



GENETIC DIVERSITY OF TILLETIA SPP. CAUSES OF COVERED SMUT DISEASE OF WHEAT AND IT RESISTANCES

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Article info	Abstract
Received: 2025-01-05 Accepted: 2025-02-26 Published: 2025-06-30	The research was conducted in the Department of Plant Protection- College of Agriculture- Anbar University. Aimed to detect the genetic diversity of fungal species responsible for covered smut disease in wheat and evaluate the effectiveness of inducers, hydrogen peroxide, melatonin, and salicylic acid, in combating the disease and improving growth parameters. The genetic analysis revealed that the isolates R14 (Anbar - Al Jazeera) and O21 (Nineveh - Mosul) were closely related to the isolate identified as MH855829.1, showing a 99% identity with <i>T. caries</i> fungal isolates. Similarly, the isolates R5 (Salah al-Din - Makishifa) and R16 (Anbar - Al-Karma) similarity to the isolate MH114992.1, with a 99% match to <i>T. controversa</i> . Furthermore, the isolates R3 (Baghdad - Al-Ridwaniyah) and S21 (Salah al-Din - Samarra) were closely related to the isolate MH4980301.1, with a 99% match to <i>T. indica</i> . The results showed that the Nazar variety, without of pathogen, exhibited the highest absorption rate of 60%, outperforming all other varieties. Additionally, the Pora variety achieved the highest wet gluten percentage, reaching 45.50%. Using inducers (hydrogen peroxide, melatonin, and salicylic acid) at concentrations of 300, 400, and 500 ppm significantly reduced the infection of covered smut disease. The infection rate for hydrogen peroxide treatment was 15.93%, with the 500 ppm concentration being the most effective, reducing the
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infection rate to 17.96%. The best interaction was observed between hydrogen peroxide and the 500 ppm concentration, resulting in an infection rate of just 13.50%.

Keywords: Wheat, Teleospores, Tilletia spp, Genetic diversity, Stimulating agent.

التباين الوراثي للفطر Tilletia spp المسبب لمرض التفحم المغطى في الحنطة ومقاومته

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الخلاصة

نفذت الدراسة في قسم وقاية النبات - كلية الزراعة - جامعة الأنبار وهدفت الى تحديد التباين الوراثي لبعض انواع الفطر المسبب لمرض التفحم المغطى في الحنطة وتقييم كفاءة بعض الاستحثاث (بيروكسيد الهيدروجين والميلاتونين وحامض السالسليلك) في مقاومة المرض وبعض صفات النمو. بينت نتائج القرابة الوراثية ان العزلتين (R14 الانبار - الجزيرة و O21 نينوى- الموصل) كانت قريبة الصلة للعزلة التي تحمل الرمز (MH855829.1) بنسبة تطابق بلغت 99% مع عزلات الفطر *T.carries*. وان العزلتين (R5 صلاح الدين - مكشيفة و R16 الانبار - الكرمة) كانت قريبة الصلة للعزلة التي تحمل الرمز (MH114992.1) وبنسب تطابق بلغت 99% مع عزلات الفطر *T.controversa*، والعزلتين (R3 بغداد - الرضوانية و S21 صلاح الدين - سامراء) كانت قريبة الصلة للعزلة التي تحمل الرمز (MH4980301.1). وبنسب تطابق بلغت 99% مع عزلات الفطر *T.indica*، وبينت النتائج ان الصنف نزار بدون الممرض سجل نسبة الامتصاصية بلغت 60% متفوقاً على الأصناف جميعها. تفوق الصنف بورا معنوياً بنسبة الكلوتين الرطب اذ بلغ 45.50% على بقية الاصناف، وظهرت النتائج ان استعمال عوامل الاستحثاث (بيروكسيد الهيدروجين، الميلاتونين، حامض السالسليلك) بالتراكيز 300 و 400 ppm والتداخل بين عوامل الاستحثاث وتراكيز استعمالها حقق خفض معنوي في نسبة الإصابة بمرض التفحم المغطى اذ بلغت نسبة الإصابة في معاملة بيروكسيد الهيدروجين 15.93% وكان أفضل تركيز في خفض نسبة الإصابة 500 ppm وبلغت 17.96% اما أفضل تداخل بين بيروكسيد الهيدروجين وتركيز 500 ppm اذ بلغت نسبة الإصابة 13.50%.

كلمات مفتاحية: الحنطة، السبور التيلي، Tilletia spp، التباين الوراثي، عوامل الاستحثاث.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops used as a basic food source for humans, as it is used by more than a third of the global population, about 35% (19). The area cultivated with wheat in the world reached about 222 million hectares, with a productivity of approximately 777 million tons annually. In Iraq, the cultivated area reached 37,856,000 hectares, with an average production rate of 423,400 tons of wheat in 2021 (13). The crop is exposed to obstacles, the most prominent of which are plant diseases (1 and 4). especially wheat smut disease caused by the fungus *Tilletia* spp (8). Three types of smut affect wheat, the most important of which are common smut, dwarf smut, and partial smut (14). Covering smut in wheat is caused by several species belonging to the genus *Tilletia*, which are *T. caries* and the synonym name is *T. tritici*, *T. laevis*, and the synonym is *T. foetidae*, and the third type is *T. controversa* and the synonym name *T. brevifaciens*, which causes dwarf smut disease dwarf bunt (4 and 21). The three types are closely related. There is also a fourth type, *T. indica*, and its synonym, *Neovossia indica*, which causes Karnal bunt, which also causes partial bunt (11 and 14). The ITS (Internal Transcribed Spacer) region was adopted, which is considered more suitable for molecular diagnosis and is used in diagnosing fungi due to its high heterogeneity (16). This region is found in DNA and is considered the best code for using fungi (15). Since this variation in the ITS1 and ITS4 regions is a taxonomic characteristic of any fungus, it is similar to a human fingerprint (5 and 20). In order to reduce the damage resulting from the disease, several environmentally friendly methods were used, the most important of which is induced resistance, the use of which has expanded recently because it has achieved results in laboratory or field experiments (10 and 12). Several factors were used, the most important of which were salicylic acid, peroxides, and glutathione, which work to increase the induced resistance in the plant. Salicylic acid (SA) in plants is a hormone that plays a crucial role in activating plant defenses against pathogens. It also actively contributes to the plant's response to various abiotic stresses, including cold, drought, salinity, and heavy metals. Furthermore, recent studies have highlighted the significant role of SA in plant morphogenesis (23). Melatonin acts as an anti-stress agent, helping plants adapt to various biotic and abiotic stresses. Additionally, gene regulation associated with the primary role of melatonin highlights its function as an antioxidant (2). This, in turn, enhances the growth and quality of fruits and vegetables. This study aimed to detect the genetic diversity of types of fungi that cause common smut disease in wheat, the role of melatonin, Hydrogen peroxide, and salicylic acid against the pathogenic fungus, and some growth parameters were studied.

Materials and Methods

Determining the phylogeny of some types of *Tilletia* spp fungi that cause covered smut disease in wheat spread in Iraq: Gene Viewer Snap software was used to draw a phylogenetic tree according to the protocol described in (17 and 19). To determine genetic diversity and draw the genetic evolutionary tree of isolates registered in GenBank (9). The tree contained *Tilletia* spp. Which infects wheat plants in some governorates of Iraq and is indicated by the symbols (S21, R16, R14, R3, R5, and O21)

and compared with the global isolates that were identified based on the ITS region after determining the sequences of the isolates taken from NCBI and GenBank.

Table 1: Accession numbers in the Gene Bank for some species of *Tilletia* spp.

Sample	Collection place	Diagnosed fungus	The bank number registered with NCBI
S21	Saladin - Samarra	<i>Tilletia indica</i>	OQ657050
R16	Anbar-Al-Karmah	<i>T.controversa</i>	OQ657048
R14	Anbar - Al Jazeera	<i>T.caries</i>	OQ657045
R3	Baghdad - Al-Ridwaniyah	<i>T.indica</i>	OQ657049
R5	Saladin - Makishifa	<i>T.controversa</i>	OQ657047
O21	Nineveh - Mosul	<i>T.caries</i>	OQ657046



Fig. 1: Symptoms and signs of covered smut disease on plants and seeds of wheat.

Testing susceptibility of some wheat varieties to covered smut disease caused by *Tilletia caries* and its effect in some qualitative characteristics of bread flour: The experiment was carried out in the agricultural season 2022 - 2023 and on 15/11/2022 in the field belonging to the Department of Plant Protection - College of Agriculture - Anbar University. Soil used mixed with a ratio of (1:1) (river sand: clay soil), sterilized with formalin solution (the active ingredient). 37% diluted at a concentration of 5%. The soil was moistened with the diluted solution in layers. The soil was covered tightly with polyethylene for 15 days. The polyethylene cover was opened to ventilate the soil and stirred for another 15 days under the sun's rays to aerate and rid the soil of formalin residues. The anvils, 20 cm in diameter, were filled with a 10 kg capacity of sterilized soil and prepared for planting. The experiment was carried out according to a Completely Random Design Using a simple experiment system using five varieties of wheat found in Iraq (Al-arbia, Maya, Nazar, Pora, and Abo-Graib), wheat seeds for the varieties (Al-arbia, Maya, and Nazar) were obtained from the Saqlawiyah- Tharthar Agriculture Division, and the varieties (Pora and Abo-Graib) were obtained. From Karkh Agriculture - Baghdad Agriculture Directorate, the center, seeds were planted at a rate of 5 for each anvil. Three anvils were used in each replicate, and at a rate of 3 replicates for each variety. The seeds for each variety were divided into two parts. One part of the seeds was contaminated with the teliospores of the fungus *Tilletia Caries*, while the other was left as a comparison treatment. It was planted with Seeds 5 cm deep. The experiment was carried out in the following method. The wheat grains of

each mentioned type were washed with water to remove dust and impurities. The wheat grains of each type were sterilized. Sodium hypochlorite (NaOCl) at a concentration of 2% for 3 minutes. The sterilized grains were soaked with a molasses solution to increase the adhesion of the teliospores to the seeds by 5% for 3 minutes. For each variety, the control treatment was planted in the anvils prepared for that purpose at a depth of 5 cm. The wheat grains treated with the molasses solution were placed in polyethylene bags, each variety separately, and were contaminated with teliospores at a rate of 3 g/kg and shaken well to mix the grains with the teliospores. The seeds contaminated with teliospores were planted at a depth of 5 cm in the pots prepared to ensure regular irrigation. In order for water to reach all experimental units equally, a drip irrigation system was installed using 16 mm GR pipes, draining 8 liters per hour, according to the plant's need, in addition to service work for the plant, such as fertilizing, weeding, and maintaining the plants. After the crop fully matured, the following data were recorded for each variety.

Calculating the water absorption rate: The percentage of water absorption was calculated for each of the studied varieties, contaminated and uncontaminated with the fungus *T. caries*. After grinding it with a blender, each type was taken separately, and 10 g of flour was taken from each type. Water was added with a buret and mixed to form a non-sticky, cohesive dough without leaving any dough residue. The water absorption rate inside the container was calculated using the following equation

$$\text{Water absorption rate} = \text{Water absorption} / \text{Weight of flour sample} \times 100 \quad (7)$$

Calculating the percentage of wet gluten: The percentage of wet gluten in the flour of each wheat variety contaminated and uncontaminated with the fungus *T. caries* was calculated. I followed the method (3). Ten grams of flour were taken for each variety separately, and after obtaining the dough, it was ball-shaped and then placed in a container containing tap water. Until submersion and left for an hour. After that, the dough was removed from the water and placed by hand between the fingers while stirring it quietly and washing it under a continuous stream of water at a slow flow of about 3-4 ml s⁻¹. Place a sieve under the running water to avoid losing the dough. The washing process continued until the starch present in the dough was eliminated. The gluten was squeezed into a transparent bowl containing clear water to ensure this. If the water was turbid, it indicated the presence of starch, and the washing must continue after completion. Squeeze the gluten into a ball and weigh it after six hours. After that, the calculations were made. The percentage of wet gluten is calculated using the following equation:

$$\text{Percentage of wet gluten} = \text{weight of wet gluten} / \text{weight of flour sample} \times 100 \quad (3).$$

Effect of some induction agents and concentration on germination percentage and infection of wheat caused by *T. caries*: The test was carried out using a completely randomized design (CRD) in a factorial experiment system. The wheat seeds of the Nazar variety were washed with running tap water for 30 minutes to remove impurities and suspended dust. Then, the seeds were left on a flat surface until dry. Then, the wheat seeds were placed in a 25 ml sodium hypochlorite sterilization solution with 75 ml Sterile distilled water for five minutes. Then, the sterilization solution was poured, and the seeds were washed with sterile water to remove the Effects of sterilization; wheat seeds were divided into 100 grams for each of the concentrations of the tested

substances individually and 100 grams for the comparison treatment. I used three dishes for each of the concentrations and three for the comparison treatment, after which the seeds were placed in Petri dishes and immersed in the concentrations of the tested substances individually. As for the comparison treatment, its seeds were immersed in sterile distilled water. Soaking the seeds continued for 8 hours, after which the solution for all treatments was poured. One hundred fifty seeds were taken from each treatment, divided into three dishes, and left for five days under laboratory conditions. Then, the percentage germination rate was calculated using the following equation: % germination = number of germinated seeds for each treatment / total number of seeds * 100%. The remaining seeds were planted after being contaminated with fungus *T. caries* fungus spores, which were 3 grams per kilogram in pots of 10 kilograms in a sterilized soil container with five seeds. As for the comparison treatment, wheat seeds were planted without contamination, and after the crop matured, the infection rate was calculated according to the following equation: % of infection = number of infected plants per treatment / total number of plants in the treatment * 100% (4).

Results and Discussion

Determine genetic kinship Phylogenetic analysis: The results in Figures 1 and 2 showed that the two isolates (R14 and O21) were closely related to the strain bearing the symbol (MH855829.1), with an identity rate of 99% with the *T. caries* isolates. The two isolates (R5 and R16) were closely related to the strain bearing the symbol (MH114992.1), with identity rates reaching 99% with isolates of the fungus *T. controversa*, and the two isolates (R3 and S21) were closely related to the strain bearing the symbol. (MH4980301.1) with an identity rate of 99% with the isolates of the fungus *T. indica*. The results showed that all branches arising in the tree show locations close to each other. It is clear from the results above that the isolates analyzed were closely related to the fungi *T. caries* *T. controversa* and *T. indica*. This gives evidence of the ability of these special primers to describe the sequences of *Tilletia* spp., which were verified according to their genetic location. The high degree of relatedness and genetic similarity of the locally isolated fungal community may be due to the emerging evolution of species across According to their genetic location, the high degree of relatedness and genetic similarity to the locally isolated fungal community, perhaps due to the emerging evolution of the species over time, indicates the presence of this evolutionary trait, which indicates the high accuracy of the ITS2-ITS1 used for PCR sequences in detecting the fungal sequences that were currently examined. Moreover, the tree drawing shows the isolates as somewhat heterogeneous, which could be due to genetic mixing by introducing varieties from plant families carrying different strains, leading to genetic diversity among the fungi studied (17).

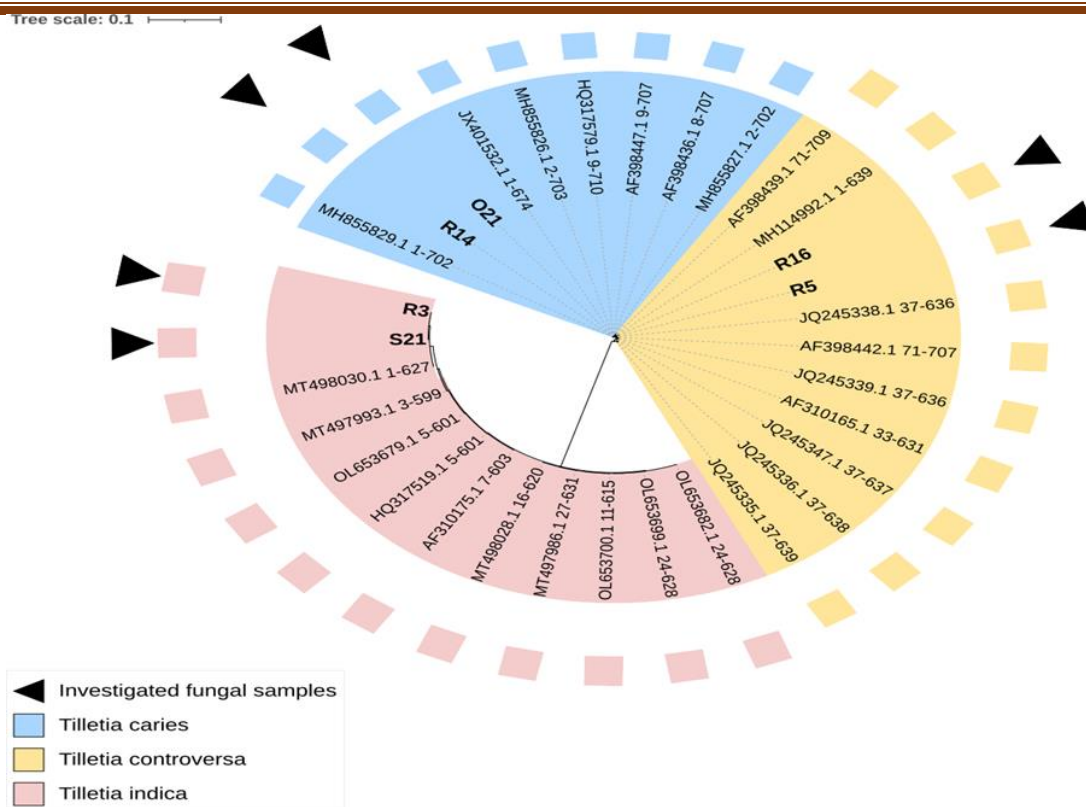


Fig. 2: Comprehensive circular phylogenetic tree of genetic variants of the ITS1-ITS2 sequences for six isolates of *Tilletia caris*, *Tilletia controversa*, and *Tilletia indica*. The black triangle indicates the isolates analyzed.

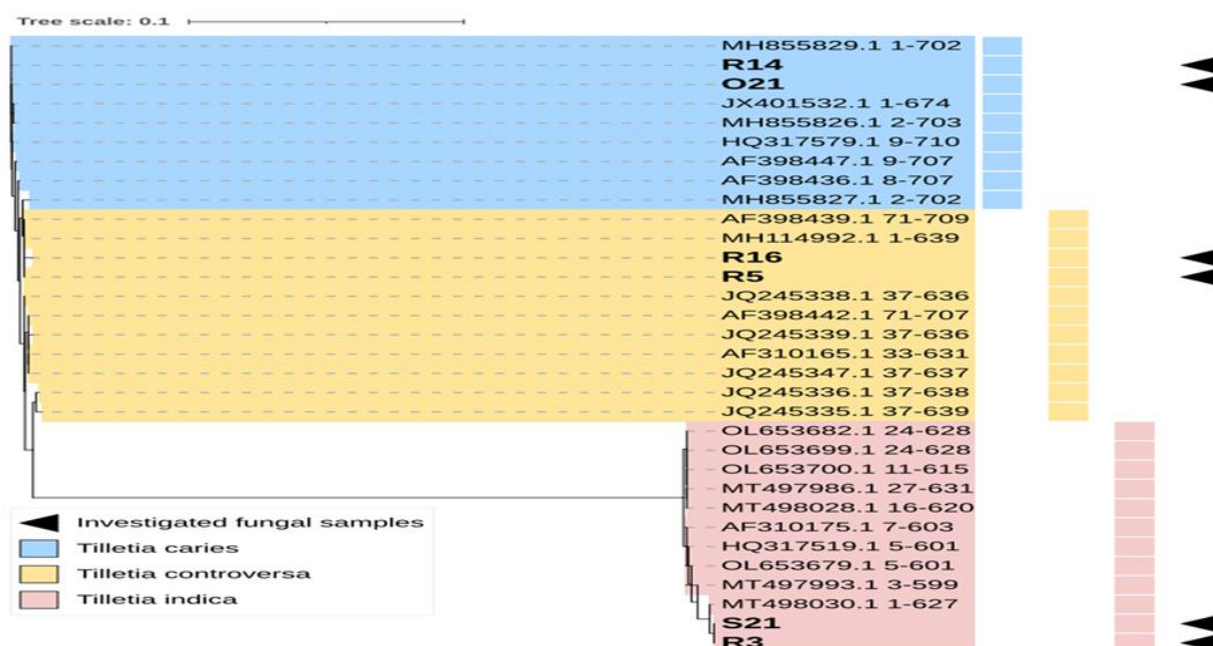


Fig. 3: Comprehensive rectangular phylogenetic tree of genetic variants of the ITS1-ITS2 sequences for six isolates of *Tilletia caris*, *Tilletia controversa*, and *Tilletia indica*. The black triangle indicates the isolates analyzed.

Effect of covering smut disease on the qualitative characteristics of some wheat varieties (absorption percentage and wet gluten): Results showed in Table 2 that the Nazar variety without the pathogen recorded an absorption rate of 60.00 % superior to

all the varieties Al-arbia, Maya, Pora, and Abo-Graib, which reached 61.50, 59.10, 61.00, and 62.83%, respectively, and the variety Maya recorded the higher absorption rate. 97.10%, significantly superior to all varieties Nizai, Pora, Al-arbia, and Abo-Garib, as the absorption reached 70.50, 71.00, 71.70, and 80.00%, respectively. In the presence of the pathogen. All varieties with the pathogen's presence recorded superiority over those without the pathogen in the absorption rate. These results are consistent with what was stated by (6), which is that flour whose absorbency rate is between 60.1 - 69.7% in the various types of wheat is considered first-class flour, and commercial flour has water absorption. About 60 - 70%. The high percentage of absorption due to the presence of the pathogen reflects negatively on the quality of the flour. The results of Table 2 showed that the Pora variety was significantly superior in wet gluten percentage by 45.50% over all the varieties Al-arbia, Nizai, Abo-Garibe, and Maya, which recorded 31.40, 34.00, 33.90, and 37.70% respectively without the pathogen, and the Pora variety recorded a superiority of 29.00% in wet gluten percentage over the varieties Maya and Abo-Garibe, as it reached 25.06 and 28.00%. Sequencing in the presence of the pathogen, the varieties Abo-Garibe, Al-arbia, and Nazar recorded a wet gluten percentage of 28.00, 31.35, and 29.30%. Respectively, it was superior to the Maya variety, which recorded 25.06% in the presence of the pathogen. All varieties without the pathogen confirmed a significant superiority over the other varieties, which led to the presence of the pathogen in the percentage of wet gluten. The difference in the absolute absorption rate of the two mixtures may be due to the difference in the content of the varieties and wet gluten due to the difference between the varieties in their protein and carbohydrate content, which affects the nature of the interaction between the pathogen and the studied varieties, and this agrees with (6 and 18) that the superiority of wheat varieties in gluten is the presence of protein at a high level in addition to the genetic difference in the varieties.

Table 2: Effect of covering smut disease on the qualitative characteristics of some wheat varieties.

varieties	Absorbency	Wet gluten percentage
Al-arbia + <i>Tilletia caries</i>	71.70	28.35
Al-arbia without <i>Tilletia caries</i>	60.50	31.40
Abo-Garib + <i>Tilletia caries</i>	80.00	28.00
Abo-Garibe without <i>Tilletia caries</i>	62.53	33.90
Nazar+ <i>Tilletia caries</i>	70.50	29.30
Nazar without <i>caries Tilletia</i>	60.00	34.00
Pora+ <i>Tilletia caries</i>	71.00	29.80
Pora without <i>Tilletia caries</i>	61.00	45.50
Maya+ <i>Tilletia caries</i>	97.10	25.06
Maya without <i>Tilletia caries</i>	59.10	37.70
LSD 5%	2.74	2.04

*Each number in the table represents the average of three replicates, with each replicate having three pots and five seeds in each pot.

Effect of chemical induction factors on the germination rate of wheat plants in the presence of the fungus *Tilletia caries*: The results of Table 3 showed that all treatments (hydrogen peroxide, melatonin, salicylic acid) achieved a significant increase in wheat seed germination, reaching 83.74, 82.30, and 80.93%, respectively, compared to the

control treatment contaminated with the fungus *Tilletia caries*, in which the percentage germination reached 78.64%. The results showed that all concentrations led to a significant increase in the germination of wheat seeds, reaching 80.51, 81.07, 81.57%. The results showed that the best interaction between the induction factors and the concentrations used was between the hydrogen peroxide treatment and the concentrations of 400, 300, and 500 ppm, as it reached 84.27, 81.97, 85.00. Sequentially, the effect of the induction factors, the concentrations of their use, and the interaction between the treatments and concentrations is attributed to the ability of these factors to activate physiological processes within the seeds, thus breaking the seed dormancy period, which helped increase the speed of germination in wheat seeds (9 and 19).

Table 3: Effect of some chemical stimulating factors on the germination of wheat seeds contaminated with the fungus *T. caries*.

Transactions	Effect of the interaction between treatments and their addition concentrations on the percentage of germination of wheat seeds			Effect of Treat A
	Soak the seeds in a concentration PPM300	Soak the seeds in a concentration PPM400	Soak the seeds in a concentration PPM500	
Hydrogen peroxide	81.97	84.27	85.00	83.74
Melatonin	81.80	82.23	82.87	82.30
Salicylic	79.70	81.93	81.17	80.93
con- compared without the pathogen	79.73	79.13	79.97	79.68
con+ compared to the presence of the pathogen	79.13	77.93	78.87	78.64
Effect of concentrations	80.51	81.07	81.57	
LSD B=1.12	LSD A*B=2.52		LSD A=1.45	

Each number in the table represents an average of 4 replicates. In each replicate, three pots were planted. In each pot, five seeds were planted.

Effect of chemical induction agents in reducing the percentage of infection with covered smut disease of wheat plants caused by the fungus *T. caries*: Table 4. Showed that all induction factors reduced the infection pathogen, in which the infection rate reached 32.52%. The results showed that the concentration of ppm500 seed soaking was superior to all treatments, as the infection rate reached 17.96%. The two concrete, reaching 15.93, 23.31, and 25.07% compared to the comparison treatment in the presence of the pathogen, 300 and 400 ppm, significantly reduced the percentage of infection, amounting to 20.84 and 19.36%, respectively. The results showed that the best interaction was between the hydrogen peroxide treatment and the concentration of 500 ppm, as the percentage of infection reached 13.50%. The frequency of treatments, the concentrations of their use, and the interactions between the treatments and their concentrations are attributed to the ability of these factors to induce resistance in the plant by raising the concentration of phenols, peroxidases, and other metabolic compounds that play a role in increasing the plant's resistance to infection (8 and 22).

Table 4: Effect of some chemical inducers on resistance to covered smut disease in wheat.

Transactions A	Effect of the interaction between treatments and their added concentrations on the percentage of infection			Treatment effect
	Soak the seeds in concentration PPM300	Soak the seeds in concentration PPM400	Soak the seeds in concentration PPM500	
Hydrogen peroxide	18.23	16.07	13.50	15.93
Melatonin	25.20	23.37	21.37	23.31
Salicylic acid	26.37	25.20	23.93	25.07
Control without pathogen	0.00	0.00	0.00	0.00
control with pathogen	34.40	32.17	31.00	32.52
concentrations	20.84	19.36	17.96	
LSD A=1.56		LSD A*B=2.05		LS,D B=0.89

*Each number in the table represents an average of 4 replicates. In each replicate, three pots were planted. In each pot, five seeds were planted.

Conclusions

The results of study the genetic variation of *Tilletia* spp that was registered in GenBank showed that it is closely related to the global isolates registered in GenBank that belong to the species *T.caries*, *T.controversa*, and *Tilletia indica*; It is widespread in some governorates of Iraq (Anbar, Salah al-Din, Mosul). It causes covered smut disease, and infection with the disease negatively affects the qualitative characteristics of bread, such as absorbency. And the percentage of wet gluten. The results also showed the efficiency of the induction factors and their concentrations in reducing the incidence of the disease and increasing the percentage of seed germination.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Theyab A. Farhan: Methodology and writing the original manuscript. Jasim M. Abed: Methodology and original draft preparation. Ali A. Khadum: Software and statistical analysis, Rowida G. Al-Ani: Data collection, and formatted the final manuscript. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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