fig. 1. Barley plants used in the study at different stages.

2.3. Studied Characteristics

For each experimental unit, ten plants were randomly chosen for each pot and were used to determine all studied characteristics as follows: Germination percent was measured by the equation mentioned by [] as follows: [14]

$$G = \frac{L}{S} \times 100 \quad (\text{seed day}^{-1}) \quad (1)$$

where G is the germination percent, L is the germinated seeds, S is the total sown seeds

Germination velocity was measured by the equation mentioned by (2) [15] as follows:

$$GV = \frac{\sum_{n=1}^n D}{n} \quad (\text{day}) \quad (2)$$

Where, GV is the germination velocity, n is the number of seeds, which were germinated corresponding to the day D

observation (not the accumulated number) and D is number of days counted from the beginning of germination.

Flag leaf area (cm^2) was measured by the equation applied by Al-Hassnawi and Al-Burki [16]. Plant height was measured by a measuring tape line from the soil surface to the barley spike base at physiological maturity [17]. The number of emerged tillers was recorded. The ratio of 20 mL of 80% acetone to 0.4 g fresh sample was used to estimate chlorophyll a (Chl.a), chlorophyll b (Chl.b), and total carotenoids as reported by Wellburn [18]. The activity of peroxidase (POD) enzyme (absorbing units g^{-1} fresh weight [FW]) in leaves was estimated according to Müftügil [19] spectrophotometrically at 420 nm using guaiacol and H_2O_2 . The proline content in leaves (mM/g FW) was estimated using spectrophotometer at 520 nm which was calibrated with a proline standard curve using sulfosalicylic acid and ninhydrin as it mentioned by Bates *et al.* [20]. Total carbohydrate in dry leaves was determined using concentrated sulfuric acid (H_2SO_4) and 5% phenol spectrophotometrically at 488 nm as reported by Herbert *et al.* [21].

Yield and yield components were measured at harvest, as it described by Shemi *et al.* [22] including number, length (cm), and weight (g) of spikes, spike grain number, weight of 1,000 grains (g), whereas grain yield (ton hectare^{-1}) which was calculated from the yield of each experimental unit and converted to ton hectare^{-1} , biological yield (ton hectare^{-1}) was calculated from the weight of the entire above soil harvested dry plants for an area of 0.1256 m^2 (pot area) then converted to ton hectare^{-1} , biological yield without grain yield was the straw yield. Harvest index (%) is the ratio of grain yield to biological yield as mentioned by Sharma and Smith [23] as it appears in the following equation:

$$\text{Harvest index} = (\text{Grain yield/biological yield}) \times 100 \quad (3)$$

2.4. Statistical Analysis

This study was conducted as a factorial experiment in a CRD with three replications. Analysis of variance was used for

data analysis, and the test of Duncan's multiple range at a 5% probability level was used for the comparison between the experiment means, using the statistical program SAS version 9.1 [24].

3. RESULTS AND DISCUSSION

3.1. Vegetative Growth

Results in (Table 2) show increasing Cd concentrations significantly delayed germination velocity from 11.51 days for the control treatment to 12.38 days for the highest Cd concentration (30 mg/kg¹ soil). Cd concentration of 20 mg kg¹ soil was the best for the significant increase in plant height, number of tillers, and flag leaf area to 66.06 cm, 6.96, and 6.21 cm² compared to the control treatment (73.52 cm, 6.87, and 5.49 cm²), whereas increasing Cd concentration to 30 mg kg¹ soil decreased these characteristics. Regarding the SA effects, it was seen (Table 2) that earlier seed germination was in the control treatment (11.60 days), whereas using SA in both concentrations retarded seed germination to 12.03 and 11.94 days for the SA86 and SA172 treatments. Adding 86 mg/kg soil of SA increased each plant height, number of tillers, and flag leaf area to 56.50 cm, 7.10, and 6.01 cm² compared to the control and SA172 treatments.

In general, interactions of no Cd application regardless the SA concentration led to earlier seed emergence, and increasing Cd

concentration to 20 and 30 mg/kg soil, increased the period for seed germination, where the longest period was for the interaction Cd30 × SA86, whereas the earlier germination was in the Cd20 × SA0 treatment not significantly different from the control treatment (11.53 days). Plant height increased significantly to 68.80 cm in the Cd20 × SA86 treatment compared to other treatments except the interactions Cd0 × SA0, Cd0 × SA86, Cd10 × SA86, and Cd30 × SA172, respectively. The lowest plant height was reported in the interaction Cd0 × SA172 significantly compared to all other treatments. The superior interaction for plant height was the same for the number of tillers which recorded 8.16 tillers, significantly higher compared to all other treatments, and the lowest value (5.70) was recorded for the interaction Cd10 × SA0 treatment. It was observed from the results (Table 2) that the effect of higher SA concentration on the vegetative growth characteristics was clear in the high Cd concentration (Cd30 mg/kg soil). Flag leaf area increased significantly in the interactions of Cd10 with 86 and 172 SA, and Cd20 with 0, and SA86 significantly compared to all other interaction treatments. This indicates that SA applied without Cd or at high Cd concentrations, regardless of SA level, led to decreasing most vegetative growth.

Germination percent remained high (97.66–100%) without any significant differences across all treatments; Cd and SA concentrations, and their interactions (Table 2).

TABLE 2: Effects of Cd, SA, and their interactions on some vegetative growth parameters of barley

Treatments	Germination velocity (day)	Germination percent	Plant height (cm)	Number of tillers	Flag leaf area (cm ²)
Cd concentration (mg kg ⁻¹ soil)					
Cd 0	11.51 c*	100.00 a	63.52b	6.87 ab	5.49 c
Cd10	11.92 b	100.00 a	62.85 b	6.50 c	6.05 a
Cd20	11.63 bc	99.22 a	66.06 a	6.96 a	6.21 a
Cd30	12.38 a	99.22 a	62.82 b	6.73 b	5.71 b
SA concentration (mg/kg soil)					
SA0	11.60 b	100.00 a	63.22 b	6.38 c	5.73 b
SA86	12.03 a	99.41 a	65.50 a	7.10 a	6.01 a
SA172	11.94 a	99.41 a	62.72 b	6.82 b	5.86 ab
Interactions between Cd and SA					
Cd0×SA0	11.53 d-f	100.00 a	66.66 a-c	7.30 b	5.58 de
Cd0×SA86	11.49 d-f	100.00 a	66.55 a-c	6.73 c	5.50 de
Cd0×SA172	11.51 d-f	100.00 a	57.35 f	6.60 cd	5.41 e
Cd10×SA0	11.36 ef	100.00 a	61.80 de	5.70 f	5.33 e
Cd10×SA86	11.83 c-e	100.00 a	66.20 a-c	6.90 c	6.35 a
Cd10×SA172	12.56 b	100.00 a	60.55 e	6.90 c	6.49 a
Cd20×SA0	11.12 f	100.00 a	63.80 cd	6.33 de	6.22 ab
Cd20×SA86	11.65 d-f	97.66 a	68.80 a	8.16 a	6.43 a
Cd20×SA172	12.13 b-d	100.00 a	65.60 bc	6.40 de	5.97 bc
Cd30×SA0	12.40 bc	100.00 a	60.63 e	6.20 e	5.79 cd
Cd30×SA86	13.17 a	100.00 a	60.45 e	6.60 cd	5.76 cd
Cd30×SA172	11.57 d-f	97.66 a	67.40 ab	7.40 b	5.59 de

*The means followed by the same letter for each factor and their interactions separately are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test

3.2. Photosynthetic Pigments

Results presented (Table 3) show that chlorophyll a content was significantly affected by Cd and SA treatments. The highest chlorophyll a (0.85 and 0.84 mg/g FW) was observed at Cd10 and Cd20, respectively, while the lowest value (0.82 mg/g FW) occurred in both the control (Cd0) and Cd30 treatments. Chlorophyll b content increased significantly with Cd20 (0.69 mg/g FW) compared to other Cd levels, while Cd30 caused a marked reduction (0.55 mg/g FW). Interestingly, total carotenoids were highest (0.15 mg/g FW) for Cd30, whereas a significant decrease was recorded at Cd20 (0.10 mg/g FW). Regarding SA effects, SA86 treatment reduced chlorophyll a to 0.82 mg/g FW, while the highest value (0.84 mg/g FW) was recorded under the control (SA0). Chlorophyll b content was significantly enhanced in the control SA treatment (0.70 mg/g FW), while both SA treatments reduced it to 0.58 and 0.56 mg/g FW for SA86 and SA172, respectively. Carotenoid content, on the other hand, increased significantly with rising SA concentration compared to the SA0 treatment, peaking at 0.16 mg/g FW in the SA172 treatment.

Significant interaction effects between Cd and SA concentrations were also evident (Table 3), where the

TABLE 3: Effects of Cd, SA, and their interactions on photosynthetic pigments of barley

Treatments	Chlorophyll a	Chlorophyll b	Total carotenoids
Pigments (mg/g fresh weight)			
Cd concentration (mg kg ⁻¹ soil)			
Cd 0	0.82 b*	0.61 b	0.14a
Cd10	0.85 a	0.60 b	0.14 a
Cd20	0.84 a	0.69 a	0.10 b
Cd30	0.82 b	0.55 b	0.15 a
SA concentration (mg/kg soil)			
SA0	0.84 a	0.70 a	0.10 b
SA86	0.82 b	0.58 b	0.14 a
SA172	0.83 ab	0.56 b	0.16 a
Interactions between Cd and SA			
Cd0×SA0	0.85 a-c	0.72 a	0.09 ef
Cd0×SA86	0.80 f	0.52 de	0.17 b
Cd0×SA172	0.81 d-f	0.59 b-d	0.15 b
Cd10×SA0	0.83 c-e	0.66 a-c	0.11 c-f
Cd10×SA86	0.86 ab	0.61 b-d	0.13 b-e
Cd10×SA172	0.87 a	0.55 cd	0.17 b
Cd20×SA0	0.84 bc	0.75 a	0.08 f
Cd20×SA86	0.83 b-d	0.66 a-c	0.11 c-f
Cd20×SA172	0.85 a-c	0.66 a-c	0.11 c-f
Cd30×SA0	0.84 a-c	0.67 ab	0.11 d-f
Cd30×SA86	0.81 d-f	0.55 cd	0.15 b-d
Cd30×SA172	0.80 ef	0.44 e	0.21 a

*The means followed by the same letter for each factor and their interactions separately are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test

highest chlorophyll a (0.87 mg/g FW) was recorded under the Cd10 × SA172 treatment, followed closely by Cd10 × SA86 (0.86 mg/g FW), while the lowest (0.80 mg/g FW) was observed in Cd0 × SA86 and Cd30 × SA172. Chlorophyll b reached a maximum of 0.75 mg/g FW in the Cd20 × SA0 treatment and was significantly reduced under Cd30 × SA172 (0.44 mg/g FW). Carotenoid content increased significantly in Cd30 × SA172 (0.21 mg/g FW), whereas the lowest value (0.08 mg/g FW) was found in the Cd20 × SA0 interaction. These results indicate that Cd and SA treatments have complex and sometimes opposing effects on pigment concentrations, indicating that high SA and Cd levels may mitigate or exacerbate pigment decline depending on the specific combination.

3.3. Enzymatic and Non-enzymatic Antioxidants

Results (Table 4) indicate that the application of Cd significantly influenced antioxidant activity in barley plants. The application of 10 mg/kg soil (Cd10) increased POD activity to 251.22 units/g FW, which was the highest among all Cd treatments and significantly greater than the control (239.11 units/g FW). However, higher Cd levels

TABLE 4: Effects of Cd, SA, and their interactions on some enzymatic and non-enzymatic antioxidants of barley

Treatments	Peroxidase absorbing (units/g fresh weight)	Proline (µg/g fresh weight)	Carbohydrates (%)
Cd concentration (mg/kg soil)			
Cd 0	239.11 b*	0.25 b	1.91 a
Cd10	251.22 a	0.22 c	0.90 d
Cd20	235.55 c	0.40 a	1.54 c
Cd30	236.44 c	0.38 a	1.74 b
SA concentration (mg/kg soil)			
SA0	246.41 a	0.35 b	1.34 c
SA86	232.16 c	0.23 c	1.74 a
SA172	243.16 b	0.37 a	1.50 b
Interactions between Cd and SA			
Cd0×SA0	230.66 e	0.32 d	1.87 e
Cd0×SA86	222.00 f	0.21 e	3.21 a
Cd0×SA172	264.66 a	0.24 e	0.67 h
Cd10×SA0	263.00 a	0.12 f	1.13 g
Cd10×SA86	236.00 d	0.08 g	1.37 f
Cd10×SA172	254.66 b	0.46 b	0.22 i
Cd20×SA0	236.33 d	0.42 c	2.13 d
Cd20×SA86	228.33 e	0.31 d	0.26 i
Cd20×SA172	242.00 c	0.46 b	2.22 c
Cd30×SA0	255.66 b	0.53 a	0.23 i
Cd30×SA86	242.33 c	0.31 d	2.11 d
Cd30×SA172	211.33 g	0.31 d	2.90 b

*The means followed by the same letter for each factor and their interactions separately are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test

(20 and 30 mg/kg) reduced POD activity to 235.55 and 236.44 units/g FW, respectively. Proline content increased with high Cd treatments, where the highest values were recorded at Cd20 (0.40 µg/g FW) and Cd30 (0.38 µg/g FW), significantly higher than the control (0.25 µg/g FW), while the lowest value was at Cd10 (0.22 µg/g FW), the same treatment, which gave the lowest total carbohydrate content significantly reduced to 0.90% compared to the control (1.91%), but recovery was observed at Cd20 and Cd30 (1.54 and 1.74%), respectively. POD activity was highest in the absence of SA (246.41 units/g FW), followed by SA172 (243.16 units/g FW), and lowest in SA86 (232.16 units/g FW). Applying SA at 172 mg/kg led to an increase in proline accumulation to 0.37 µg/g FW, followed by SA0 (0.35 µg/g FW), while SA86 had the lowest value (0.23 µg/g FW), the same treatment that recorded highest carbohydrate content percentage (1.74%), while SA0 had the lowest value (1.34%).

Concerning the effects of interactions between Cd and SA, the highest POD activity was observed in Cd0 × SA172 (264.66 units/g FW) and Cd10 × SA0 (263.00 units/g FW), significantly higher than all other combinations. The lowest POD value was recorded in Cd30 × SA172 (211.33 units/g FW). The highest proline content (0.53 µg/g FW) occurred under the Cd30 × SA0 treatment, while Cd10 × SA86 showed the lowest (0.08). Carbohydrate content reached a maximum

(3.21%) in Cd0 × SA86, whereas the lowest value (0.22%) appeared in Cd10 × SA172. Notably, combinations with high Cd levels (Cd20 and Cd30) and low or no SA exhibited higher proline and carbohydrate accumulation, likely reflecting a stress-response mechanism. Meanwhile, the SA application under low Cd (Cd10) reduced antioxidant metabolite levels, suggesting SA's modulating role under mild stress conditions.

3.4. Yield and Yield Components

Results (Table 5) show that increasing Cd concentration of 30 mg/kg soil was the best for a significant increase in the number of spikes, weight of spike, grain yield, biological yield, and harvest index to 5.48, 4.97 g, 5.11 ton/ha, 10.02 ton/ha, and 50.99%, respectively, compared to the control treatment, which recorded 5.28, 4.67 g, 4.87 ton/ha, 9.74 ton/ha, and 50.03%. In comparison to the control and higher Cd concentrations, the application of low Cd concentration (10 mg/kg soil) resulted in a significant decrease in the number of spikes (5.15), spike weight (4.67 g), grain yield (4.81 ton/ha), number of grains per spike (20.86), and harvest index (48.95%), whereas, moderate Cd concentration of 20 mg/kg demonstrated improvements in several yield traits, particularly the number of grains per spike (22.13) and 1,000-grain weight (45.33 g), while the harvest index (50.56%) and biological yield (9.84 ton/ha) remained comparatively stable.

TABLE 5: Effects of Cd, SA, and their interactions on yield and yield components of barley

Treatments	Number of spikes	Length of spikes (cm)	Weight of spike (g)	Number of grain spike ⁻¹	Weight of 1,000 grains (g)	Grain yield (ton/ha)	Straw yield (ton/ha)	Biological yield (ton/ha)	Harvest index (%)
Cd concentration (mg/kg soil)									
Cd 0	5.28b*	7.63a	4.67b	21.46b	42.60c	4.87bc	4.86b	9.74b	50.03b
Cd10	5.15b	7.43ab	4.67b	20.86c	45.40a	4.81c	5.02a	9.84ab	48.95c
Cd20	5.28b	7.47ab	4.90a	22.13a	45.33a	4.97b	4.87b	9.84ab	50.56ab
Cd30	5.48a	7.32b	4.97a	21.83ab	44.03b	5.11a	4.91b	10.02a	50.99a
SA concentration (mg/kg soil)									
SA0	5.30ab	7.07c	4.83b	19.97c	44.90a	4.94b	4.94b	9.89b	49.95a
SA86	5.37a	7.80a	4.97a	22.85a	44.27b	5.14a	5.07a	10.22a	50.32a
SA172	5.22b	7.52b	4.61c	21.89b	43.85c	4.74c	4.73c	9.48c	50.12a
Interactions between Cd and SA									
Cd0×SA0	5.03d	7.31d	4.72cd	20.10d	42.90b	4.84de	4.89bc	9.73cd	49.73cd
Cd0×SA86	5.56b	7.83bc	4.94a-c	23.50b	41.20c	5.18b	5.03ab	10.21ab	50.73bc
Cd0×SA172	5.27cd	7.76cd	4.36e	20.80d	43.70b	4.61f	4.68c	9.29ef	49.62d
Cd10×SA0	5.10d	5.98f	4.45e	16.70f	45.70a	4.57f	4.71c	9.29ef	49.20d
Cd10×SA86	5.23cd	7.92bc	4.87bc	22.40c	45.30a	5.07bc	5.17a	10.24ab	49.49d
Cd10×SA172	5.13d	8.39a	4.69cd	23.50b	45.20a	4.81de	5.18a	9.99bc	48.17e
Cd20×SA0	5.16d	8.25ab	4.99ab	23.20bc	45.60a	4.99cd	5.04ab	10.03bc	49.73cd
Cd20×SA86	5.46bc	6.67e	5.15a	18.59e	45.50a	5.21ab	5.22a	10.43a	49.95cd
Cd20×SA172	5.23cd	7.49cd	4.57de	24.60a	44.90a	4.72ef	4.36d	9.08f	51.99a
Cd30×SA0	5.93a	6.74e	5.17a	19.90d	45.40a	5.37a	5.13a	10.51a	51.14ab
Cd30×SA86	5.23cd	7.66cd	4.93a-c	23.10bc	45.10a	5.11bc	4.89bc	10.00bc	51.12ab
Cd30×SA172	5.28cd	7.56cd	4.82b-d	22.50bc	41.60c	4.85de	4.71c	9.56de	50.72bc

*The means followed by the same letter for each factor and their interactions separately are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test

According to the influence of SA, the application of 86 mg/kg soil resulted in the best performance in yield traits, as it significantly increased the number of spikes, spike length, spike weight, grain number per spike, grain yield, and biological yield to 5.37, 7.80 cm, 4.97 g, 22.85, 5.14 ton/ha, and 10.22 ton/ha, respectively, compared to the control (SA0) and the higher SA concentration (172 mg/kg), while, the SA172 treatment showed a significant reduction in most reproductive traits, particularly spike weight (4.61 g), grain yield (4.74 ton/ha), and biological yield (9.48 ton/ha), although the harvest index remained relatively unaffected among the SA treatments. Regarding the interaction between Cd and SA concentrations, the best results in terms of number of spikes (5.93), spike weight (5.17 g), grain yield (5.37 ton/ha), and biological yield (10.51 ton/ha) were recorded in the Cd30 × SA0 treatment. The highest number of grains per spike (24.60) and harvest index (51.99%) were observed in Cd20 × SA172. The greatest 1,000-grain weight (45.70 g) was recorded in Cd10 × SA0, whereas the longest spike length (8.39 cm) appeared in Cd10 × SA172. Conversely, the Cd10 × SA0 and Cd0 × SA172 combinations showed the lowest values for the majority of traits.

4. DISCUSSION

4.1. Vegetative Growth

According to the results (Table 2), which show complex reactions to Cd stress and SA application, Cd30 considerably lengthened the germination period in comparison to the control. Oxidative stress brought on by Cd may be the cause of this delay since it reduces metabolic activity in the early growing stages [7]. Barley's seed tolerance to Cd at the tested levels was demonstrated by the minimal impact of Cd on seed germination which may possibly be due to adding Cd as a dry powder before sowing, where at the first period may Cd not perfectly dissolved that influences were not appeared, which is in agreement with findings of Ayachi *et al.* [25]. A hormetic effect, in which low-to-moderate stress stimulates growth, as shown in some barley studies, while higher doses inhibit it, may be the reason why moderate Cd levels unexpectedly increased plant height, number of tillers, and flag leaf area [26], in contrast, a higher concentration of Cd decreased these parameters, which agrees with research showing that elevated Cd impairs barley growth and biomass by disrupting nutrient uptake and enhancing oxidative stress [27].

According to the current study, SA application did not speed up germination velocity; rather, it delayed it when compared to the control. This may indicate that SA contributes to

stress priming by, in some conditions, delaying germination, which may later improve the seedling's ability to handle stress. Before growing quickly, slower germination might enable seeds to evaluate the surrounding environments and activate defenses. This corresponds with research showing that SA increases stress tolerance later on but slows germination in non-stressful situations [28], [29]. Plant height, number of tillers, and flag leaf area were all significantly increased by moderate SA (SA86). Higher SA doses, however, may inhibit growth by overstimulating defense mechanisms or upsetting the equilibrium of other plant hormones, such as auxin, which is critical for healthy growth and development. Therefore, when exposed to high doses of SA, plants may grow more slowly or become smaller [29]. The interaction data suggest that SA is most effective at moderate Cd levels, possibly by modulating stress signaling pathways and promoting detoxification processes. Vegetative growth traits such as plant height and leaf area often show high plasticity and may even be enhanced (a phenomenon known as hormesis) under moderate stress or when SA is applied [30]. In general, the results appear to show the potential of SA to enhance barley resilience and productivity in Cd-contaminated soils, supporting its use in sustainable crop management under heavy metal stress [31].

4.2. Photosynthetic Pigments

The results presented (Table 3) clearly demonstrate that Cd and SA treatments significantly affect the photosynthetic pigments in barley. The results showed that at high levels of Cd, chlorophyll decreased, at low and moderate Cd levels, chlorophyll a and b slightly increased, which possibly due to a mild stress that actually induced the plant to produce more pigments, a phenomenon known as hormesis [32]. However, when Cd increased to 30 mg kg⁻¹ soil, chlorophyll decreased, indicating that higher Cd levels became toxic and damaged pigment formation or possibly because chlorophyll is more sensitive to this stress [33]. At Cd20, carotenoids which aid in shielding chlorophyll and other photosynthetic components from oxidative stress decreased; however, at Cd30, they rose once more, which could be the plant's reaction to more extreme stress [34]. SA raised the carotenoid content, particularly at the higher dose (SA172), which demonstrates how SA helps plants' antioxidant defenses [31].

The results of the interaction between Cd and SA were intriguing. The Cd10 × SA172 treatment had the highest levels of chlorophyll a. This implies that SA can support pigment production by mitigating Cd-induced damage at lower levels. However, Cd30 × SA172 had the lowest chlorophyll b, indicating that SA may worsen pigment loss in

the presence of high Cd stress. The highest carotenoid level was found in this treatment, indicating that SA protects the plant in another way by increasing antioxidant levels during periods of extreme stress. The photosynthetic response to SA is both dose- and species-dependent [35]. These results suggest that under high Cd stress, plants treated with SA focus more on survival and stress protection (like producing antioxidants) than on keeping high levels of chlorophyll. This strategy helps plants cope with damage but may reduce their photosynthetic capacity. Similar effects of SA were seen in other studies on wheat [36]. The different results in this study compared to others in which SA increased chlorophyll (e.g., in wheat and maize) may be due to plant species differences, growing conditions, or how SA was applied [12].

4.3. Enzymatic and Non-enzymatic Antioxidants

This study shows that Cd and SA application significantly influence the antioxidant defense system and metabolic responses in barley (Table 4), indicating a complex but coordinated physiological response to heavy metal toxicity. At low Cd levels (Cd10), a significantly increase in POD activity suggests that barley activates its enzymatic antioxidant defense to detoxify reactive oxygen species (ROS), which are generated as a result of Cd-induced oxidative stress [37]. PODs help break down hydrogen peroxide (H_2O_2), a major ROS, into harmless molecules, thus reducing oxidative damage to proteins, lipids, and DNA. However, at the highest Cd level (Cd30), the reduction in POD activity may indicate that the antioxidant system is overwhelmed, or the enzymes themselves have been inactivated due to severe stress or oxidative injury. This aligns with earlier findings in rice and barley that moderate levels of heavy metals can stimulate enzymatic defenses as part of a hormetic response levels but inhibit them at high doses [12], [38].

In parallel with antioxidant enzyme activity, the accumulation of proline, a non-enzymatic osmoprotectant, was significantly enhanced, especially under Cd20 and Cd30 treatments. Proline plays a dual role: not only does it help in osmotic adjustment (by retaining cellular water) but also acts as a ROS scavenger and a stabilizer of proteins and membranes. The elevation in proline suggests that barley adopts osmotic and antioxidant strategies simultaneously to mitigate Cd-induced damage. The fact that SA application, particularly at SA172, further enhanced proline levels under stress indicates that SA may boost stress tolerance by modulating both hormonal signaling and metabolite accumulation, aligning with the findings of [13]. The idea that mild stress may not yet cause full-scale osmolyte accumulation because the antioxidant system may still be able to handle it well at this point is

further supported by the low proline level at Cd10. The stress severity-dependent proline accumulation model seen in a variety of crops under heavy metal stress is supported by these findings [39].

Under all Cd levels, particularly at Cd10, carbohydrates dropped. It may come as a surprise, but when plants are under stress, they frequently use up sugars for energy or to create defense molecules rather than storing them. Moreover, photosynthesis is known to be harmed by Cd, resulting in lower sugar production (Table 3). The increased carbohydrate accumulation in the Cd0 $\times$ SA86 interaction may reflect a temporary metabolic adjustment, where photosynthesis and sugar metabolism are redirected to support defense mechanisms such as the activity of antioxidant enzymes and osmolyte synthesis. On the other hand, reduced carbohydrate levels in certain treatments may indicate that stress was too severe or that SA disrupted normal carbohydrate metabolism, possibly by affecting photosynthetic efficiency or sugar transport. This supports the idea that sugar balance under stress is highly dynamic and influenced by both stress severity and hormonal modulation [40]. Importantly, the coordination between POD activity, proline accumulation, and carbohydrate levels suggests a systemic stress adaptation strategy in barley. Under moderate stress, all three responses, enzymatic defense, osmolyte production, and energy mobilization, appear to function synergistically to protect the plant. SA plays a regulatory role in fine-tuning this balance, but its effect is highly dose-dependent and influenced by the stress intensity. At lower or optimal doses, SA enhances tolerance by boosting antioxidant capacity and osmolyte accumulation; however, at higher doses or under severe Cd stress, its effectiveness may diminish or even become detrimental due to interference with metabolic pathways.

4.4. Yield and Yield Components

In general, the plants were under the effects of the Cd and SA treatments only, and meteorological data shown in (Table 1) confirm that environmental conditions especially the temperature were within the normal range of barley growth and did not cause stress, where cereals are cultivated in temperate, the average of optimum temperature for maximum grain yields ranged between 14 and 18 C (Chowdhury and Wardlaw, 1978). The results showed (Table 5) appear the significant effects of Cd and SA on the barley yield and its component, revealing both adaptive and stress-related responses. Under Cd stress, barley exhibits a notable shift in growth strategy characterized by reproductive compensation, a form of adaptive plasticity where the plant reallocates limited resources to ensure reproductive success. At the

highest Cd concentration (Cd30), the spike number increased significantly, despite Cd's known phytotoxic effects. This reflects a phenomenon often described as "stress-induced reproductive compensation" or "terminal investment," wherein plants under severe stress select seed production over vegetative expansion to safeguard generational survival [41]. Conversely, spike length decreased at Cd30, indicating a trade-off likely due to Cd-induced inhibition of cell elongation and meristematic activity through disruption of calcium-dependent signaling pathways and cytoskeletal organization [42]. Interestingly, despite shorter spikes, spike weight increased at Cd20 and Cd30, suggesting a reallocation of biomass into compact reproductive structures. The elevated harvest index (HI) at Cd30 further highlights that, under high Cd stress, the plant reallocates assimilates from vegetative parts toward grain filling, likely involving remobilization of stored carbohydrates and nitrogenous compounds [43].

SA had a clear controlling effect on barley's reproductive performance. The moderate SA level (SA86) produced the best results, significantly increasing spike number, length, and weight, grain number, grain, and the biological yield. These effects are consistent with the role of SA as an important molecule signaling key that enhances stress tolerance by activating different antioxidant enzymes, stabilizing membranes, and maintaining hormonal balance under metal stress [22]. However, the highest SA dose (SA172) reduced most parameters, such as spike number and weight, weight of 1,000 seeds, grain, straw, and biological yield (ton ha^{-1}), indicating that excess SA may lead to oxidative imbalance or disrupt growth-promoting hormonal pathways [44]. Interactions between Cd and SA revealed nuanced outcomes. For instance, Cd10 $\times$ SA0 produced the poorest results with spike length and spike grains, likely due to the absence of SA-mediated antioxidant support at a moderately stressful Cd level. In contrast, Cd20 $\times$ SA86 resulted in the highest number of spike grains, highest weight of 1,000 grain, and the highest HI but reduced straw yield, suggesting a shift in carbon partitioning toward reproductive sinks at the expense of vegetative growth. This could be due to SA-induced suppression of auxin biosynthesis or signaling, which is essential for tillering and stem elongation [44]. Surprisingly, Cd30 $\times$ SA0 delivered the highest grain and biological yields, indicating that at severe Cd stress, endogenous defense pathways might already be maximally engaged, and external SA supplementation could disrupt hormonal balance or create redundancy in signaling these results disagree with [36], where Cd stress led to a noticeable decline in plant height, number of spikes, length and grain number and weight,

hundred-grain weight, straw, biological and economic yield of two wheat varieties. Biologically, these findings emphasize that barley possesses inherent mechanisms to prioritize reproduction over growth under toxic conditions, a strategy reflected in high HI values, especially in Cd30 $\times$ SA0 and SA86 and Cd20 $\times$ SA172 treatments. Agronomically, low-dose SA (SA86) supports yield stability under low-to-moderate Cd stress but becomes counterproductive at higher concentrations, potentially due to antagonistic crosstalk with stress-induced hormones such as abscisic acid and ethylene. Therefore, SA application should be context-specific, while SA86 is beneficial at Cd0 to Cd20, it should be avoided at Cd30 to prevent yield reduction.

5. CONCLUSION

Cd is a toxic heavy metal that disrupts plant growth and metabolism. At low levels, however, it may trigger protective responses in barley. This study shows that barley adapts to Cd stress by altering biomass distribution, enhancing antioxidant activity, and prioritizing reproduction over vegetative growth at high Cd levels, where SA modulated these responses in a dose-dependent mode. SA at 86 mg/kg improved barley tolerance and yield under moderate Cd stress, while at 172 mg/kg, it reduced productivity. Under severe Cd stress, plants performed better without SA. Therefore, application of 86 mg/kg SA is suitable for moderately contaminated soils, while avoiding SA in highly Cd-polluted soils is advisable. This study was conducted under pot conditions; field trials are necessary to confirm these findings and suggest future work: Gene regulation, long-term soil studies.

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