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Effect of germination stimulants and *Trichoderma harzianum* on germination indicators of stored wheat seeds

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ABSTRACT

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Wheat is one of the most important strategic cereal crops and one of the most widely produced and consumed in the world. A field experiment was conducted during the 2024/2025 growing season at Thi-Qar University, in the Physiology Laboratory of the College of Agriculture and Marshes, on December 12, 2024, to study the effect of the *T. harzianum* and gilibric and salicylic acids on wheat seeds of the Research 22 variety, stored for one and six years. Twenty-eight Petri dishes were used, with three replicates and three treatments, in which 10 seeds of the Research 22 wheat variety, stored for one and six years, were planted. The rate of germination and mortality of seedling were as well determined in plastic pots. The germination rate' results of the seeds of wheat by using seeds stored for one and six years respectively demonstrated significant differences between treatments. Gilibric and salicylic acids treatment in seeds stored for 1 year gave the highest average germination rate compared to the other treatments, reaching 100%, joined by the treatment of gilibric acid and a *T.harzianum* , where the rate of germination reached 93.33%. On the other hand, gilibric acid and *T. harzianum* for seed of 6-year storage had the lowest average germination percentage (10 %). The longest average length of plumule after 11 days for 6-year-stored seed was 15.67 cm, in *T. harzianum* treatment while the lowest was found to be (4.00 cm) for the salicylic acid treatment both for the 1-year-and 6 -year stored seedlings. In results the 1-year-stored seed had an average of radicle length (cm) for *T. harzianum* treatment 9.67 cm, and the lowest average radicle length for the 1-year-stored seed was 4.00 cm for the combination treatment between *T. harzianum* and salicylic acid.

1-Introduction

Wheat is one of the most important strategic grain crops and the most produced and consumed in the world. A third of the world's population depends on it for food. Due to the global population explosion, wheat production has not kept pace with this increase. Global wheat production reached 761.7 million tons in 2020, compared to 732.4 million tons in 2019 [1]. In Iraq, wheat production in 2021 reached 4,234,000 tons, a decrease of 32.1% compared to the previous year's production of 6,238,000 tons. This is despite the fact that the area cultivated with wheat in Iraq reached 9,464,000 dunams in 2021, an increase of 10.4% compared to 2020, when it was 8,574,000 dunams. In terms of productivity, the average yield per dunam, based on the total cultivated area, was 447.3 kg per dunam, a decrease of 38.5% compared to the 2020 wheat yield of 727.6 kg per dunam [1].

The increasing worldwide population requires enhanced production methods to boost both numerical output and product quality by using innovative crop species together with contemporary farming techniques. The plant hormone gibberellic acid serves as a vital tool for triggering seed germination during its essential role in plant development. The process becomes faster during seed emergence and germination when plants face various environmental conditions [2]. The plant hormone functions to stimulate growth because it promotes both cell division and cell elongation processes. The distribution of hemicellulose fibrils in cell walls becomes affected by this process which reduces their stiffness while increasing their flexibility to enable cell growth [3]. Gibberellic acid raises cell contents, driving to raised swell and osmotic pressure, proliferating water influx, and consequently, cell expansion. Gibberellic acid impacts germination through two processes: reducing the outer tissues mechanical resistance surrounding

the embryo and increasing the embryo's potential for development and growth. Gibberellic acid plays a role in water absorption and cell division, which leads to an increase in cell size by increasing protoplasmic content, thus increasing the surface area of plant and overall size. The gibberellic acid natural presence within the seed reduces the dormancy and inhibitory effect of abscisic acid during seed development [4].

Salicylic acid is a plant hormone of a phenolic nature, which affects many physiological processes, including regulation of ion uptake, flowering induction, stomata opening and closing, hormonal balance, and photosynthesis. Also, it has an important role in regulating the response of plant to environmental stress conditions. This compound provides protection against types of environmental stress such as salt, drought, heat and heavy metals stress. It has physiological roles in ethylene synthesis, and an opposite effect to the growth inhibitor, and accelerates the formation of chlorophyll and carotene pigments, speeds up photosynthesis, and increases the activity of some important enzymes. Also, it has a role in thermoregulation in some plants, and numerous studies have indicated that salicylic acid may contribute to the regulation of gene expression signaling during leaf aging. Given the many physiological roles of salicylic acid in plant growth, development, and maturation, this compound has been added to the list of known plant hormones (auxins, gibberellins, and cytokinins). Currently, it is considered a natural plant hormone [5].

Fungi are highly diverse organisms and play a crucial role in ecosystems. In addition to being decomposers, they have numerous relationships with their hosts and competitors, the medium on which they grow, and even with other factors. Some of these relationships may be beneficial to organisms in the abiotic environment, while

others are limiting to the growth of at least one organism. Through these relationships, fungi can be identified as potential biological control agents. These relationships within and between biotic communities are multifaceted, ranging from synergistic to mutualistic. *Trichoderma harzianum* is primarily used to control soil-borne diseases, as well as some leaf and spike diseases affecting various plants. Its role extends beyond disease prevention; it also promotes plant growth, improves nutrient utilization efficiency, enhances plant resistance, and improves the environment for chemical agricultural pollution. *T. harzianum* is a safe, low-cost, effective, and environmentally friendly biological control agent for various crops [6].

The study investigates how *T. harzianum* controls plant diseases through its biological mechanisms which include competition, antagonism, mycoparasitism, and its ability to enhance plant development and activate systemic defense responses in plants. The researchers tested *T. harzianum* to see how it affected wheat seeds which had been stored for either one year or six years. The researchers applied gibberellic acid together with salicylic acid to test their impact on wheat seeds which had been stored for different durations including one year and six years.

2.MATERIALS AND METHODS

Research Site

The field experiment took place during the 2024/2025 growing season at Thi-Qar University within the Physiology Laboratory of the College of Agriculture and Marshes. The investigation of *T. harzianum* effects on Research 22 wheat seeds stored for six years and one year began on December 12, 2024. The study utilized twenty-four Petri dishes which contained three repetitions and three different experimental conditions. The

stored wheat variety Research 22 required ten seeds to be planted inside the Petri dishes. The research team studied seedling death rates together with germination percentages by planting the seeds into 0.5 kilogram plastic containers which they filled with peat moss and soil mixed at a 1:3 ratio.

Preparation of *T. harzianum*

The laboratory prepared *T. harzianum* which had been cultured in its natural state using PDA medium during the experiment. The PDA medium spores underwent dilution through distilled water before use for three separate treatment applications [7].

Preparation of Salicylic Acid

Different salicylic acid concentrations were prepared by first dissolving 1 gram of salicylic acid into 50 milliliters of distilled water and then mixing it with 50 milliliters of ethyl alcohol. The solution required ten minutes of stirring while being heated on a hot stirrer before adding distilled water to reach a target concentration of 250 mg/L. The dilution method served as the basis for preparing the different levels of solution [8].

Preparation of Gibberellic Acid

To create various concentrations of gibberellic acid, one gram of gibberellic acid was mixed with distilled water before adding two drops of sodium hydroxide at 1 N concentration, and then the solution was completed to reach 1000 milliliters using distilled water [9].

Seed Treatment and Studied Traits

Wheat seeds stored for one year and six years were soaked in the treatments shown in Table 1 for one hour to determine the effect on wheat seeds germinated in Petri dishes. Ten seeds were planted in each Petri dish, and each dish was treated separately.

The number of germinated seeds was counted after 10 days, and the germination percentage was calculated in the Petri dishes. The same calculation was repeated after another 10 days, and the overall germination percentage, plumule length, and radicle length were determined [9]. The treatments were repeated in the half-kilogram plastic pot experiment with a 3:1 peat moss:soil mixture ratio. Germination was measured after 10 days, and seedling mortality was measured after 30 days. A completely randomized block design with the least significant difference test was used.

3.RESULTS AND DISCUSSION

Germination Percentage

The results in Tables 2 and 3 indicate the germination percentage of wheat seeds using seeds stored for one year and six years respectively. A significant difference was observed between the treatments, as the treatment combining gibberellic acid and salicylic acid in seeds stored for one year gave the highest percentage of germination compared to the other treatments, reaching 100%. This was followed by the treatment of gibberellic acid and *T. harzianum*, where the percentage of germination reached 93.33%. In contrast, gibberellic acid and *T. harzianum* treatments for seeds stored for six years gave the lowest percentage of germination, which was 10%.

The reason for this superiority is attributed to the gibberellic acid and *T. harzianum* treatment. Gibberellic acid functions to enhance plant development and germination through activation of cell division and elongation, regulating seed germination and germination speed [10]. The fungus *T. harzianum* functions to decrease seed germination time while boosting the process of seed germination. The combination of gibberellic acid with *T. harzianum* produced beneficial effects which enhanced plant growth and boosted

seed sprouting success. The process led to better radicle and plumule development and higher germination success rates.

Plumule Length

The highest average plumule length after eleven days for one-year-stored seeds was noticed in the *T. harzianum* treatment, reaching 15.67 cm. The lowest average plumule length was noticed in the salicylic acid treatment for both one-year and six-year-stored seeds, reaching 4.00 cm, as shown in Tables 4 and 5.

The production of cell walls and cell division processes become more efficient because gibberellic acid promotes these functions which enhance photosynthetic performance and nutrient movement throughout the plant. The process creates longer cells which produce additional leaf base structures that result in longer plumule and radicle growth and better seed germination success. The plant uses this process to generate more cells which then grow longer in its developing sections while it also drives its overall growth. The process results in longer plumule development together with extended radicle growth and improved seed germination success rates. This aligns with Weaver's [11] finding that soaking seeds with gibberellic acid resulted in a significant increase in height.

Radicle Length

The results in Tables 6 and 7 showed the average radicle length, with the highest average length (9.67 cm) noticed in the *T. harzianum* treatment for one-year-stored seeds, and the lowest average radicle length (4.00 cm) observed in the combination treatment of *T. harzianum* and salicylic acid for one-year-stored seeds.

T. harzianum has a positive role in supporting and nourishing the plant and elevating the rate of germination. This was also confirmed by the study by Amal and Shammari [12], which showed an increase in the biomass of plant. This encourages the use of *T. harzianum* as a biofertilizer, as

well as its effect on stimulating plant growth. This is attributed to the secretion of plant growth regulators that work synergistically with other plant growth regulators, increasing the availability and absorption of nutrients by plants and thus enhancing plant growth characteristics. It also has the ability to compete with other fungi harmful to plants, competing for space and nutrients. Furthermore, it secretes enzymes that break down the cell walls of harmful fungi and directly parasitizes other fungi, absorbing their contents.

Plastic Pot Experiment Results

The results of the plastic pot experiment showed the highest germination rates for the treatments of salicylic acid and *T. harzianum*, gibberellic acid, the combination of salicylic acid and *T. harzianum*, the combination of salicylic acid and gibberellic acid, and the control group. The germination rate reached 100% for seeds stored for one year. The highest seedling mortality rate for the same seed was observed in the treatment combining salicylic acid and *T. harzianum*, reaching 2.6%, as shown in Table 8.

Similarly, the results for seeds stored for six years showed the highest germination rate in the salicylic acid treatment, as well as in the combination of gibberellic acid and *T. harzianum*, reaching 83.66%. The seedling mortality rates were 5.3% and 3.5%, respectively, in the control group and the *T. harzianum* treatment. The reason for the increased germination rate and seedling death is attributed to the ability to increase the delivery of water and salts to the leaves, in addition to the fact that *T. harzianum* has the ability to secrete enzymes that strengthen the plant cell walls and enter and strengthen the tissues of the roots and seeds [13].

4. DISCUSSION

The results clearly demonstrate that seed storage duration significantly affects wheat germination and early seedling growth. Seeds stored for one year consistently

outperformed those stored for six years across all measured parameters. This reduction in viability with prolonged storage is attributed to the deterioration of seed reserves, membrane damage, and reduced enzymatic activity essential for germination [4].

The positive effects of gibberellic acid on germination parameters align with its established role in mobilizing seed reserves and promoting embryo growth [2, 3]. Gibberellic acid stimulates the production of hydrolytic enzymes such as α -amylase, which break down stored starch into sugars available for the developing embryo. This explains the improved germination percentages and enhanced plumule and radicle growth observed in GA₃-treated seeds.

The stimulatory effect of *T. harzianum* on germination and seedling growth parameters is consistent with previous studies [6, 12]. *Trichoderma* species are known to produce plant growth-promoting substances including auxins, gibberellins, and cytokinins, as well as enzymes that facilitate nutrient availability. The fungus also enhances plant defense mechanisms and improves root development, leading to better nutrient and water uptake [7].

Salicylic acid showed variable effects depending on concentration and seed age. While it improved germination in some treatments, higher concentrations or combinations with other treatments occasionally reduced growth parameters. This dual effect of salicylic acid has been documented, where low concentrations promote growth while higher concentrations may induce stress responses [5, 8].

The superior performance of combination treatments, particularly GA₃ + SA for germination percentage and GA₃ + *T. harzianum* for growth parameters, suggests synergistic interactions among these treatments. Gibberellic acid and *T. harzianum* may complement each other by providing both hormonal stimulation and

enhanced nutrient availability, leading to improved seedling vigor [10].

The reduced effectiveness of all treatments in six-year-stored seeds indicates that prolonged storage leads to irreversible seed deterioration that cannot be fully overcome by exogenous growth regulators or beneficial microorganisms. This emphasizes the importance of using fresh, high-quality seed for optimal crop establishment.

5. CONCLUSION

The study concludes that seed storage duration significantly affects wheat germination and seedling growth, with one-year-stored seeds performing better than six-year-stored seeds. Treatments with gibberellic acid, salicylic acid, and *T. harzianum* improved germination and early growth parameters, especially in recently stored seeds. The combination of gibberellic acid and salicylic acid produced the highest germination percentage, while *T. harzianum* treatment resulted in the longest plumule and radicle lengths. However, prolonged storage decreased seed viability and limited the effectiveness of these treatments. The study recommends using fresh wheat seeds (stored for one year or less) for optimal germination and seedling establishment, and suggests that seed treatment with gibberellic acid, salicylic acid, or *T. harzianum* can enhance germination and early growth, particularly when used in appropriate combinations.

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AUTHORS' CONTRIBUTIONS

All activities were performed by author.

COMPETING INTEREST

The author affirm that he has no financial conflicts of interest or competing interests in this study.

6. REFERENCES

- [1] Directorate of Agricultural Statistics. (2021). Estimation of wheat and barley production. Ministry of Planning, Central Statistical Organization of Iraq.
- [2] Asif Mahamud, M., Imran, S., Paul, N.C., Rabbi, R.H.M., Jahan, N., Sarker, P., & Rhaman, M.S. (2025). An overview and current progress of gibberellic acid-mediated abiotic stress alleviation in plants. *Plant, Soil and Environment*, 71(7), 453-479. <https://doi.org/10.17221/137/2025-PSE>
- [3] Dai, L., Zhao, X., Liu, S., Keerthana, K., Vijayakanth, V., Zhi, Y., Chen, M., Que, F., Ramakrishnan, M., Ahmad, Z., & Wei, Q. (2025). Gibberellin-mediated internode elongation in grasses with a focus on bamboo: molecular pathways and regulatory networks. *Frontiers in Plant Science*, 16, 1665328. <https://doi.org/10.3389/fpls.2025.1665328>
- [4] Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant Physiology and Development* (6th ed.). Sinauer Associates.
- [5] Spoel, S. H., & Dong, X. (2024). Salicylic acid in plant immunity and beyond. *The Plant Cell*, 36(5), 1451–1464. <https://doi.org/10.1093/plcell/koac058>
- [6] Adss, I., Amer, G. M., Bayoumy, S. R., & Eid, A. R. (2021). Effect of Abscisic Acid, Salicylic Acid, Potassium Silicate, and *Trichoderma harzianum* As Biocontrol Agent to Induce the Tomato Resistance Against Early Blight Disease Caused by *Alternaria solani*. *Alexandria Science Exchange Journal*, 42(3), 773-788. <https://doi.org/10.21608/asejaiqisae.2021.19641>
- [7] Silva, R. L., Pereira, F. N., & Souza, A. C. (2025). Increase of *Trichoderma harzianum* production using mixed-level fractional factorial design. *Applied Sciences*, 13(16), 9244. <https://doi.org/10.3390/app13169244>
- [8] Hayat, Q., Hayat, S., Irfan, M., & Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: A review. *Environmental and Experimental Botany*, 68(1), 14–25. <https://doi.org/10.1016/j.envexpbot.2009.12.005>

[9] Al-Rubaie, A. H., & Al-Saadi, A. H. (2013). Effect of gibberellic acid and seeding depth on germination and seedling growth of wheat (*Triticum aestivum* L.). *Basrah Journal of Agricultural Sciences*, 26(1), 123-134.

[10] Zhong, C., Xu, H., Ye, S., Wang, S., Li, L., Zhang, S., & Wang, X. (2015). Gibberellic Acid-Stimulated Arabidopsis6 Serves as an Integrator of Gibberellin, Abscisic Acid, and Glucose Signaling during Seed Germination in *Arabidopsis*. *Plant Physiology*, 169(3), 2288-2303. <https://doi.org/10.1104/pp.15.00858>

[11] Weaver, R. J. (1972). *Plant Growth Substances in Agriculture*. W.H. Freeman and Company, San Francisco.

[12] Amal, A. M., & Shammari, A. A. (2005). Effect of *Trichoderma harzianum* on growth and yield of tomato. *Iraqi Journal of Agricultural Sciences*, 36(4), 45-52.

[13] Al-Maliki, A. A. T. (2009). A study of cucumber seedling death and root rot caused by the fungus *Pythium aphanidermatum* (Edson) Fitz and its control and integrated control potential [Master's thesis]. University of Basrah, College of Agriculture.

Table 1: Treatments used in the Experiment

Treatments	Meaning
S	Salicylic Acid
T	<i>T. harzianum</i>
G	Gilibric Acid
T.G	<i>T. harzianum</i> + Gilibric Acid
T.S	<i>T. harzianum</i> + Salicylic Acid
G.S	Salicylic Acid + Gilibric Acid
T.G.S	<i>T. harzianum</i> + Gilibric Acid + Salicylic Acid
C	Control

Table 2: Germination rate of wheat seeds using seeds stored for one year

Treatments	Germination rate mean after 3 days	Germination rate mean after 6 days
S	43.33	86.66
T	66.66	86.66
G	90.00	86.67
T.G	66.66	93.33
T.S	66.66	90.00
G.S	73.33	100.00
T.G.S	63.33	90.00
C	86.66	90.00

G	4.33	6.67
T.G	6.00	10.00
T.S	5.00	7.33
G.S	7.33	9.00
T.G.S	6.33	8.00
C	5.66	8.33
L.S.D	2.529	3.462

Table 6: Radicle length of wheat seeds using seeds stored for one year

Treatments	Radicle length mean after 8 Days	Radicle length mean after 11 Days
S	5.00	4.33
T	7.00	8.00
G	7.00	7.33
T.G	7.17	8.00
T.S	5.83	5.33
G.S	5.33	6.67
T.G.S	6.50	6.67
C	6.50	7.33
L.S.D	1.767	2.596

Table 7: Radicle length of wheat seeds using seeds stored for 6 years

Treatments	Radicle length mean after 8 Days	Radicle length mean after 11 Days		
S	3.66	4.33		
T	6.50	7.00		
G	4.33	7.67		
T.G	6.67	8.33		
T.S	4.17	4.00		
G.S	4.33	4.67		
T.G.S	6.00	L.S.D	6.00	3.706
C	8.33	5.33		

Table 8: Germination and seedling mortality rates for wheat seeds stored for one year in pots

Treatments	Germination rate %	Mortality rate %
S	100	0
T	100	0
G	100	1.6
T.G	96.66	0
T.S	100	2.6
G.S	100	1.6
T.G.S	96.66	0
C	100	0
L.S.D	5.21	3.14

Table 9: Germination and seedling mortality rates for wheat seeds stored for 6 years in pots

Treatments	Germination rate %	Mortality rate %
S	86.66	0
T	83.33	3.5
G	83.33	1.3
T.G	86.66	0
T.S	73.33	2.6
G.S	83.33	2.6
T.G.S	83.33	0
C	73.33	5.3
L.S.D	7.11	1.62