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RESEARCH ARTICLE
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# Physiological and Morphological Responses of Wheat (*Triticum aestivum* L.) and Tomato (*Solanum lycopersicum* L.) to Seed Priming with Indole Acetic Acid as Plant Growth Regulator

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## ABSTRACT

The use of plant growth hormones has become increasingly important in agriculture as they have the potential to improve plant growth, act as slow-release fertilizers, and facilitate targeted delivery of agrochemicals for sustainable crop production. In our study, we aimed to investigate the effects of different concentrations of Indole acetic acid (IAA) on the growth rate and antioxidant enzyme activities of wheat and tomato plants. Different concentrations of IAA (control, 0.1, 1, 2.5, 5, 10, and 20mmol.L$^{-1}$) were applied for 24 hours using seed priming techniques for wheat and tomato seeds. Our results showed that the application of IAA resulted in enhanced plant height, shoot and root biomass, and leaf area. Chlorophyll (a, b) and total chlorophyll contents were also promoted in wheat and tomato with varying responses. The highest growth level in wheat was recorded at 2.5mmol.L$^{-1}$ treatment, whereas in tomato, it was recorded at 5mmol.L$^{-1}$ followed by 10mmol.L$^{-1}$ treatment. Moreover, the application of IAA significantly increased antioxidant enzyme activities such as Glutathione, Nitric Oxide, and malondialdehyde. These results suggest that IAA has a different effect on wheat and tomato seed priming, indicating that it may increase plant growth and development in different responses.

**Keywords:** Growth regulators; Antioxidant Enzymes; Growth Rate; Chlorophyll content

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## INTRODUCTION

Indole acetic acid (IAA) which is known as a plant growth regulator (PGR) is essential for controlling several physiological functions in plants, including growth, development, and stress reactions. Plant growth regulators (PGRs) are commonly utilized in horticulture to improve plant growth and yield by enhancing fruit number and size (Serrani et al., 2007; Batlang, 2008). The application of PGRs has shown significant improvement in the production of tomatoes, wheat, and other vegetables, leading to better growth and quality (Saha et al., 2009; Nawaz et al., 2023). In crops like tomatoes, pepper and others, the application of PGRs can induce artificial parthenocarpy, enabling fertilization-independent fruit development and reducing yield fluctuation (Heuvelink & Körner, 2001; Saeed et al., 2023; Zafar et al., 2023). Synthetic gibberellins and auxins are frequently employed to enhance fruit setting in various fruit and vegetable cultivation, such as citrus and tomatoes. This is due to their recognized influence on parthenocarpy, fruit setting, and fruit size. By using PGRs, yields can be dramatically increased, up to four times in some cases.

As natural auxin moves from the stem to the roots, it prompts the overall growth of the root system. This leads to the formation of longer and more branched roots, which enhances the absorption of nutrients from the soil. Consequently, these nutrients are stored in the plant's storage organs, resulting in increased yield (Wang et al., 2005; Anwar et al., 2023). When the source of IAA is eliminated, root stimulation decreases accordingly. IAA is essential for fruit growth and development, retards fruit aging, and plays a minor role in the initiation of flowering and the development of reproductive structures (Asahira, 1967; Ahmed et al., 2023).

IAA plays a major role in regulating various aspects of plant growth, including the elongation of cells, apical dominance, and the formation of vascular tissue (Wang et al., 2021). The process known as lipid peroxidation (LPO) is

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happened when lipids are oxidized by reactive oxygen species (ROS), which damages cell membranes. Cell damage and eventually cell death may result from this (Celik & Tuluce, 2006; Ali et al., 2023). Depending on the IAA concentration and the plant species, IAA treatment can raise LPO levels in plant tissues (Yemelyanov et al., 2020; Zafar et al., 2022). Furthermore, by scavenging ROS and shielding cell membranes from LPO, IAA has been demonstrated to have antioxidant qualities and may aid in the reduction of oxidative stress in plants. This implies that IAA might shield plants from oxidative damage (Das & Roychoudhury, 2014; Rehman et al., 2023). Furthermore, IAA and NO are significant signaling molecules in tomatoes that are crucial for controlling a range of physiological processes, such as defense mechanisms, growth and development regulation, seed germination, root development, and reaction to biotic and abiotic stimuli. Nevertheless, the buildup of ROS may have numerous detrimental effects, such as color bleaching, LPO, and protein degradation (Ali et al., 2023; Singh et al., 2024).

Three amino acids make up the tripeptide molecule glutathione (GSH): glutamic acid, glycine, and cysteine. It is a potent antioxidant that aids in shielding plant cells from synthetic phytohormones, and oxidative stress brought on by pollution (Banerjee & Roychoudhury, 2019; Hossain et al., 2022). In addition, GSH helps detoxify toxic compounds and controls the growth and development of plants. Although the exact nature of the relationship between IAA and GSH in plants is unknown, research has indicated that IAA production and metabolism-related enzyme activity can be influenced by GSH levels (Jozefczak et al., 2012; Hasanuzzaman et al., 2017). By raising the activity of SOD in wheat, exogenous administration of phytohormones is one of the greatest ways to reduce oxidative stress (Guerreiro et al., 2013; Muhammad et al., 2016).

Our study aimed to evaluate the different responses in productivity of two important crops tomatoes and wheat due to treatment by different concentrations of IAA (control, 0.1, 1, 2.5, 5, 10, and 20mmol.$L^{-1}$) using seed priming technique, by measuring the change in the morphological and physiological characteristics as the germination percentage, root length, shoot length, fresh weight, dry weight, and measuring some antioxidant activities.

## MATERIALS & METHODS

The research was performed to study the morphological and physiological response of wheat and tomato plant growth to Indole acetic acid as a growth hormone treatment. Wheat accession ID number (181) with local name Baldy Burr, tomato accession ID number (548). The seeds were collected from the National Gene Bank, Agricultural Research Center (ARC), Minister of Environment, Water and Agriculture, Riyadh, Kingdom of Saudi Arabia. Pre-sterilized seeds of the under-study plants were sown in different concentrations of IAA (Control, 0.1, 1, 2.5, 5, 10, and 20mmol.$L^{-1}$) for 24H, treated seeds were washed in distilled water and then placed in petri dishes, followed by three layers of filter paper that were moistened with distilled water. The control treatment was distilled water. After one week, the germinated seedlings were transferred to plastic pots of 30cm length containing 8kg of clayed soil. The sandy loam soil composition consisted of around 60% sand, 10% clay, and 30% silt particles. Organic manure was incorporated into the soil at a 1:1 ratio; synthetic fertilizers were not applied, and no signs of disease were detected throughout the experimental duration.

A completely randomized design with three replications was used. To assess the possible impacts of various concentrations of IAA on wheat and tomato growth attributes, plant height (cm), shoot fresh and dry mass (g), root length (cm), and root fresh and dry masses (g) were recorded. The leaf area was recorded with a leaf area meter (CI-202 Portable Laser Leaf Area Meter) after four weeks, six weeks, and eight weeks from selected plants of each treatment.

Physiological attributes, such as chlorophyll contents (chlorophyll a, chlorophyll b, and total chlorophyll), were measured by following protocol of Arnon (1949) and antioxidant enzyme activities (GSH, GST, GPX, and MDA) were also analyzed.

### Antioxidant Enzyme Activities Determination

Ten mL of 100mM phosphate buffer was used to grind 0.5g of wheatgrass and tomato seeds, which were then submerged in an ice bath to estimate MDA, NO, GSH, and their corresponding enzymes (glutathione peroxidase, GPx and glutathione-S-transferase, GST). The homogenate was centrifuged for 20min at 4°C at 5000rpm. The various parameters were conducted using the supernatant. The biodiagnostic kit's brochure states that the method, which is based on the determination of thiobarbituric acid reactive substance (TBARS) as a product of lipid peroxidation (LPO), can be modified slightly and is described as follows: thiobarbituric acid reacts with malondialdehyde (MAD) in an acidic medium at 95°C for 30min to form TBARS; the absorbance of this pink product can be measured at 534nm (Buege & Aust, 1978). With a few minor adjustments, the nitric oxide assay was measured using Montgomery and Dymock's (1962) method. With a few minor adjustments, the procedure was reported by Beutler et al. (1963) for the interaction of GSH with 5, 5-dithiobis-2-nitrobenzoic acid, DTNB, to get a yellow color (Beutler et al., 1963). According to the biodiagnostic kit's brochure, the decreased chromogen's absorbance can be evaluated at 450 nm, with a direct correlation to the GSH concentration. Glutathione peroxidase activity is measured using the Paglia and Valentine (1967) method. With very few adjustments, the Habig et al. (1974) approach was applied, and it is reliant on quantifying the conjugation of reduced glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) to estimate the activity of GST. At 340nm, absorbance increases in tandem with conjugation (Habig et al., 1974). The rate of rise is directly correlated with the sample's GST activity as per the biodiagnostic kit's booklet.

**Statistical Analysis**

For this study, all experiments were conducted in triplicates. The mean and SD of the results were calculated. The investigated parameters were calculated by comparing the different gradual concentrations of Indole acetic acid (IAA) groups, 0.1, 1, 2.5, 5, 10, and 20mmol.L$^{-1}$ to the control group. The ANOVA test was performed, and the data are presented as mean±SE from three independent biological replicates. Mean values with distinct letters indicate significant differences as determined by Tukey's multiple range test (P≤0.05).

## RESULTS

**Impact of IAA on Morphological Characteristics of Wheat and Tomato**

The experiment aimed to assess the impact of various concentrations of IAA on the growth of wheat and tomato plants after 24-hour seed priming. Pre-sterilized seeds of the wheat variety and tomato variety were subjected to various IAA concentrations (Control, 0.1, 1, 2.5, 5, 10, and 20mmol.L$^{-1}$) and then germinated using the seed priming technique. The plants were later transplanted to clay soil-filled pots. and data regarding growth parameters (plant heights) were collected and presented in Table 1 and Table 2 and illustrated in Fig. 1 and Fig. 2 for wheat and tomato plants, respectively.

**Impact of IAA on Morphological Characteristics of Wheat**

The measured growth parameters of wheat were collected and presented in Table 1. The data in Table 1 exhibited that the highest root fresh weight (1.98±0.66)) was recorded with 2.5mmol.L$^{-1}$ IAA treatment, followed by 5mmol.L$^{-1}$ treatment which was 1.66±0.72 compared to the control (1.56±0.83). The application of IAA also significantly boosted shoot fresh weight with the same concentrations of IAA. The highest shoot fresh weight (2.53±0.73) was recorded with 2.5mmol.L$^{-1}$ IAA treatment, followed by 5mmol.L$^{-1}$ which was (2.41±0.66) compared to the control (2.11±0.93). After one month of germination, the highest shoot length was recorded with 2.5mmol.L$^{-1}$ IAA treatment, followed by 1mmol.L$^{-1}$ IAA treatment compared to the control Table 1.

The maximum dry mass of the shoot was recorded (0.91±0.20g) with 2.5mmol.L$^{-1}$ IAA treatment, followed by 5mmol.L$^{-1}$ which was (0.82±0.09g) compared to the control (0.66±0.09g). The minimum dry mass of the shoot was recorded with 20 5mmol.L$^{-1}$ IAA treatment (0.31±0.05g). The highest root length after one month of germination was recorded with 2.5mmol.L$^{-1}$ IAA treatment, followed by 5mmol.L-1 compared to the control. The maximum root fresh weight (1.98±0.66g) was recorded with 2.5mmol.L$^{-1}$ IAA treatment, followed by 5mmol.L-1 (1.66±0.72g) compared to the control. The minimum root fresh weight was recorded with 20mmol.L$^{-1}$ IAA treatment. Similarly, the maximum root dry weight was recorded with the same treatment concentrations of 2.5mmol.L$^{-1}$ and 5mmol.L$^{-1}$ as shown in Table 1.

Plant shoot height was monitored during the growth period for two, three, four, and eight weeks to evaluate the effectiveness of the different concentrations of IAA, these results are presented in Table 2 and illustrated in Fig. 1. After two weeks of germination, the induced growth effect was regarded only with 2.5mmol.L$^{-1}$ treatment with plant height was 20.4cm compared with the height of the control treatment which was 18.26cm. Although the other treatments had plant lengths less than the control where the lowest plant height was recorded with 20mmol.L$^{-1}$. After three, four, and eight weeks of germination, the highest plant height was recorded with 2.5 followed by 5mmol.L$^{-1}$ treatment, and the lowest plant height was recorded with 20mmol.L$^{-1}$ treatment. Fig. 2 shows the difference in wheat growth rate due to the application of different concentrations of IAA.

**Table 1:** Effect of different treatments of Indole acetic acid (IAA) on morphological attributes of wheat

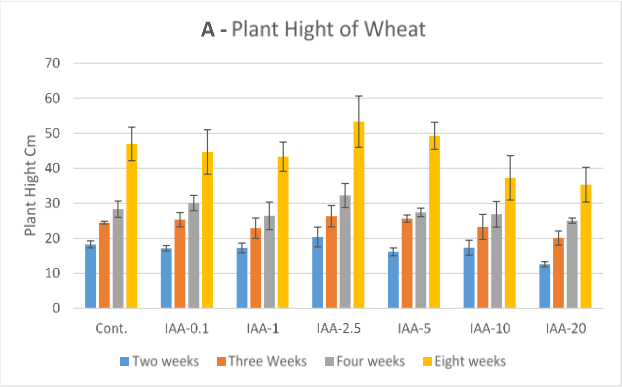
| IAA Treatment | Root length | Root fresh weight | Root dry weight | Shoot length | Shoot fresh weight | Shoot dry weight |
|---|---|---|---|---|---|---|
| Control | 11.24±2.78a | 1.56±0.83ab | 0.26±0.27b | 28.33±2.35a | 2.11±0.93ab | 0.66±0.09ab |
| 0.1 | 9.56±2.34b | 1.29±0.56b | 0.20±0.29b | 30.06±2.2a | 2.29±0.08ab | 0.61± 0.13ab |
| 1 | 12.23±3.12ab | 1.31±0.79b | 0.24±0.18b | 26.43±3.93b | 1.63±0.05b | 0.55±0.18b |
| 2.5 | 15.33±3.84a | 1.98±0.66a | 0.35±0.22a | 32.26±3.44a | 2.53±0.73a | 0.91±0.20a |
| 5 | 13.3±2.95a | 1.66±0.72ab | 0.29±0.09b | 27.43±1.23b | 2.41±0.66a | 0.82±0.09a |
| 10 | 8.32±1.24b | 0.96±0.05c | 0.19±0.04b | 26.89±3.67b | 1.34±0.87b | 0.57±0.07b |
| 20 | 7.34±1.08b | 0.35±0.08c | 0.13±0.09b | 25.06±0.736ab | 0.97±0.06c | 0.31±0.05c |

The data are presented as mean±SE from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test (P≤0.05).

**Table 2:** Plant height of wheat monitoring for 2, 3, 4, and 8 weeks after IAA treatment

| Experimental weeks | IAA Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.1 | 1 | 2.5 | 5 | 10 | 20 |
| 2 | 18.26±1.01ab | 17.16±0.78ab | 17.26±1.38ab | 20.40±2.84a | 16.16±1.13ab | 17.33±2.16ab | 12.6±074c |
| 3 | 24.50±0.4ab | 25.33±2.05a | 22.90±2.9c | 26.33±3.06a | 25.63±1.06a | 23.26±3.56ab | 20.1±2.02ab |
| 4 | 28.33±2.35ab | 30.06±2.2ab | 26.43±3.93ab | 32.26±3.44a | 27.43±1.23ab | 26.89±3.67ab | 25.06±0.736c |
| 8 | 47.00±4.78ab | 44.66±6.34ab | 43.33±4.18ab | 53.33±7.34a | 49.30±3.85a | 37.30±6.34c | 35.33±4.98c |

The data are presented as mean±SE from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test (P≤0.05).



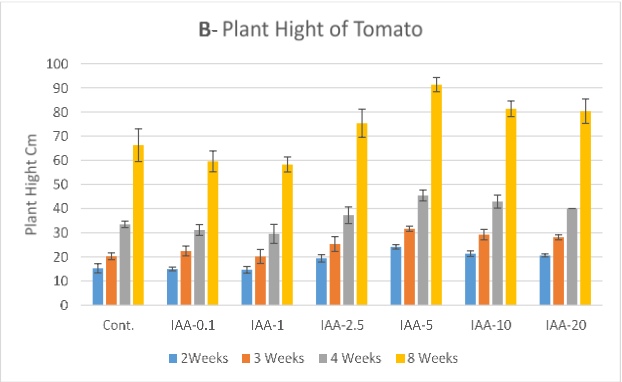




**Fig. 1:** Shows the different responses in plant heights of (A) wheat and (B) tomato after 2, 3, 4, and 8 weeks of treatment with different concentrations of Indole acetic acid (IAA).

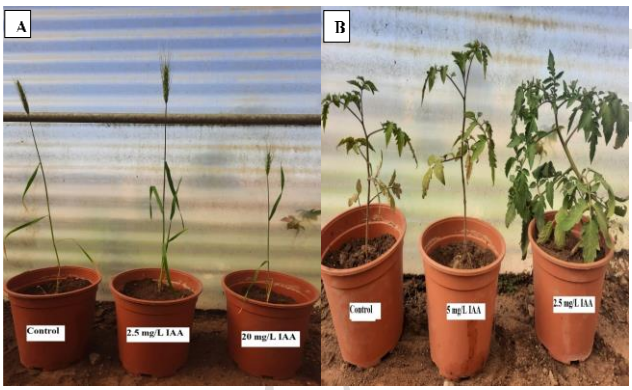
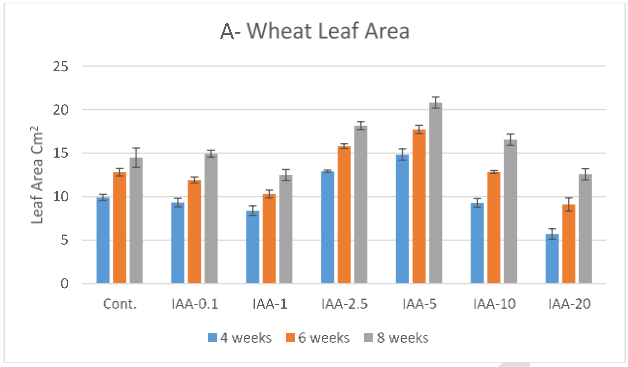



**Fig. 2:** Show the difference in growth rate response for A-wheat and B-tomato with the treatment of Indole acetic acid (IAA).

The leaf area was also observed during the growth periods for four, six, and eight weeks and the results are presented in Table 3 and illustrated in Fig. 3. The highest leaf area was recorded with 2.5 followed by 5mmol.L$^{-1}$ treatment compared with control.

Information regarding the influence of IAA on wheat chlorophyll content is provided in Table 4. The findings reveal significant discrepancies among different treatments. The highest values for chlorophyll a, b, and total chlorophyll were recorded with 2.5mmol.L$^{-1}$ treatment followed by 5mmol.L$^{-1}$ treatment. Similarly, minimum chlorophyll contents' were obtained with 20mmol.L$^{-1}$ treatment, as compared with the control.



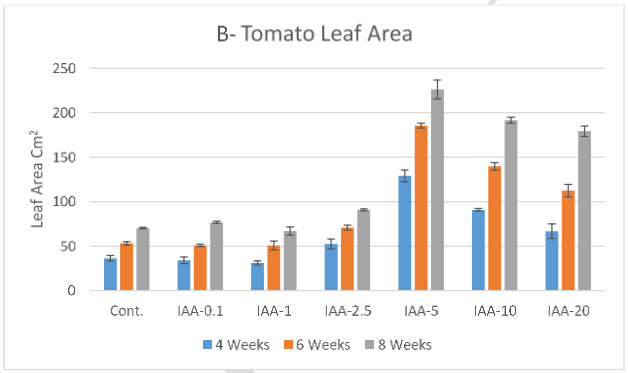




**Fig. 3:** Shows the different responses in the leaf area of (A) wheat and (B) tomato after 4, 6, and 8 weeks of treatment with different concentrations of Indole acetic acid (IAA).

**Impact of IAA on Morphological Characteristics of Tomato**

The measured growth parameters of tomato (Table 5) show that the highest root length after one month of germination was recorded with 5mmol.L$^{-1}$ IAA treatment, followed by 10mmol.L-1 compared to the control. The highest root fresh weight (2.59g) was recorded with 5mmol.L$^{-1}$ IAA treatment, followed by 10mmol.L$^{-1}$ treatment which was (2.22g) compared to the control (2.06g). The minimum root fresh weight was recorded with 20mmol.L$^{-1}$ IAA treatment. Additionally, the maximum root dry weight was recorded with 5mmol.L$^{-1}$. The minimum root dry weight was recorded with 20mmol.L$^{-1}$ as shown in Table 5.

The application of IAA also significantly improved shoot length, after one month of germination, the highest shoot length (45.43cm) was recorded at 5mmol.L$^{-1}$ IAA treatment, followed by 10mmol.L$^{-1}$ IAA treatment which was 39.89cm compared to the control. The minimum shoot length was recorded with 1mmol.L$^{-1}$ IAA treatment. The highest shoot fresh weight was 9.01g which was recorded with 5mmol.L$^{-1}$ IAA treatment, followed by 10mmol.L$^{-1}$ which was 8.53g compared to the control 8.02gm. The maximum dry mass of the shoot was 2.42g recorded with 5mmol.L$^{-1}$ IAA treatment, followed by 10mmol.L$^{-1}$ which was 2.19g compared to the control 2.05g. The minimum dry mass of the shoot was recorded at 20mmol.L$^{-1}$ IAA treatment 1.77g.

Plant shoot height was examined during the growth period for two, three, four, and eight weeks to evaluate the effectiveness of the different concentrations of IAA, these results are presented in Table 6 and illustrated in Fig. 1.

**Table 3:** Wheat leaf area monitoring a long 4, 6, and 8 weeks after IAA treatment

| Experimental weeks | IAA Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.1 | 1 | 2.5 | 5 | 10 | 20 |
| 4 | 9.92±0.358ab | 9.33±0.495ab | 8.38±0.555ab | 14.84±0.649a | 12.92±0.128a | 9.28±0.512ab | 5.7±0.610c |
| 6 | 12.81±0.44ab | 11.91±0.335ab | 10.31±0.45c | 17.72±0.477a | 15.81±0.277a | 12.85±0.162ab | 9.11±0.753c |
| 8 | 14.48±1.118ab | 14.93±0.405ab | 12.5±0.639c | 20.81±0.642a | 18.15±0.455a | 16.55±0.645a | 12.58±0.652c |

The data are presented as mean±SE from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test (P≤0.05).

**Table 4:** Effect of different treatments of IAA on chlorophyll contents of wheat and tomato

| | Chlorophyll in Wheat | | | Chlorophyll in Tomato | | |
|---|---|---|---|---|---|---|
| IAA Treatments | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Total Chlorophyll (mg/g) | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Total Chlorophyll (mg/g) |
| Control | 0.21±0.02b | 0.25±0.02b | 0.46±0.01b | 0.41±0.04ab | 0.46± 0.03ab | 0.87±0.03ab |
| 0.1 | 0.26±0.02a | 0.30±0.02ab | 0.56±0.01ab | 0.35±0.03c | 0.39±0.01c | 0.74±0.04c |
| 1 | 0.28±0.03a | 0.29±0.02ab | 0.57±0.02ab | 0.29±0.01c | 0.32±0.02c | 0.61±0.03c |
| 2.5 | 0.41±0.03a | 0.49±0.03a | 0.90±0.03a | 0.45±0.02ab | 0.57±0.03ab | 1.02±0.06a |
| 5 | 0.32±0.03a | 0.40±0.03a | 0.72±0.02a | 0.58±0.05a | 0.64±0.07a | 1.22± 0.05a |
| 10 | 0.25±0.02a | 0.31± 0.02ab | 0.56±0.01ab | 0.53±0.01a | 0.59±0.08a | 1.12±0.05a |
| 20 | 0.15±0.02c | 0.19±0.02c | 0.34±0.02c | 0.44±0.06ab | 0.50±0.04ab | 0.94±0.03ab |

The data are presented as mean±SE from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test (P≤0.05).

**Table 5:** Effect of different treatments of Indole acetic acid (IAA) on morphological attributes of tomato after one month of treatment

| IAA Treatments | Root length | Root fresh weight | Root dry weight | Shoot length | Shoot fresh weight | Shoot dry weight |
|---|---|---|---|---|---|---|
| Control | 13.24±6.78ab | 2.06± 0.22ab | 0.30±0.06ab | 33.43±1.32ab | 8.02±0.28ab | 2.05±0.19a |
| 0.1 | 11.56±4.34b | 1.99±0.17b | 0.28±0.05ab | 31.16±2.23b | 7.83±0.22ab | 1.91±0.07b |
| 1 | 12.23±3.12b | 1.82±0.28b | 0.24±0.06b | 29.53±3.93b | 7.94±0.56ab | 1.85±0.03b |
| 2.5 | 15.33±5.84a | 2.02±0.27ab | 0.27±0.09b | 37.26±3.44a | 8.33±0.38ab | 2.04±0.13a |
| 5 | 17.3±2.95a | 2.59± 0.22a | 0.42±0.05a | 45.43±2.23a | 9.01±0.63a | 2.42±0.33a |
| 10 | 15.42±3.24a | 2.22±0.37a | 0.33±0.08ab | 39.89±2.67a | 8.53±0.44a | 2.19±0.17a |
| 20 | 10.34±5.08c | 0.95±0.07c | 0.19±0.09c | 38.06±2.36a | 7.27±0.22b | 1.77±0.08c |

The data are presented as mean±SE from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test (P≤0.05).

**Table 6:** Plant height of Tomato monitoring for 2, 3, 4, and 8 weeks after IAA treatment

| Experimental weeks | IAA Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.1 | 1 | 2.5 | 5 | 10 | 20 |
| 2 | 15.26±1.91ab | 14.96±0.78c | 14.66±1.38c | 19.40±1.54a | 24.16±0.93a | 21.33±1.16a | 20.6±0.64a |
| 3 | 20.25±1.4ab | 22.43±2.05ab | 20.19±2.9ab | 25.30±3.06ab | 31.63±1.06a | 29.26±2.16a | 28.1±1.02a |
| 4 | 33.43±1.32ab | 31.16±2.23c | 29.53±3.93c | 37.26±3.44ab | 45.43±2.23a | 42.89±2.67a | 40.06±2.36a |
| 8 | 66.24±6.78ab | 59.56±4.34c | 58.23±3.12c | 75.33±5.84a | 91.3±2.95a | 81.32±3.24a | 80.34±5.08a |

The data are presented as mean±SE from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test (P≤0.05).

The highest shoot length was recorded with 5mmol.L$^{-1}$ IAA treatment followed by 10mmol.L$^{-1}$ at all examination periods. The lowest shoot length was recorded with 1mmol.L$^{-1}$ IAA treatment at all examination periods.

The leaf area was also observed during the growth periods for four, six, and eight weeks and the results are presented in Table 6. And illustrated in Fig. 3. The highest leaf area was recorded with 5mmol.L$^{-1}$ treatment followed by 10mmol.L$^{-1}$ treatment compared with control.

Table 4 displays the impact of IAA on the chlorophyll levels of tomato plants. The outcomes demonstrate notable distinctions among the different treatments. The highest values for chlorophyll contents were recorded with 5mmol.L$^{-1}$ treatment followed by 10mmol.L$^{-1}$ treatment. Additionally, minimum chlorophyll contents' were obtained with 1mmol.L$^{-1}$ treatment, as compared with the control.

**Antioxidant Enzyme Activities Determination**

**Impact of IAA Treatments on Antioxidant Enzyme Activity**

The results of the study on the effect of Indole-3-Acetic Acid (IAA) application on different antioxidant enzyme activities are presented in Table 7. The application of IAA has been found to promote antioxidant enzyme activities. Table 7 and Fig. 4 show that all plant groups exhibited a significant increase in malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH), Glutathione Peroxidase (GPx), and glutathione -S-transferase (GST)

**Table 7:** Antioxidant activities of Wheat (W) and Tomato (T) as Lipid Peroxidation Products (malondialdehyde, MDA), nitric oxide (NO), Glutathione (GSH), Glutathione peroxidase (GPx), and Glutathione-S-transferase (GST) Concentration (µmol/g. tissue) at Different Indole acetic acid (IAA) treatments

| Lipid Peroxidation Product | Control | IAA-0.1 | IAA-1 | IAA-2.5 | IAA-5 | IAA-10 | IAA-20 |
|---|---|---|---|---|---|---|---|
| MDA-W | 17.37±0.41ab | 18.07±0.38ab | 19.73±0.25ab | 30.7±0.51a | 27.22±0.53a | 20.03±1.26) a | 23.87±1.36a |
| MDA-T | 8.57±0.5ab | 10.8±0.72ab | 16.7±0.36a | 21.9±1.69a | 77.9±2.1a | 44.4±3.47a | 35.87±0.76a |
| NO-W | 145±5ab | 150.3±3.51ab | 160±3.6ab | 201.3±1.53a | 311.7±7.64a | 262.3±8.6a | 228.7±3.21a |
| NO-T | 89.3±5.4ab | 95.2±1.6ab | 99.1±0.79ab | 210±1.3a | 177.6±2.5a | 112.7±2.6a | 153±2.7a |
| GSH-W | 2.21±5c | 3.76±0.15ab | 6.71±0.28ab | 7.47±0.47a | 14.22±0.63a | 13.68±0.19a | 9.12±0.75a |
| GSH-T | 14.22±0.63ab | 18.68±0.82ab | 57.39±3.05a | 69.28±0.75a | 122±4.76a | 115.7±4.76a | 91.88±3.46a |
| GPx-W | 13.3±3.6ab | 17.5±0.86ab | 28.9±2.8a | 95.4±5.5a | 84.2±5a | 63.1±3.1 a | 48.2±2.1a |
| GPx-T | 92.3±0.72ab | 102.7±2.2ab | 100.9±2.8ab | 133.5±13.6a | 193.4±5.9a | 194.4±5.5a | 164±4.1a |
| GST-W | 4.22±0.21ab | 6.14±0.48ab | 9.1±2ab | 14.7±1.2a | 12.56±0.68a | 10.4±0.13a | 11.1±0.42a |
| GST-T | 30.5±0.79ab | 41±1.9a | 46.2±2a | 48.5±2.5a | 64.9±3.2) a | 56.6±0.68a | 50.7±0.42a |

The data are presented as mean±SE from three independent biological replicates. Mean representative values with different letters are significantly different in a row according to Tukey's multiple range test (P≤0.05).

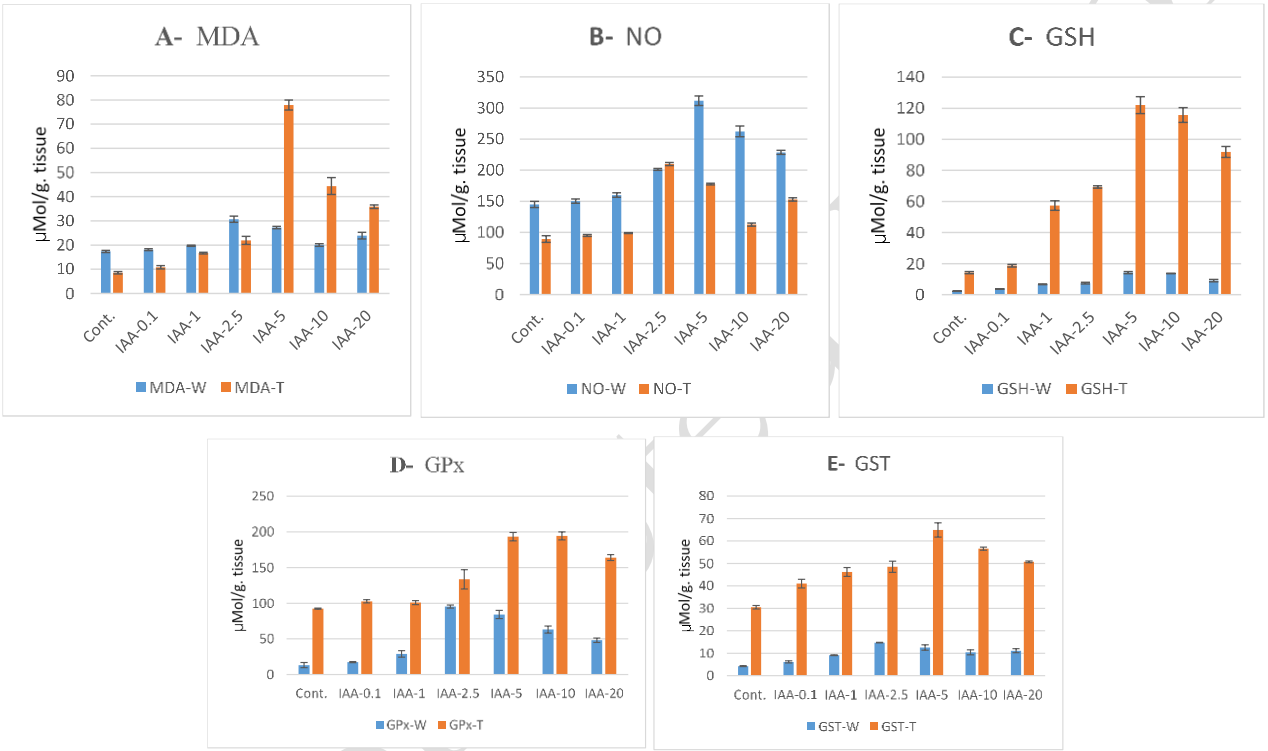



**Fig. 4:** Antioxidant activities of Wheat and Tomato as **A-** Lipid Peroxidation Products (malondialdehyde, MDA), **B-** Nitric oxide (NO), **C-** Glutathione (GSH), **D-** Glutathione peroxidase (GPx), and **E-** Glutathione-S-transferase (GST) Concentration at Different IAA treatments.

activities in both wheat and tomato plants up to a certain concentration, after which there was a decrease in activity compared to the control group.

For wheat seeds, the ideal concentration for (MDA, GPx, and GST) activities was 2.5mmol.L$^{-1}$ followed by 5mmol.L$^{-1}$. In the case of (NO and GSH), it was 5mmol.L$^{-1}$ treatment followed by 10mmol.L$^{-1}$. For tomato seeds, the ideal concentration for the examined antioxidant enzyme activities was 5mmol.L$^{-1}$ treatment followed by 10mmol.L$^{-1}$, except for NO, where the ideal concentration was 2.5mmol.L$^{-1}$ followed by 5mmol.L$^{-1}$.

## DISCUSSION

The purpose of this study was to determine the potential impact of IAA on the growth of wheat and tomato plants. The application of various concentrations of IAA through seed priming significantly enhanced the growth of both wheat and tomato plants in comparison to the control group. The analysis revealed that IAA had a significant impact on the development of roots, shoots, and leaves of both wheat and tomato plants, including root and shoot length, root, and shoot fresh and dry weight, and leaf area (Table 1-5).

The study revealed that the most effective concentration of IAA for wheat plants was 2.5mmol.L$^{-1}$, while for tomato plants, the ideal concentration was 5mmol.L$^{-1}$, followed by 10mmol.L$^{-1}$. It was observed that 2.5mmol.L$^{-1}$ treatment concentration enhanced all measured morphological attributes such as root length, root fresh and dry weight, shoot length and shoot fresh and dry weight in the wheat plant (Table 1). Our results agrees with the findings of Al-haidary and AL-zubaidy (2019), who revealed that Indole-3-acetic acid (IAA) is a

plant hormone that promotes wheat growth. They found that foliar application of IAA at different growth stages of wheat (*Triticum aestivum* L.) resulted in increased plant height, chlorophyll content, spike length, number of spikes per square meter, 1000-grain weight, grain yield, and biological yield. The study concluded that the best results were achieved with a 100-ppm concentration of IAA. Our study revealed that IAA seed priming of wheat increases wheat chlorophyll content (chlorophyll a and chlorophyll b) with a treatment concentration of 2.5mmol.$L^{-1}$.

The highest root length was observed with a 2.5mmol.L-1 treatment, followed by a 5mmol.L-1 treatment. However, the 10 and 20mmol.L-1 treatments caused root dwarfism Table 1. These results support the findings of Kumar et al. (2017), who concluded that the role of IAA in plant physiology is important in stimulating root thickness, and root number, increasing the plant's nutrient and mineral uptake, and promoting plant growth (Kumar et al., 2017).

Our results disagree with the findings of Edelmann (2022), who indicated that the effect of IAA on root length is still being studied. He suggests that the classical theory for auxin-regulated root growth, which posits the inhibitory action of IAA on the elongation growth of root cells, may not be accurate (Edelmann, 2022).

There is a noticeable effect of IAA 5mmol.$L^{-1}$ treatments on the morphological and physiological attributes of tomato plants as leaf surface area, plant height, shoot dry and fresh weight, root length, root dry and fresh weight, Chlorophyll content followed by 10mmol.$L^{-1}$ treatments. These findings could be ascribed to IAA's function in promoting plant growth and development through its stimulation of various processes, such as cell division, tissue growth, phototropism, gravitropism, apical dominance, lateral root initiation, vascular tissue differentiation, embryogenesis, senescence, and ripening (Naeem et al., 2004). Additionally, it leads to increased cell elongation and the accumulation of building blocks, along with higher saccharide content (Mostafa & Alhamd, 2011).

Our findings align with the findings of Fu et al. (2015), who elucidated the significant role of IAA in regulating plant growth. For instance, it governs the development of vascular tissues, cell elongation, and apical dominance. Similarly, Yurekli et al. (2003) elucidated that IAA stimulates cell elongation by modifying conditions such as an increase in osmotic cell contents and enhanced water permeability into the cell (Yurekli et al., 2003).

Commercially, auxins are employed to enhance crop production and regulate various aspects of plant growth and development. While they facilitate rapid growth, such as in shoot tissues, young leaves, and developing seeds, they may not directly promote lateral root development.

IAA can either remain in its free state or form a conjugated complex with sugars or amino acids in developing leaves and cotyledons (Olatunji et al., 2017). It participates in the ripening of fruit, root growth, and cell elongation activities. It also promotes cell division. Furthermore, it is distributed throughout the plant to regulate several physiological processes and is synthesized in the apical meristem of the plant (Pustovoitova & Zholkevich, 1992; Donoso et al., 2017).

Our findings suggest that the application of IAA can enhance the antioxidant enzyme activities of wheat and tomato plants up to certain concentrations, after which there is a reduction in activity. The study provides insights into the optimal concentration of IAA application for promoting antioxidant enzyme activities in wheat and tomato plants. The increase in oxidants like MDA and NO concentration in tomato (Kamran et al., 2021) and wheat (Bashri & Prasad, 2016) tissues is determined by the dose-dependent nature of IAA as in Table 7 and Fig. 4. Drought-induced oxidative stress is prevented in soybean plants by IAA, which controls NO levels and enhances heme oxygenase-1 production and activity (Lecube et al., 2014). According to Spaepen et al. (2007), there is evidence that IAA and NO can interact and affect each other's signaling pathways in plants (Spaepen et al., 2007). It was demonstrated that the procedure involves a NO-mediated cGMP-dependent pathway (Pagnussat et al., 2003). Likewise, a study has shown that NO plays a critical role in signaling plant responses to biotic and abiotic stimuli (Siddiqui et al., 2011). Furthermore, NO has been linked to the upregulation of heme oxygenase-1 in soybean plants exposed to UVB light (Pagnussat et al., 2002). Research has demonstrated that IAA can cause plant tissues to produce NO, and that NO can alter how IAA affects a plant's ability to grow and develop (Mora et al., 2014). Additionally, in response to shifting environmental conditions, they can cooperate to control nutrient intake and root development. Reactive oxygen species cause LPO, which damages and kills cells by rupturing their membranes. This implies that the effects of IAA on LPO can differ based on the circumstances and be dose-dependent Table 7 and Fig. 4.

ROS levels in plant cells are regulated by GSH and related enzymes, which may have an impact on IAA signaling pathways. IAA and plant GSH have a complicated and intertwined connection overall, with both molecules being crucial to plant growth, development, and stress responses (Raza et al., 2022). Antioxidant enzymes such as GPx help to prevent LPO in wheat and tomato plants. NO is a signaling molecule found in plants, particularly tomatoes, that regulates a few physiological processes and stress responses. Together with IAA, it might influence the growth, development, and stress tolerance of plants. It regulates the development and growth of tomato and wheat plants. It can influence the expression of antioxidant enzymes and alter the plant's defense mechanisms, which in turn influences the plant's reaction to stress. According to reports, IAA can stimulate the development of antioxidant enzymes in response to oxidative stress, which helps shield plant cells from reactive oxygen species-induced damage. Furthermore, it has been demonstrated that IAA controls GSH levels, a crucial cofactor for GPx. IAA may indirectly affect GPx activity and plant cells' total antioxidant capacity by adjusting GSH levels (Bela et al., 2015; Yang et al., 2017). Utilizing GSH, enzyme GPx catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides, protecting tomato and wheat plants from oxidative damage as investigated in Table 7 and Fig. 4.

The increase in GST enzyme activity in tomatoes and wheat helps in detoxification by combining GSH with toxic chemicals to make them more soluble in water and make removal easier. The main antioxidant defense systems in plants that combat oxidative stress are non-enzymatic ones like GSH and enzymatic ones like GST (Hasanuzzaman et al., 2017). The antioxidant defense system and ROS accumulation in plant cells should ideally maintain a steady-state equilibrium to maintain proper redox biology responses, which regulate various processes necessary for plant growth and development (Sharma et al., 2019; Zandi & Schnug, 2022). This equilibrium is upset by an abundance of ROS, such as LPO and NO, which causes cellular damage, programmed cell death, and an increase in ROS production (Tanwir et al., 2022; Faizan et al., 2023).

**Conclusion**

From the present experiment, it can be concluded that various levels of plant growth regulators as indole acetic acid (IAA) significantly affected the various growth stages of wheat and tomato. The application of IAA as a seed priming, concentration of 2.5mmol.$L^{-1}$ enhanced the morphological and physiological parameters of wheat, despite Tomato having different responses for IAA treatment where 5mmol.$L^{-1}$ treatment was the ideal concentration of IAA followed by 10mmol.$L^{-1}$ treatment. Our results suggest that the application of IAA can also enhance the antioxidant enzyme activities of wheat and tomato plants up to certain concentrations, after which there is a reduction in activity. Further, insight analysis will be helpful in determining the correct mode of action for indole acetic acid.

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